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.

ATTENDEES

COMMITTEE MEMBERS	
Lisa Butterfield, Ph.D.	University of California, San Francisco
Kenneth Berns, M.D., M.P.H.	University of Florida
Eric Crombez, M.D.	Ultragenyx Gene Therapies
Tabassum (Taby) Ahsan, Ph.D.	Therapeutic Discovery at MD Anderson Cancer Center
Bernard Fox, Jr., Ph.D.	Providence Portland Medical Center
Randy Hawkins, M.D.	Private Practice
Christopher K. Breuer, M.D.	Nationwide Children's Hospital
Jeannette Yen Lee, Ph.D.	University of Arkansas for Medical Sciences
Joseph Wu, M.D., Ph.D.	Stanford University
John A. Zaia, M.D.	Beckman Research Institute of City of Hope
Mark C. Walters, M.D.	USCF Benioff Children's Hospital Oakland
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Theo Heller, M.D.	National institutes of Health
LaTasha Crawford, V.M.D., Ph.D., D.A.C.V.P.	University of Wisconsin-Madison School of Veterinary Medicine

Roland W. Herzog, Ph.D.	**Indiana University School of Medicine**
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Raymond Roos, M.D.	**University of Chicago**
Carlos Sanchez, M.D.	**Children's National Hospital**
Carles Vite, B.S., D.V.M., Ph.D.	**University of Pennsylvania**
Charles P. Venditti, M.D., Ph.D.	**National Institute of Health**
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SPEAKERS AND GUEST SPEAKERS	
Deepa Chand, M.D.	**Novartis Gene Therapies**
Roland Crystal, M.D.	**Weill Cornell Medicine**
Lindsey A. George, M.D.	**The Children's Hospital of Philadelphia**
Mark S. Sands, Ph.D.	**Washington University School of Medicine**
Denise E. Sabatino, Ph.D.	**The Children's Hospital of Philadelphia**
James (Jim) Wilson, M.D., Ph.D.	**University of Pennsylvania**
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DAY 2

OPENING REMARKS: CALL TO ORDER, INTRO OF COMMITTEE

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

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

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

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

ADMINISTRATIVE ANNOUNCEMENTS, ROLL CALL, INTRODUCTION OF COMMITTEE, CONFLICT OF INTEREST STATEMENT

MR. JARROD COLLIER: Thank you, Dr.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

tissue engineering and stem cells in gene therapy.

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

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

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

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

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

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

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

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

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

MR. JARROD COLLIER: That's perfect.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

We have the following consultants serving as temporary voting members. Dr. Frederic Bushman, Dr. Barry Byrne, Dr. Latasha Crawford, Mr. James DeFilippi, Ms. Peggy DiCapua, Dr. Theo Heller, Dr. Roland Herzog, Dr. Raymond Roos, Dr. Carlos Sanchez, Dr. Charles Venditti, Dr. Charles Vite, and Dr. Caroline Zeiss. Based on today's agenda and all financial interests reported by committee members and consultants, there have been four conflicts of interest waivers issued under 18 U.S. Code Section 208 in connection with this meeting.

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

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

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

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

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

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

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

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

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

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

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

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

Butterfield. Thank you.

FDA OPENING REMARKS

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

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

DR. LISA BUTTERFIELD: Dr. Bryan.

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

therapy products.

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

SESSION 3: THROMBOTIC MICROANGIOPATHY

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

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

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

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

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

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

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

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

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

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

alternative pathway of complements.

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

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

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

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

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

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

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

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

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

the real-world setting.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Other questions that I often get asked are

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

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

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

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

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

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

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

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

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

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

INVITED SPEAKER PRESENTATION Q&A

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.

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?

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

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

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

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

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

variety there which I think raises questions as well.

DR. KENNETH BERNS: Yeah, I guess the question to me is to what extent can we attribute the TMA to the AAV vector versus the oligotherapy, in these cases? Anyway, thank you very much.

DR. LISA BUTTERFIELD: Thank you, Dr. Berns. Next question from Dr. Wu.

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

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

DR. JOSEPH WU: Thank you.

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

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

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

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

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

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

denominator, is that right?

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

DR. RAYMOND ROOS: Thanks.

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

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

The second question would be with respect to

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

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

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

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

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

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

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

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

individualized level.

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

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

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

DR. LISA BUTTERFIELD: Thank you very much.

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

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

DR. CHARLES VENDITTI: Thank you.

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

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

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

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

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

DR. RANDY HAWKINS: Thank you very much.

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

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

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

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

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

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

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

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

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

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

DR. MARK WALTERS: Thank you.

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

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

[BREAK]

SESSION 3: OPEN PUBLIC HEARING

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

DR. LISA BUTTERFIELD: Thank you very much. So welcome to the Open Public Hearing session. 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 of the Advisory Committee meeting, FDA believes that it's important to understand the context of an

individual's presentation.

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

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

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

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

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

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

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

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

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

seen promptly.

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

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

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

So there I was speaking about a pretty typical

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

COMMITTEE DISCUSSION OF QUESTIONS

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

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

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

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

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

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

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

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

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

DR. BARRY BYRNE: Yeah.

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

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

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

MR. MICHAEL KAWCZYNSKI: Well, I hear you. Let's see here.

DR. LISA BUTTERFIELD: For Dr. Byrne.

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

DR. LISA BUTTERFIELD: Yes.

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

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

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

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

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

DR. BARRY BYRNE: Will do. Yep.

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

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

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

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

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

MR. MICHAEL KAWCZYNSKI: Yes, go ahead.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

So, when we see the extreme risk manifest of

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

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

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

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

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

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

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

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

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

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

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

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

I'll stop there.

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

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

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

patients this pathway is active.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

And I'll just mention one other thought is

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

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

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

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

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

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

adding the rapamycin to- (audio skip).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

contain full AAV genomes with two IgRs.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

would normally schedule. It looks okay.

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

[LUNCH BREAK]

SESSION 4: NEUROTOXICITY: DORSAL ROOT GANGLION (DRG) TOXICITIES

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

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

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

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

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

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

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

peripheral nerves.

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

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

It turns out we had not recovered DRGs because

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

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

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

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

Now, what about background lesions which are

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

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

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

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

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

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

away, and it's not a progressive finding.

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

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

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

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

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

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

There's a way to evaluate a DRG toxicity or

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

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

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

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

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

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

duration of the study bilaterally.

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

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

But interestingly, the two in which we saw

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

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

high dose AAV.

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

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

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

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

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

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

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

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

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

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

And so the initial study of this was an enzyme

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

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

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

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

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

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

So just to conclude, we do believe the

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

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

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

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

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

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

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

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

INVITED SPEAKER PRESENTATION Q&A

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

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

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

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

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

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

DR. CHARLES VITE: Okay. Thank you.

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

Crombez.

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

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

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

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

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

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

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

DR. BARRY BYRNE: Okay. Helpful. Thanks.

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

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

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

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

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

DR. JAMES WILSON: How what?

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. BERNARD FOX: Great. Thank you.

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

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

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

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

DR. JAMES WILSON: Right.

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

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

are you referring to clinical measures or histopathologic analysis?

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

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

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

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

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

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

DR. JAMES WILSON: Right.

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

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

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

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

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

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

[BREAK]

SESSION 4: OPEN PUBLIC HEARING

Mr. MICHAEL KAWCYNSKI: 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?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

AEs across trial.

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

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

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

MR. JARROD COLLIER: Yes we can.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

COMMITTEE DISCUSSION OF QUESTIONS

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

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

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

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

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

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

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

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

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

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

cooperation between different groups involved in gene therapy trials.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. KENNETH BERNS: Hello?

DR. LISA BUTTERFIELD: There you go.

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

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

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

The second part and related to that -- and

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

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

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

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

And we have some evidence that even at doses

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

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

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

We still have time for additional comment.

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

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

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

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

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

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

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

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

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

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

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

So, these are important issues with respect to

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. BARRY BYRNE: No additional comments, actually.

DR. LISA BUTTERFIELD: Right. Dr. Zeiss.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. LISA BUTTERFIELD: Great thanks very much.

Dr. Kenneth Berns.

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

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

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

DR. KENNETH BERNS: Thank you.

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

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

In terms of animals, dogs were raised as a

model that would be a naive state to the AAV vector. Porcine models were mentioned that would also be excellent for asking questions of DRG toxicities.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

patients.

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

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

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

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

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

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

for clinical trials. Recommendations to mitigate the risks,

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

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

Selective use of MRI testing, while costly,

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

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

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

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

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

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

large animal species.

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

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

I think these are important issues, complex

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

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

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

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

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

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

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

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

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

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

SESSION 5: NEUROTOXICITY: BRAIN MRI FINDINGS

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

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

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

INVITED SPEAKER PRESENTATION: CLINICAL AND NONCLINICAL CONSEQUENCES OF DIRECT CNS PARENCHYMAL ADMINISTRATION OF AAV VECTORS

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

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

The disease onset is ages 2 to 4. These

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Those were the lesions that we saw. This is

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

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

time.

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

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

examples as well.

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

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

this is the same vector AAVrh.10 but now coding for hARSA. It had a FLAG tag on it. And was administered to the cortex of African Green nonhuman primates. The doses where 2.85 times 10 to the tenth gc total, 2.4 times 10 to the ninth per site, and another series of animals at a higher dose of 1.5 times 10 to the twelfth gc and 1.3 times 10 to the eleventh gc per site. And nonhuman primates were evaluated at 0, 13, 26 and 52 weeks.

And we saw similar lesion we saw in humans, but we also saw it with the capsid itself, that's on the second row. That's a Null vector, an AAVrh.10Null vector, at 1.5 times 10 to the twelfth. And we saw lesions. And then the row after that is the lower dose of the hARSA vector, and then the higher dose is shown next. So, it's dose dependent and seen in nonhuman primate as well as humans.

In terms of histology, the PBS control, you

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

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

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

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

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

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

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

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

the mode of administration.

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

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

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

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

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

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

INVITED SPEAKER Q&A

DR. LISA BUTTERFIELD: Terrific. Thank you

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

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

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

vector tip it still maybe useful.

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

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

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

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

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

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

catheter tip goes in?

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

MS. PEGGY DICAPUA: All right. Thank you for

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

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

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

DR. RONALD CRYSTAL: That's correct.

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

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

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

DR. BERNARD FOX: Thank you.

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

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

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

DR. KENNETH BERNS: Good afternoon, Ron. The

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

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

DR. KENNETH BERNS: Thank you.

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

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

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

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

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

MR. JAMES DeFILIPPI: Thank you.

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

[BREAK]

COMMITTEE DISCUSSION OF QUESTIONS

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

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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. LISA BUTTERFIELD: Thank you very much. Dr. Fox?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. LISA BUTTERFIELD: Thank you for that

addition. Dr. Zeiss?

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

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

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

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

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

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

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

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

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

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

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

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

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

invasive than a vector distribution by an LP.

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

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

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

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

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

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

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

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

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

diffusion changes, those are pretty subtle.

The point I was trying to make is that there's always a footprint with the neurosurgical procedure beyond the purpose of the procedure itself. Let's say removing the tumor, the tumors gone. But the frequency of, let's say, T2 changes due to the placement of a retractor or something else like that, are very common. As neurosurgeons we typically measure our success post-operatively, not so much on the asymptomatic radiological changes post-operatively, but really more the symptomatic, transient or permanent neurological deficits.

I was just trying to contextualize that, given that this is a much more invasive procedure relative to a cisternal tap or an LP, it's somewhat expected that we'll see changes like this. And the real measure is whether the patient's changed neurologically after this procedure, even despite if they remain on imaging for

months or years afterwards.

DR. LISA BUTTERFIELD: Thanks very much for that further clarification.

DR. CARLOS SANCHEZ: Sure.

DR. LISA BUTTERFIELD: All right, other comments, questions and points to be raised by committee members? Dr. Crystal, anything you wanted to add at this point from the first few comments from the committee?

DR. RONALD CRYSTAL: We would agree with all the comments that were made. As I said, the methodology, as we just heard from Dr. Sanchez, I mean, putting catheters in the brain is something that's standard in the neurosurgical world. And the issue is really, can we effectively deliver the product of our vectors throughout the brain with this technology? I think the answer is, you can do it in a limited area but not throughout the brain.

DR. LISA BUTTERFIELD: All right, thank you. All right, other thoughts? Other questions or points to raise from the committee? If not, I can summarize a couple of points made in response to this question. All right, so the clinical significance to date seems to be very minor. These MRI findings, these T2, diffusion changes seem to be quite minor, clinically silent. And while they have resulted in the lessons learned about gene transfer with the adenoviral vectors, that delivery procedure may not be as efficacious as others under current investigation. Other thoughts to add to that?

All right, then we are going to efficiently move to the third question. Our third of four questions for the committee to discuss and provide recommendations on, we're asked to provide recommendations on strategies that could be implemented before and after vector administration to prevent or

mitigate the risk of central nervous system injury. We'll start with Dr. Roos to initiate the discussion.

DR. RAYMOND ROOS: First, I wonder about features of the vector or the transgene that make them especially prone to trigger pathology, be it inflammatory pathology or edema. Maybe that's something that could be clarified in animal studies because an issue with human intraparenchymal injections is that we invariably lack all the controls that would be appropriate, because we're dealing with human patients. I think obtaining MRIs is clearly important, probably in regular intervals.

In addition, I don’t know whether the use of immunosuppression has been clarified as well as it could be. And perhaps this should be initiated with intraparenchymal injections in animals and, then if it looks promising, in human studies as well. The issue with respect to strategies in humans really probably

relates to localized injections for somewhat localized disease. And so, having animal models that might mirror those particular instances would be valuable in the study. Thanks.

DR. LISA BUTTERFIELD: Thank you for starting us off. Now looking to the committee members to weigh in on their thoughts on recommendations on strategies to prevent or mitigate risk of CNS injury. Thank you, Dr. Sanchez.

DR. CARLOS SANCHEZ: I would start by saying that any neurosurgeons that are working for a CED trial will apply general principles that we apply for passing a catheter through the brain. So the trajectories that they pick, the avoidance of vessels, the avoidance of sulci where we'll lose the vector, will be in their mind. And then with that would follow routine post-op management with routine post-op imaging and seizure control if needed. Then you can layer on sort of

technical aspects, so the convection enhanced delivery that's been worked up by multiple groups, and I would sort of parse that into different cannula designs, different settings for the actual infusion with CED or otherwise, and then use of adjuncts like mannitol for brain swelling if needed.

Then I think one thing that some groups are using is the use of interoperative MRI as well as the co-infusion of gadolinium to actually see the infusion in real time. I think that that will provide in the future a lot more safety in terms of seeing where the cannula tip is, where is the infusion starting. It allows us to actually adjust the cannula position in real time as the infusion is occurring. It's seeing our target, it's identifying infusion leak around the catheter, reflux if that happens. Then one of the challenges of injecting deeper spaces is this idea of losing some of the vector with perivascular

distribution. If we see that, we can actually advance a catheter as the infusions happening.

So, there's more of a cost with that, of using interoperative MRI suite. And there's more of a technical aspect in using MRI-compatible equipment for a procedure like that, but I think the future of CED, again, understanding that it's not a whole brain vector distribution but a localized one, that we'll benefit from using those strategies.

DR. LISA BUTTERFIELD: Thanks very much for those specific recommendations. All right, additional thoughts from the committee on recommendations to mitigate this risk? What we've heard thus far are that certainly the surgical techniques in place mitigate a lot of the risk, but there are new approaches to modifying the infusion that can be adopted. And consideration of MRI-guided real-time imaging during infusion could help minimize some of this risk and

track the infusion to optimize those outcomes.

Then, further study of the viral vector responses and transgene responses in animal models, those could then identify new mechanisms that would then shed light on additional mechanisms that might be employed, including a possible mitigation role for immune suppression. Thoughts in addition to what I've just summed up? Assuming, Dr. Sanchez, that is the previous hand up and not a new hand up. But let me know if you have something to add. Okay.

All right, if we have no further recommendations from the committee on question three, then this is our final question for discussion along the committee members. "Please recommend a duration of monitoring for subjects who have abnormal brain MRI findings, or factors to consider for the determination of an appropriate duration of monitoring." We'll start with, again, Dr. Roos, our discussant.

DR. RAYMOND ROOS: One has concern about intraparenchymal delivery of AAV vectors after seeing MRIs that Dr. Crystal showed. I think monitoring on a regular basis, patients that have abnormal MRIs, or just intraparenchymal injections would be important. Perhaps 1 month, 3 months, 6 months, 12 months and maybe yearly.

The duration of monitoring and interventions might depend a little bit on the MRI abnormality. For example, there's a hemorrhage that's visualized that's of concern, maybe this patient needs to be hospitalized and maybe further interventions might be necessary. The use of steroids or some immunosuppression also might be entertained. I have the feeling that intraparenchymal injections, especially new catheter design, is a little bit of a pioneer area with respect to AAV delivery, and maybe a dynamic and changing one. And for that reason, I think that monitoring patients

in a rigorous way would be suggested. Thanks.

DR. LISA BUTTERFIELD: Great, thank you very much. We have now some initial thoughts on that monitoring frequency. Professor Fox.

DR. BERNARD FOX, JR.: Yeah, I didn't mention it in the last question because it seemed like it'd been covered before, but in thinking about, as Dr. Roos was presenting this data, the idea that when you have these abnormal MRI findings that it's going to be relatively easy to have an overlapping (inaudible) library of the gene of interest and to be able to go back and look at whether or not that correlates with systemic immunity for blood assays. Those are pretty straightforward. And then identifying what were the profiles of those immune cells that might be recognizing it, or if they're not recognizing that, versus the viral or the capsids. Thank you.

DR. LISA BUTTERFIELD: Great, thank you for

that perspective. I guess, so if I understood Dr. Crystals data correctly, these effects have not been disappearing. So they were not reversible, but they have been clinically silent to date. So I guess the question for Dr. Crystal would be, how long of a follow-up has your group been performing to perhaps guide a recommendation in that area?

DR. RONALD CRYSTAL: The longest we've followed the nonhuman primates has been one year. The duration of the study with the children was 18 months, since we had that at 18 months. In a small proportion, the lesions disappear. In most, they continue, but they're not increased in terms of size or extent.

In the trial itself we did several months and one year and 18 months, so 6 months, 18 months, done. In terms of the answer to the question of how long you should follow them, probably, if you see the lesions, a yearly basis would be a rational thing to think about.

DR. LISA BUTTERFIELD: Great, thank you for that. Ms. DiCapua.

MS. PEGGY DICAPUA: I was just going to say, yeah, I would think you would have to come out for a couple of years to see and follow it. In the cases that I know for degenerative, I mean, it also depends on how fast the brain -- there's progression of the degeneration, how fast that's progressing. So, you're looking at that -- so you're seeing deterioration, the MRI, you're seeing that, but possibly you could see where the injections were and you could see that disappearing, I don't know, but, yeah, I would think you'd have to go out at least a couple of years if you can, if the subject is able to.

DR. LISA BUTTERFIELD: Yes, thank you for that. Dr. Roos.

DR. RAYMOND ROOS: Yeah, I just wanted to get back to the topic of whether these MRI abnormalities

are clinically symptomatic. And I wanted to ask Dr. Crystal whether that was clearly the case in these patients with ceroid lipofuscinoses?

DR. RONALD CRYSTAL: The question was did we see them progress at all?

DR. RAYMOND ROOS: My question was whether it was clear that these MRI lesions from the intraparenchymal injections had no clinical effect on the patients.

DR. RONALD CRYSTAL: There's a caveat to my answer, I mean, the answer is we saw no clinical correlates. However, these children clearly have neurologic disease and so, whatever I'm looking at is what you're looking at is something superimposed. And of course, I'm sure you would agree, it's challenging to see new lesions. But as far as we could determine, there was no clinical correlates beyond the primary disease itself.

DR. LISA BUTTERFIELD: Thank you. Dr. Roos, any follow-up? Did that answer your question?

DR. RAYMOND ROOS: Yes.

DR. LISA BUTTERFIELD: All right, thank you. Dr. Kenneth Berns, please.

DR. KENNETH BERNS: Well, again, this is just an informational question. Ron, what's the life expectancy of the patients that you're treating this way? How long are they really going to survive? I can't hear.

DR. RONALD CRYSTAL: Their after life expectancy is 8 to 10 years.

DR. KENNETH BERNS: Okay, thank you. In that case, I guess long-term monitoring might be useful.

DR. RONALD CRYSTAL: The one caveat I'd make in terms of trying to follow those children, is that to do an MRI in a child with one of these disorders, you'd have to use general anesthesia. That's another

complication that is challenging in terms of how often you want to do that.

DR. LISA BUTTERFIELD: All right. Thank you for that discussion. Okay. Let's check the hands up and see if indeed the hands up are current hands up for new comments. All right, so any additional comments from members of our panel today on the topic of duration of monitoring and what factors to consider to determine that? Okay, so what I've heard is that initially perhaps one, three, and six months, but then following annually would be appropriate. One year follow-up in the nonhuman primates has been done in the past, but we have to keep in mind that in a number of pediatric patient settings that the MRI follow-up is challenging in that setting.

Then the other note is that if an immune component is found to be important in these MRI findings, that that could be something that might be

monitored in the blood, and then to understand that immune mechanism might allow for a blood biomarker to follow that immune response over time.

Anything else to add to that quick summary of the recommendations and discussion points? All right. Then, with that, we have these response to the four questions. Now, we have an opportunity, I'll ask Dr. Wilson Bryan and see if our FDA colleagues have other clarifications they would like.

DR. WILSON BRYAN: Thank you. The discussions been great. I do have one follow-up question. And I don't like to dwell too much on hypotheticals, but we are really very early, I think, in the therapeutic world for gene therapies. And in the years ahead, I wouldn't be surprised if we do see some patients who have MRI findings with associated clinical manifestations.

In that event, any thoughts from the

committee? Would their recommendations change in any way with regard to those specific subjects, or to the field? Would the recommendations change in any way if in the years ahead we do have examples of clinical manifestations?

DR. LISA BUTTERFIELD: I'm watching for a show of hands from members of the committee that have weighed in on these recommendations. Dr. Roos.

DR. RAYMOND ROOS: I think that certainly that would be a concern and perhaps the details would be important with respect to what the disease was, what risk/benefits were involved, was there a mistake in the implementation of the intraparenchymal delivery. It is a bit hypothetical, but I have no question that there would be concerns, and maybe investigations of that particular clinical case. And whether one was dealing with some idiosyncratic reaction rather than something that might recur.

I don't know whether I've answered the question, but I think having significant issues with gene therapy approaches is going to impact the field, no question. Looking carefully at that case and the details would be important as far as learning and perhaps modifying the route of entry and distribution and catheter design features.

DR. LISA BUTTERFIELD: Thank you. And Dr. Fox, did you also want to weigh in on this question?

DR. BERNARD FOX, JR.: Yeah. I think, to Dr. Bryan's point and coming back to Dr. Wilson's comments about GFP being immunogenic. I think in cancer we're really excited to be studying the surfaceome now and the different proteins, whether or not proteins are short-lived or long-lived. If they're short-lived, they tend to end up more into the proteosome and get through the ER to be presented more in class one molecules where they can be targets of immune response.

I think as we look at the different gene therapies and the different transgenes, whether or not those transgenes, as part of their function, end up more as a long-lived protein or as a short-lived protein, might end up -- will certainly affect their expression on the surface and their ability to be targeted, as well as the degree of dissimilarity with other gene families in the body.

So just thinking about it from the immune side, the potential there is pretty strong to see something that might be a unique epitope and represents a neoantigen that there's a strong response against. I would be looking for those kinds of things as the trials progress and if you saw a series of adverse events.

DR. LISA BUTTERFIELD: Thank you. Dr. Bryan. Other questions or clarifications from the FDA to the session, or anything else over our last two days,

really?

DR. WILSON BRYAN: This is very helpful. The discussion has actually been very invigorating for me. Very thorough and helpful, so I really appreciate the perspective of the committee, and want to re-emphasize how important it is that all the stakeholders communicate with each other as we go forward in thinking about these safety issues. So, thank you very much.

DR. LISA BUTTERFIELD: All right then, I will hand the meeting to Jarrod to start the closing procedures, and here we have Dr. Peter Marks.

CLOSING REMARKS

DR. PETER MARKS: I just wanted to thank everyone on the committee for a really excellent discussion. I was able to actually listen to most of

today and at least a part of yesterday. Really, I completely agree with Dr. Bryan, it was an incredibly robust discussion, so I think it was exactly what we were looking for. Thank you so much for that.

I also want to thank the staff of the Office of Tissues and Advanced Therapy for preparing everything so well, and also the advisory committee staff for putting this together and all of the work that goes into this.

Just to finish up by saying that I think for those who may be listening in who aren't as familiar with gene therapies, some might be concerned that we have all of these different side effects that we've been talking about regarding gene therapies. And they may wonder, are these things going to become a reality? I would say the fact that we're discussing these is evidence that they very much are becoming a reality.

And it's actually a good sign because every

medical therapy, as it comes along, we have to deal with the side effects that may come up and address them. So, I think that this robust discussion will help us address these. And the fact that we're having to address them is a very good sign that we're actually making progress here.

We'll obviously have a lot to take back from the discussion to think about, and we really appreciate that. Given that this is the end a long day prior to a holiday weekend, I'm going to keep my remarks relatively short and just finish by saying, thank you so much to everyone once again, and I wish you a very good holiday weekend.

ADJOURN

MR. JARROD COLLIER: Thank you very much, Dr. Marks, for your closing remarks. At this point that

concludes the 70th meeting of the Cellular Tissue and Gene Therapies Advisory Committee. I would like to thank all participants for their time and effort to conduct today's meeting, and I would also like to give special thanks to Mr. Michael Kawcynski for managing the Adobe Connect platform throughout the two-day meeting, and also, our wonderful chair, Dr. Lisa Butterfield, for facilitating the discussion questions and all relevant information regarding this meeting. At this point the meeting is now adjourned. Thank you all for your participation and have a great evening and holiday weekend. Thank you very much.

[WHEREAS MEETING ADJOURNED FOR THE DAY]