

**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
TEMPORARY VOTING MEMBERS	
Frederic Bushman, Ph.D.	Perelman School of Medicine University of Pennsylvania
Peggy DiCapua	Advanced Real Estate, LLC
Barry J. Byrne, M.D., Ph.D.	University of Florida
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
James DeFilippi, M.B.A., P.M.P.	DeFilippi Consulting, LLC

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
Caroline Zeiss, D.A.C.V.P., D.A.C.L.A.M., Ph.D.	Yale University School of Medicine
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
FDA PARTICIPANTS/SPEAKERS	
Peter W. Marks, M.D., Ph.D.	Food and Drug Administration
Wilson Bryan, M.D.	Food and Drug Administration
Leila Hann	Food and Drug Administration
Raj Puri, M.D., Ph.D.	Food and Drug Administration
Denise Gavin, Ph.D.	Food and Drug Administration
Celia Witten, Ph.D., M.D.	Food and Drug Administration
Rachael F. Anatol, Ph.D.	Food and Drug Administration
Tejashri Purohit-Sheth, M.D.	Food and Drug Administration

Andrew Byrnes, Ph.D.	Food and Drug Administration
Dan Urban, Ph.D.	Food and Drug Administration
Mercedes Serbian, M.S.	Food and Drug Administration
Rosa Sherafat-Kazemzadeh, M.D.	Food and Drug Administration
Vijay Kumar, M.D.	Food and Drug Administration
Lei Xu, M.D., Ph.D.	Food and Drug Administration
Zenobia F. Taraporewala, Ph.D.	Food and Drug Administration
Gaya Hettiarachchi, Ph.D.	Food and Drug Administration
FDA ADMINISTRATIVE STAFF	
Prabhakara Atreya, Ph.D.	Food and Drug Administration
Joanne Lipkind, M.S.	Food and Drug Administration
Jarrod Collier, M.S.	Food and Drug Administration
Christina Vert, M.S.	Food and Drug Administration

TABLE OF CONTENTS

1	DAY 1	6
2	OPENING REMARKS: CALL TO ORDER, WELCOME.....	6
3	ADMINISTRATIVE ANNOUNCEMENTS, ROLL CALL, INTRODUCTION OF	
4	COMMITTEE, CONFLICT OF INTEREST STATEMENT	7
5	FDA OPENING REMARKS	28
6	FDA PRESENTATION "TOXICITY RISKS OF ADENO-ASSOCIATED VIRUS (AAV)	
7	VECTORS FOR GENE THERAPY (GT)"	30
8	FDA PRESENTATION Q&A	58
9	SESSION 1: VECTOR INTEGRATION AND ONCOGENICITY RISKS.....	60
10	INVITED SPEAKER PRESENTATION: "rAAV INTEGRATION: In Vitro & MICE"	60
11	INVITED SPEAKER PRESENTATION Q&A.....	80
12	INVITED SPEAKER PRESENTATION: "AAV INTEGRATION STUDIES IN LARGE	
13	ANIMAL MODELS: NON-HUMAN PRIMATES AND DOGS"	88
14	INVITED SPEAKER PRESENTATION Q&A.....	107
15	OPEN PUBLIC HEARING SESSION	117
16	COMMITTEE DISCUSSION OF QUESTIONS.....	137
17	SESSION 2: HEPATOTOXICITY	215
18	INVITED SPEAKER PRESENTATION: "CLINICAL FINDINGS OF HEPATOTOXICITY OF	
19	AAV VECTORS FOR GT"	215
20	INVITED SPEAKER PRESENTATION Q&A.....	237
21	INVITED SPEAKER PRESENTATION: "HEPATOTOXICITIES AND OTHER RELEVANT	
22	FINDINGS IN ANIMAL STUDIES"	251
23	INVITED SPEAKER PRESENTATION Q&A.....	277
24	OPEN PUBLIC HEARING	281
25	COMMITTEE DISCUSSION OF QUESTIONS.....	295
26	ADJOURN	364

1

DAY 1

2

OPENING REMARKS: CALL TO ORDER, WELCOME

3

4

MR. MICHAEL KAWCZYNSKI: Welcome to the 70th

5

meeting of the Cellular, Tissue and Gene Therapy

6

Advisor Committee meeting. My name is Mike Kawczynski

7

and I will be today's moderator, running things from

8

the backend. I do know that, due to hurricanes and all

9

that, we do have members that may be impacted as well.

10

If we do run into any technical problems we will

11

address that as we go along. Again, thank you and

12

let's get it started. Jarrod Collier is our DFO today

13

and Dr. Lisa Butterfield is our committee chair. Go

14

ahead and take it away.

15

DR. LISA BUTTERFIELD: Good morning everyone.

16

I'd like to welcome everyone to this meeting today.

17

I'd like to welcome all of the members of the

18

committee, the temporary members of the committee, the

19

presenters, all of the participants, our colleagues

20

from the FDA and the public and those in the audience

1 joining us today. We're going to spend the next two
2 days together talking over a number of very important
3 points. To bring the meeting to an opening, I'd like
4 to hand it to our Designated Federal Officer, Jarrod
5 Collier. Jarrod?

6

7

ADMINISTRATIVE ANNOUNCEMENTS, ROLL CALL,

8

INTRODUCTION OF COMMITTEE, CONFLICT OF INTEREST

9

STATEMENT

10

11

MR. JARROD COLLIER: Thank you, Dr.

12

Butterfield and good morning everyone. My name is

13

Jarrold Collier and it is my pleasure to serve as the

14

Designated Federal Officer for today's 70th CTGTAC

15

meeting. On the behalf of the FDA, the Center for

16

Biologics Evaluation and Research and the committee, I

17

would like to welcome everyone to Day 1 of a two-day

18

virtual meeting. The meeting for today will be to

19

discuss toxicity risks of adeno-associated virus

20

vector-based gene therapy products.

1 The discussion topics include oncogenicity
2 risks due to vector genome integration and safety
3 issues identified during preclinical and/or clinical
4 evaluation. Today's meeting topic was described in the
5 Federal Register Notice that was published on July 26,
6 2021. At this time, I would like to acknowledge the
7 contributions of a few other members of the Division of
8 Scientific Advisors & Consultants team, including our
9 director, Dr. Prabhakara Atreya, Joanne Lipkind, Karen
10 Thomas, Christina Vert, who will serve as the back-up
11 DFO for today's meeting, and Kathleen Hayes, who have
12 all assisted in preparing for this meeting.

13 I would also like to express many thanks to
14 Mr. Michael Kawczynski for facilitating the meeting
15 today. For any media or press-related questions, you
16 may contact FDA's Office of Media Affairs at
17 fdaoma@fda.hhs.gov. The transcriptionist for today's
18 meeting is Mr. Graham Koester.

19 We will begin today's meeting by taking a
20 formal roll call for the committee members and

1 temporary voting members. When it is your turn, please
2 turn on your video camera and unmute your phone, then
3 state your first and last name, your expertise and your
4 organization. When finished, please turn your camera
5 off and we'll proceed to the next person. Please see
6 the member roster slide in which we will begin with the
7 chair, Dr. Lisa Butterfield. Dr. Butterfield, could
8 you please introduce yourself?

9 DR. LISA BUTTERFIELD: Good morning, my name
10 is Lisa Butterfield. I'm the vice-president of
11 research and development at the Parker Institute for
12 Cancer Immunotherapy and an adjunct professor of
13 microbiology and immunology at the University of
14 California San Francisco. My expertise is in tumor
15 immunology and cancer immunotherapy.

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

18 DR. TABBASUM AHSAN: Good morning. My name is
19 Dr. Taby Ahsan. I'm the head of Analytical Development
20 and Characterization at the cell therapy manufacturing

1 facility at MD Anderson. My expertise has long been in
2 stem cell and tissue engineering and biomechanics.
3 More recently I've focused on adaptive cell therapies
4 for immuno-oncology.

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

7 DR. KENNETH BERNS: Yes, I'm Kenneth Berns.
8 I'm not the person who's in my photograph. That's the
9 stock photograph listed. I am professor emeritus of
10 molecular genetics and microbiology at the University
11 of Florida College of Medicine. I've been working with
12 AAV since 1968.

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

15 DR. CHRISTOPHER BREUR: Good morning. My
16 name's Chris Breur. I'm the director of regenerative
17 medicine at Nationwide Children's Hospital. My area of
18 expertise is tissue engineering and regenerative
19 medicine. Thank you.

20 MR. JARROD COLLIER: Thank you, Dr. Breur.

1 Next, we have Dr. Berny Fox.

2 DR. BERNARD FOX: Hi, my name is Dr. Bernard
3 Fox. I'm the Harder Family Chair for cancer research at
4 the Earl A. Chiles Research Institute, part of the
5 Providence Health System. My expertise is in tumor
6 immunology and in cancer immunotherapy.

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

9 DR. RANDY HAWKINS: Hi. Good morning. Dr.
10 Randy Hawkins, consumer representative. Expertise in
11 pulmonary and critical care medicine. Private practice
12 in the Charles R. Drew Medical Science Center.

13 MR. JARROD COLLIER: Thank you, Dr. Hawkins.
14 Next, we have Dr. Jeanette Lee.

15 DR. JEANETTE LEE: Good morning, my name is
16 Jeanette Lee. I'm a professor of biostatistics and a
17 member of the Winthrop P. Rockefeller Cancer Institute
18 at the University of Arkansas for Medical Sciences in
19 Little Rock. Thank you.

20 MR. JARROD COLLIER: Thank you, Dr. Lee. Next

1 on the list, we have Dr. Eric Crombez.

2 DR. ERIC CROMBEZ: Hello, I'm Eric Crombez.
3 I'm the chief medical officer at Ultragenyx Gene
4 Therapy. My expertise is in the development of gene
5 therapies for rare disorders.

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

8 DR. MARK WALTERS: Hi, I'm Mark Walters. I'm
9 a Professor of Pediatrics at University of California.
10 I direct the blood and marrow transplant program at
11 Children's Hospital. My expertise is in hematology and
12 oncology, blood and bone marrow transplantation.

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

15 DR. JOSEPH WU: Good morning. My name is Joe
16 Wu. I'm a professor of medicine and radiology and
17 director of the Stanford Cardiovascular Institute. My
18 expertise is in cardiac-gene therapy, tissue
19 engineering and stem cell genomics. Thank you.

20 MR. JARROD COLLIER: Thank you, Dr. Wu. Next,

1 we have Dr. John Zaia.

2 DR. JOHN ZAIA: Yes. I'm John Zaia. I'm the
3 director of the Center for Gene Therapy at City of Hope
4 in Duarte, California. My area of interest is in the
5 clinical application of genetic vectors.

6 MR. JARROD COLLIER: Okay. Thank you very
7 much, Dr. Zaia. Next, we're moving to our temporary
8 voting members, starting with Dr. Frederic Bushman.
9 Dr. Bushman, you're on mute right now.

10 MR. MICHAEL KAWCZYNSKI: He's connecting his
11 audio right now. Let's go on, and we'll go back to Dr.
12 Bushman after he gets his audio connected.

13 MR. JARROD COLLIER: Okay. Next, we have Dr.
14 Barry Byrne.

15 DR. BARRY BYRNE: Good morning, I'm Barry
16 Byrne. I'm the director of the Powell Gene Therapy
17 Center at the University of Florida College of
18 Medicine. My expertise is in clinical pediatric
19 cardiology and immunology and AAV vector translational
20 research.

1 MR. JARROD COLLIER: Thank you, Dr. Byrne.

2 Next, we have Dr. LaTasha Crawford.

3 DR. LATASHA CRAWFORD: Good morning, I'm
4 LaTasha Crawford. I'm a neuroscientist with expertise
5 in cell-specific mechanisms of pain and peripheral
6 neuropathy. I'm also a board certified veterinary
7 pathologist.

8 MR. JARROD COLLIER: Thank you, Dr. Crawford.

9 Next, we have James DeFilippi.

10 MR. JAMES DEFILIPPI: Hello, my name is James
11 DeFilippi. I'm a project manager with BR+A Consulting
12 Engineers focusing particle therapy development. I'm
13 also a patient representative and have hemophilia A.

14 MR. JARROD COLLIER: All right. Thank you,
15 Dr. DeFilippi. Next, we have Ms. Peggy DiCapua.

16 MS. PEGGY DICAPUA: Hi, I'm Peggy DiCapua.
17 I'm a patient rep, and my background is -- I spent 30
18 years in the pharmaceutical industry in administrative
19 roles. I'm the former president for New York/New
20 Jersey chapter Batten Disease Support and Research.

1 MR. JARROD COLLIER: Thank you, Peggy. Next,
2 we have Dr. Theo Heller.

3 DR. THEO HELLER: Hi, my name is Theo Heller.
4 I'm a hepatologist with the National Institutes of
5 Health. My area of expertise is liver disease.

6 MR. JARROD COLLIER: Thank you, Dr. Heller.
7 Next, we have Dr. Roland Herzog.

8 DR. ROLAND HERZOG: Good morning. My name is
9 Roland Herzog. I'm the director of the gene and cell
10 therapy program at the Herman B. Wells Center for
11 Pediatric Research at Indiana University. I run the
12 basic science lab that's primarily working on the
13 biology of AAV vectors.

14 MR. JARROD COLLIER: Okay. Thank you, Dr.
15 Herzog. Next, we have Dr. Raymond Roos. There may be
16 a connection issue, Mike, or perhaps this is a mute?

17 MR. MICHAEL KAWCZYNSKI: I just unmuted him.

18 MR. JARROD COLLIER: Okay.

19 MR. MICHAEL KAWCZYNSKI: Go ahead, Raymond.

20 DR. RAYMOND ROOS: I'm a professor in the

1 Department of Neurology at the University of Chicago
2 Medical School. My expertise is in neurodegenerative
3 diseases and unconventional virus infections. Thanks.

4 MR. JARROD COLLIER: Thank you, Dr. Roos.
5 Next, we have Dr. Carlos Sanchez.

6 DR. CARLOS SANCHEZ: Good morning, my name is
7 Carlos Sanchez. I'm a pediatric neurosurgeon at
8 Children's National Hospital in George Washington
9 University, and my interest is brain tumor
10 immunotherapy and CNS vector delivery.

11 MR. JARROD COLLIER: Okay. Thank you, Dr.
12 Sanchez. Next, we have Dr. Charles Venditti.

13 DR. CHARLES VENDITTI -- National Human Genome
14 Research Institute-- senior investigator --

15 MR. JARROD COLLIER: I'm sorry.

16 DR. CHARLES VENDITTI: Sorry.

17 MR. JARROD COLLIER: No, I'm sorry. You can
18 proceed.

19 DR. CHARLES VENDITTI: I'm the senior
20 investigator in the National Human Genome Institute.

1 I'm a pediatric biochemical geneticist. I work on
2 translational aspects of AAV gene therapy and AAV
3 integration.

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

6 DR. CHARLES VITE: Yes, hi. I'm Charles Vite.
7 I'm a veterinary neurologist and also the director of
8 the National Referral Center of Animal Models of Human
9 Genetic Disease. I'm at the University of
10 Pennsylvania. My expertise is in animal models and
11 gene therapy of the brain.

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

14 DR. CAROLINE ZEISS: Good morning, everybody.
15 I'm Caroline Zeiss. I'm a veterinarian boarded in
16 anatomic pathology and laboratory animal medicine. I'm
17 a professor in comparative medicine at Yale University.
18 My expertise is in neuropathology and translational
19 animal models. Thank you.

20 MR. JARROD COLLIER: Okay. Thank you very

1 much, Dr. Zeiss. Let's go back to Dr. Bushman. Were
2 you able to connect? Sounds like you're still muted at
3 this point.

4 DR. FREDERIC BUSHMAN: Are you able to hear
5 me?

6 MR. JARROD COLLIER: Yes. We can hear you
7 now. Thank you.

8 DR. FREDERIC BUSHMAN: Okay, my name's Ric
9 Bushman. I'm a professor of microbiology at the
10 University of Pennsylvania, chair of the department.
11 My lab has studied genomics of integration of gene
12 therapy vectors and other genomic parasites for many
13 years. I've collaborated with a number of the folks on
14 this call. Thank you.

15 MR. JARROD COLLIER: Thank you very much, Dr.
16 Bushman, and thank you all for your introductions. At
17 this time, I would like to acknowledge our leadership
18 team from the Center of Biologics Evaluation and
19 Research; Dr. Peter Marks, Director; Dr. Celia Witten,
20 Deputy Center Director; Dr. Wilson Bryan, Director of

1 Office of Tissue and Advanced Therapies, who will be
2 providing FDA opening remarks; and Dr. Rachael Anatol,
3 Deputy Director for Office of Tissues and Advanced
4 Therapies. Dr. Peter Marks will join the meeting later
5 to provide closing remarks.

6 Before we begin with reading the Conflict of
7 Interest Statement, I would like to briefly mention a
8 few housekeeping items for today's virtual meeting
9 format. For members, speakers, FDA staff and anyone
10 else joining us in the adjoining room, please keep
11 yourself on mute unless you are speaking to minimize
12 feedback. If you have raised your hand and are called
13 upon by our chair, Dr. Lisa Butterfield, please speak
14 slowly and clearly so that your comments are accurately
15 recorded for transcription and captioning. Lastly, for
16 all presenters, please try and stay in your allotted
17 presentation times.

18 Okay, and at this time, I'm going to proceed
19 with the Conflict of Interest Statement. The Food and
20 Drug Administration is convening virtually today,

1 September 2nd, for the 70th meeting of the Cellular
2 Tissue and Gene Therapies Advisory Committee under the
3 authority of the Federal Advisory Committee Act of
4 1972. Dr. Lisa Butterfield is serving as the chair for
5 today's meeting.

6 Today, September 2nd, 2021, the committee will
7 meet in open session to discuss the toxicity risks of
8 adeno-associated virus vector-based gene therapy
9 products. The discussion topics include oncogenicity
10 risks due to vector genome integration and safety
11 issues identified during preclinical and/or clinical
12 evaluation. This topic is determined to be a
13 particular matter of general applicability.

14 With the exception of the industry
15 representative member, all standing and temporary
16 voting members of the CTGTAC are appointed as special
17 government employees or regular government employees
18 from their agencies and are subject to federal Conflict
19 of Interest laws and regulation. The following
20 information on the status of this committee's

1 compliance with Federal Ethics and Conflict of Interest
2 laws include, but are not limited to, 18 U.S.C. Section
3 208 is being provided to participants in today's
4 meeting and to the public. Related to the discussion
5 at this meeting, all members, RGE, and SGE consultants
6 of this committee have been screened for potential
7 financial conflict of interest of their own as well as
8 those imputed to them, including those of their spouse
9 or minor children and, for the purposes of 18 U.S. Code
10 Section 208, their employers.

11 These interests may include investments,
12 consulting, expert witness testimony, contracts and
13 grants, cooperative research and development
14 agreements, teaching, speaking, writing, patents and
15 royalties and primary employment. These may include
16 interests that are current and/or under negotiation.
17 FDA has determined that all members of this advisory
18 committee, both regular and temporary members, are in
19 compliance with Federal Ethics and Conflict of Interest
20 laws.

1 Under 18 U.S. Code Section 208, Congress has
2 authorized FDA to grant waivers to special government
3 employees who have financial conflicts of interest when
4 it is determined that the agency's need for a Special
5 Government Employee services outweighs the potential
6 for a conflict of interest created by the financial
7 interest involved, or when the interest of a regular
8 government employee is not so substantial as to be
9 deemed likely to affect the integrity of the services
10 which the government may expect from the employee. We
11 have the following consults serving as temporary voting
12 members: Dr. Frederic Bushman, Dr. Barry Byrne, Dr.
13 LaTasha Crawford, Mr. James DeFilippi, Ms. Peggy
14 DiCapua, Dr. Theo Heller, Dr. Roland Herzog, Dr.
15 Raymond Roos, Dr. Carlos Sanchez, Dr. Charles Venditti,
16 Dr. Charles Vite, and Dr. Caroline Zeiss.

17 Based on today's agenda and all financial
18 interests reported by committee members and
19 consultants, there have been four Conflict of Interest
20 waivers issued under 18 U.S. Code Section 208 in

1 connection with this meeting. Dr. Kenneth Berns is a
2 committee member and Dr. Charles Vite, Dr. Roland
3 Herzog, and Dr. Barry Berns are special government
4 employees who have been issued a waiver for their
5 participation in today's meeting. The waivers are
6 posted on the FDA website for public disclosure.

7 Dr. Eric Crombez of Ultragenyx Gene Therapies
8 will serve as the alternate industry representative to
9 this committee. Industry representatives are not
10 appointed as special government employees and serve as
11 non-voting members of the committee. Industry
12 representatives act on behalf of all related industry
13 and bring general industry perspective to the
14 committee. Industry representatives on this committee
15 are not screened, do not participate in any closed
16 sessions if held, and do not have voting privileges.

17 Dr. Randy Hawkins is serving as the consumer
18 representative for this committee. Consumer
19 representatives are appointed special government
20 employees and are screened and cleared prior to their

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

3 The following guest speakers for this meeting
4 have been screened for conflict of interest and cleared
5 to participate as speakers for the day-one meeting; Dr.
6 Mark Sands, professor in the Department of Medicine,
7 Oncology Division, Stem Cell Biology, Washington
8 University School of Medicine, St. Louis, Missouri; Dr.
9 Denis Sabatino, research associate professor of
10 pediatrics, Perelman School of Medicine, Philadelphia,
11 Pennsylvania; Dr. Lindsey George, tenure assistant
12 professor of pediatrics, Children's Hospital of
13 Philadelphia, Philadelphia, Pennsylvania; and Dr. James
14 Wilson, director of the Gene Therapy Program at the
15 Perelman School of Medicine, Philadelphia,
16 Pennsylvania.

17 Disclosure of conflicts of interest for
18 speakers follow applicable federal laws, regulations
19 and FDA guidance. At this meeting there may also be
20 regulated industry speakers and other outside

1 organization speakers making presentations. These
2 participants may have financial interests associated
3 with their employer and support from other regulated
4 firms. The FDA asks, in the interest of fairness, that
5 they address any current or previous financial
6 involvement with any firm whose product they may wish
7 to comment upon. These individuals were not screened
8 by the FDA for conflicts of interest. FDA encourages
9 all meeting participants, including open public hearing
10 speakers, to advise the committee of any financial
11 relationships they may have with any affected firms,
12 its products and, if known, its direct competitors.

13 We would like to remind members, consultants
14 and participants that if the discussions involve any
15 other products or firms not already on the agenda, for
16 which the FDA participant has a personal or imputed
17 financial interest, the participant needs to inform the
18 DFO and exclude themselves from such involvement and
19 their exclusion will be noted for the record.

20 Additionally, I would like to provide specific

1 guidance regarding this CTGTAC, September 2nd and 3rd,
2 2021, meeting.

3 Please note that the topic of this meeting,
4 the toxicity risks of adeno-associated virus (AAV)
5 vector-based gene therapy products, is determined to be
6 of particular matter of general applicability and as
7 such does not focus its discussion on any particular
8 product, but instead focuses on the classes of product
9 under discussion. Therefore, CTGTAC's role is to
10 advice and inform the FDA, CBER, and OTAT on strategies
11 to evaluate and mitigate risks associated with AAV
12 vectors used for gene therapy.

13 Speakers will describe safety issues
14 identified during preclinical or clinical evaluation of
15 various AAV-based gene therapies. Those issues include
16 hepatotoxicity, thrombotic microangiopathy,
17 neurotoxicity and oncogenicity due to vector genome
18 integration. The examples presented by the speakers
19 will facilitate the committee's discussion of these
20 risks and possible mitigation strategies. This CTGTAC

1 meeting is not being convened to recommend any action
2 against or approval of any specific AAV-based gene
3 therapy product or clinical trial.

4 Furthermore, this CTGTAC meeting is not being
5 convened to make specific recommendations that may
6 potentially impact any specific party, entity,
7 individual or firm in a unique way, and any discussion
8 of individual products will only be to serve as an
9 example of the product class.

10 Additionally, this meeting of the CTGTAC
11 meeting will not involve the approval or disapproval,
12 labeling requirement, post-marketing requirements, or
13 related issues regarding the legal status of any
14 specific product. This concludes my reading of the
15 Conflict of Interest Statement for the Public Record.
16 At this time, I will hand the meeting back over to Dr.
17 Lisa Butterfield. Thank you.

18 DR. LISA BUTTERFIELD: Thank you, Jarrod.
19 It's now my pleasure to introduce Dr. Wilson Bryan.
20 He's the director of OTAT at CBER, and he'll be

1 providing the FDA opening remarks for our session
2 today. Dr. Bryan?

3

4

FDA OPENING REMARKS

5

6 DR. WILSON BRYAN: Good morning. On behalf of
7 the FDA, I want to thank the members of this advisory
8 committee for taking the time to consider the topic of
9 toxicity associated with adeno-associated virus, or
10 AAV, vector-based gene therapy products. Since 2017,
11 we have begun to see the fulfillment of the promise of
12 gene therapy. The FDA has now approved seven gene
13 therapies, all of which are distinguished by their
14 tremendous benefit. These are life-saving and life-
15 changing products that address unmet needs for patients
16 with rare diseases.

17 In the years ahead, I believe that the field
18 of gene therapy will provide many more such life-saving
19 and life-changing products for treatment of both rare
20 and common diseases. Our enthusiasm for this field

1 must be balanced by caution. The greatest risks in
2 drug development fall on the patients who receive an
3 investigational product. The patients who enroll into
4 these clinical trials are heroes, and we owe them a
5 great debt. We must honor their sacrifice by
6 minimizing the risks for future study subjects and
7 patients. The drug development community, including
8 scientists and regulators, has an ethical obligation to
9 minimize the risks to the study subjects.

10 Over the next two days, we are asking this
11 committee to help us to meet that obligation. Over the
12 past few years, AAV has been one of the most popular
13 gene therapy vectors. With increased preclinical and
14 clinical use of AAV, we are seeing a variety of safety
15 issues. It is those safety issues that are the topic
16 for discussion by this committee. I am very much
17 looking forward to hearing the perspectives and
18 recommendations of this committee regarding the
19 toxicities that have been associated with AAV vector-
20 based gene therapy products.

1 I am also looking forward to the presentations
2 from our guest speakers, to reviewing the public
3 comments submitted to the docket, and the statements
4 that we will hear in the open-public hearing. All of
5 your deliberations and comments will assist the FDA as
6 we work with you to advance the field of gene therapy.
7 I'll stop there and turn it back over to Dr.
8 Butterfield.

9 DR. LISA BUTTERFIELD: Thank you very much,
10 Dr. Bryan. I appreciate that perspective. Now, as we
11 begin our presentations, our first presentation is from
12 our FDA speaker, Dr. Rosa Sherafat-Kazemzadeh, who's a
13 physician for the Division for Clinical Evaluation and
14 Pharmacology/Toxicology. She'll be presenting on
15 toxicity risks of AAV vectors for gene therapy.
16 Welcome.

17

18 **FDA PRESENTATION "TOXICITY RISKS OF ADENO-**
19 **ASSOCIATED VIRUS (AAV) VECTORS FOR GENE THERAPY (GT)"**

20

1 DR. ROSA SHERAFAT-KAZEMZADEH: Thank you, Dr.
2 Butterfield. Good morning, I'm Rosa Sherafat. I'm a
3 clinical reviewer in the Office of Tissues and Advanced
4 Therapy, OTAT in CBER, FDA. Today, on behalf of the
5 Advisory Committee Planning, a working group, I will be
6 presenting FDA's perspective on risks associated with
7 adeno-associated virus, or AAV, with vector use for
8 gene therapy.

9 First, I'll read an introduction to AAV
10 vectors, including how they are currently being used
11 for gene therapies. Then, I will briefly discuss each
12 of the four toxicities based on publicly available,
13 pre-clinical and clinical data. During this two-day
14 meeting, you will hear more detailed presentations from
15 our invited guest speakers followed by advisory
16 committee discussions on each one of these topics.
17 Starting with the introduction.

18 Adeno-associated virus is a virus that can
19 target a small amount of DNA. AAV infection is
20 widespread in humans, but AAV is not associated with

1 any known disease. In fact, AAV cannot replicate on
2 its own, and it needs a helper virus, like adenovirus
3 or other viruses, to replicate. This is where the name
4 adeno-associated virus comes from. It is important to
5 know that AAV vectors are engineered so they cannot
6 replicate in humans or animals, even in the presence of
7 a helper virus. AAV is a popular reflector for
8 potential gene therapy.

9 Currently, AAV-based gene therapy products are
10 being investigated for a wide variety of diseases,
11 including neurological, ophthalmological, hematological
12 and metabolic conditions. Over the last five years, we
13 have received about 10 to 20 new investigational new
14 drug submissions, or IND, annually for AAV-based
15 products along with a much larger number of requests
16 for pre-submission advice.

17 There are currently two FDA-approved AAV-based
18 products in the United States. Voretigene neparvovec,
19 or Luxturna, was approved in 2017. It is administered
20 subretinally for treatment of retinal dystrophy due to

1 mutations in the RPE65 gene. Onasemnogene abeparvovec,
2 or Zolgensma, was approved in 2019. It is administered
3 intravenously for pediatric patients with spinal
4 muscular atrophy. Of note, onasemnogene abeparvovec
5 has a boxed warning for acute serious liver injury, and
6 patients who have preexisting liver impairments may be
7 at higher risk of hepatotoxicity.

8 In recent years, there have been multiple
9 reports of treatment emergent serious adverse events in
10 AAV vector-based gene therapy trials. As noted in this
11 slide, these TESAEs include hepatotoxicity thrombotic
12 microangiopathy, or TMA, and neurotoxicities that
13 include brain MRI findings of uncertain significance
14 and dorsal root ganglion neuronal loss. Although AAV
15 vectors are generally not considered to be pro-
16 inflammatory, AAV vectors can invade all arms of the
17 immune system, innate, humoral, and cellular immunity.

18 As illustrated in this sticker, the capsid,
19 the genome, and the transgene product have all been
20 implicated as triggers of the immune system which can

1 affect the safety and efficacy of AAV vectors. Humans
2 are exposed to natural adeno-associated viruses. Older
3 children and adults often have preexisting antibodies
4 against AAV that can inhibit activity of AAV vectors.
5 It is unknown whether these preexisting antibodies
6 might also affect toxicity.

7 Following this illustration of an AAV vector,
8 the immune system can be activated against the AAV
9 vector and the therapeutic protein that the vector
10 encodes. It is critical to find strategies to avoid or
11 mitigate these toxicities. One potential way to do
12 that is an immunosuppressive drug, such as
13 corticosteroids, as shown on the next slide. T cells
14 are one of the key mediators of AAV vector
15 hepatotoxicity. When AAV vectors reach the liver, the
16 vector may express a therapeutic protein in hepatocyte.
17 However, AAV vectors are also taken up by stimulatory
18 antigen presenting cells, such as dendritic cells.

19 Dendritic cells can prevent the antigen from
20 AAV vectors and then stimulate the T cells against AAV.

1 These T cells can then attach hepatocytes that have
2 been exposed to AAV. Clinically, this hepatotoxicity
3 may present as asymptomatic liver enzyme elevation or,
4 in severe cases, as symptomatic liver toxicity and
5 liver failure. Corticosteroids can stunt the T cell
6 reactivity and, subsequently, cytotoxicity of vector
7 transient cells.

8 Currently, corticosteroids are used
9 prophylactically in many trials that administer high
10 doses of AAV vectors. It is important to note that the
11 immune system is not responsible for all the toxicities
12 of DRG AAV-based products. For example, in animal
13 studies, high-level expression of a therapeutic protein
14 in the dorsal root ganglia was found to kill sensory
15 neurons. Also, AAV vector DNA can integrate into
16 cellular DNA both in animals and in humans. In mice,
17 integration had been shown to cause liver tumors.

18 Product quality may also play a role in the
19 toxicity of AAV vectors. Preparations of AAV vector
20 products can contain impurities, including empty

1 capsids, that lack vector DNA. The AAV protein found
2 in empty capsids might trigger antibodies, complements,
3 and T cells. Although it is possible to remove empty
4 capsids during the manufacturing process, doing so
5 makes manufacturing more complex and expensive. Many
6 manufacturers either do not remove empty capsids or
7 only partially remove them.

8 Also, during manufacturing, AAV capsids can
9 package non-vector DNA from cells or plasma. This non-
10 vector DNA may be pro-inflammatory or pro(inaudible).
11 It is not possible to remove non-vector DNA from the
12 product. FDA requires manufacturers to monitor and
13 control the amount of impurities in AAV vectors.
14 However, in most cases, what constitutes safe levels of
15 the impurities is not known.

16 Now, I will discuss hepatotoxicities associated
17 with AAV vectors. When delivered intravenously, nearly
18 all AAV vectors traffic to the liver and hepatotoxicity
19 is the most commonly-observed adverse event in clinical
20 trials involving IV administration of AAV vectors.

1 Hepatotoxicity is often T-cell mediated and has led to
2 the common use of prophylactic corticosteroids in these
3 trials. Hepatotoxicity often presents as elevated
4 levels of the liver enzymes alanine aminotransferase,
5 or LAT, and/or aspartate aminotransferase, AST.

6 Less frequently, hepatotoxicity can result in
7 drug-induced liver injury, also known as DILI. In rare
8 cases, participants in clinical trials have experienced
9 hepatic failure, and three deaths have been reported.

10 In the next several slides, I will briefly discuss
11 clinical experience of hepatotoxicity in three
12 different diseases for which AAV vector-based
13 treatments have been studied, spinal muscular atrophy,
14 hemophilia, and X-linked myotubular myopathies.

15 Spinal muscular atrophy, or SMA, is a rare
16 neurodegenerative disorder caused by irreversible loss
17 of lower motor neurons in the brain stem and spinal
18 cord. It results from mutations in the survivor motor
19 neuron one gene, or SMN1. As I mentioned, onasemnogene
20 abeparvovec, or Zolgensma, received FDA approval in

1 2019 for treatment of infants and young children with
2 this condition. To date, over 500 patients have been
3 treated.

4 Typically, hepatotoxicity associated with
5 onasemnogene abeparvovec presents with elevations as
6 seen on liver enzyme concentrations and most often
7 occurs between one week and one month after product
8 administration. About one-third of clinical trial
9 participants had at least one adverse event of
10 hepatotoxicity. Prior to treatment, these patients had
11 low titer, that is less than 1 to 50, of anti-AAV9
12 antibodies. After treatment, some patients experienced
13 liver enzyme elevations reaching as high as more than
14 20 times the upper limit of normal.

15 In most cases, liver enzyme concentrations
16 normalized following treatments with corticosteroids.
17 For some, prolonged corticosteroid treatment was
18 necessary as long as 229 days. One case of acute
19 serious liver injury was reported in a patient who
20 received onasemnogene abeparvovec prior to its

1 marketing approval. At baseline, that patient had an
2 elevated AST and ALT of unknown etiology. Other
3 indicators of liver function, such as gamma-glutamyl
4 transferase, total bilirubin, and prothrombin time,
5 were normal.

6 About seven weeks after receiving the product,
7 the patients developed jaundice. Laboratory testing
8 was consistent with acute liver failure. Liver
9 biopsies showed acute massive degeneration of
10 hepatocytes and massive mixed inflammatory infiltrates
11 primarily through the (inaudible) positive (inaudible).
12 The patient was treated with corticosteroids and
13 recovered two days later. Since FDA approval, there
14 have been additional case reports of severe
15 hepatotoxicity. Today's few cases also have been
16 resolved with post-corticosteroid testing.

17 Because of this potential serious risk with
18 onasemnogene abeparvovec, the US Prescribing
19 Information includes a boxed warning to alerts
20 prescribers to the possibility of elevated

1 aminotransferases and acute serious liver injury. In
2 addition, there are detailed instructions for
3 monitoring liver functions and treatment with systemic
4 corticosteroids. Hepatotoxicity has been a blurb in
5 clinical trials for AAV-based gene therapy for
6 hemophilia. Soon after treatment with the product,
7 some participants experienced elevated aminotransferase
8 levels which were transient and asymptomatic.

9 Variability was observed regarding AAV capsid-
10 specific cell-mediated immune responses. Many
11 participants developed elevated aminotransferases and
12 an AAV capsid-specific cell-mediated immune response.
13 However, some participants have elevated
14 aminotransferases without a detectable immune response,
15 while others had an anti-capsid immune response but
16 without elevated aminotransferases. Some participants
17 lost expression of the transgene; that is they no
18 longer produced the clotting factor. This loss
19 occurred with the onset of the aminotransferase
20 elevation.

1 A subset of these participants also was found
2 to have anti-capsid T cell responses. This suggests
3 that the observed loss of expression of the clotting
4 factor may have been due to T cell-mediated
5 destruction of transfused hepatocytes. Next, I will
6 discuss hepatotoxicities observed with AAV vector gene
7 therapy for X-linked myotubular myopathy, or XLMTM,
8 which is a serious and rare neuromuscular disease
9 characterized by muscle weakness and is typically
10 fairly early in life.

11 XLMTM is product of mutations in the
12 myotubularin 1 gene, or MTM1, resulting in lack of
13 functional myotubularin protein, which is needed for
14 the normal development, maturation, and activity of
15 skeletal muscle cells. The ASPIRO clinical trial
16 involved intravenous administration of an
17 investigational AAV-based gene therapy product which
18 utilizes an AAV8-based vector to deliver a normal copy
19 of MTM1. As of July 2020, a total of 23 pediatric
20 participants received the product.

1 Six received a low dose of 1×10 to the 14th
2 vector genome per kilogram of body weight. Seventeen
3 participants received a high dose of 3×10 to the 14th
4 vg/kg. Participants in the low-dose cohort had a lower
5 median age and generally weighed less than the
6 participants in the high-dose cohort. To dampen the
7 immune response to the product, the clinical protocol
8 included prophylactic treatment, prednisolone, 1
9 milligram per kg per day, for the first 8 weeks after
10 infusion of the product followed by a slow taper from
11 weeks 9 through 16.

12 Unfortunately, three participants in the
13 ASPIRO trial died. All three were in the high-dose
14 cohort, and their deaths occurred about 20 to 40 weeks
15 after they received the product. By the time of their
16 deaths, all three children were older than five years
17 of age. These children were among the older and
18 heavier participants in the trial, and correspondingly,
19 they received the highest total vector doses, ranging
20 from 4.8 to 7.7×10 to the 15th vg. All three

1 children had preexisting hyperbilirubinemia consistent
2 with intrahepatic cholestasis.

3 In all three cases, the development of
4 intrahepatic cholestatic liver failure began with rise
5 and total bilirubin up to 35 to 50 times the upper
6 limit of normal and direct bilirubin rise up to 90
7 times upper limit of normal. In contrast, AST and ALT
8 elevations typically lagged weeks to months behind the
9 bilirubin elevation. Treatment with ursodiol,
10 augmented corticosteroids, and various
11 immunosuppressants were inefficient in helping to ease
12 the progression in these cases. The immediate cause of
13 death was sepsis in two cases and gastrointestinal
14 bleeding in the third case.

15 Autopsy findings were consistent with
16 intrahepatic cholestatic liver failure. The
17 pathologies did not show prominent inflammatory
18 cellular infiltrates from the liver (inaudible). In a
19 natural history study, 33 patients with XLMTM between
20 the ages of 10 years and 11 months were followed for

1 one year. A substantial number showed evidence of
2 hepatobiliary disease. Elevated aminotransferases were
3 present in 22 percent, liver enlargement in 12 percent,
4 jaundice in 15, and liver hemorrhage in 6 percent.

5 In the ASPIRO trial, more than 50 percent of
6 participants had some evidence of pre-existing
7 hepatobiliary disease. While one cannot establish a
8 direct causal link between hepatotoxicity and the three
9 deaths in the ASPIRO trial, dose-related hepatotoxicity
10 was evident in the context of preexisting hepatobiliary
11 abnormalities, common in children with XLMTM. Of note,
12 several publications have reported on preclinical
13 studies accessing different AAV vectors for SMA,
14 hemophilia, and XLMTM. Neonatal FVB/NJ mice developed
15 transient elevation in aminotransferases as well as
16 minimal to moderate histopathological findings in the
17 liver following IV administration of onasemnogene
18 abeparvovec.

19 In nonhuman primates, acute liver failure,
20 thrombocytopenia, and severe coagulopathy were reported

1 following the IV administration of AAVhu68 vector
2 encoding the SMA gene, ultimately resulting in the
3 euthanasia of one the three animals. Similar findings,
4 along with transient complement activation, were also
5 reported in NHPs who were administered other AAV
6 vectors. For hemophilia preclinical studies evaluating
7 the various AAV vectors encoding factor 8 in a canine
8 disease model and in healthy nonhuman primates, the
9 (inaudible) transient elevations in aminotransferases
10 without hepatocyte (inaudible).

11 An X-linked myotubular myopathy studies
12 evaluating AAV8 vectors carrying MTM1 gene administered
13 to murine and canine models of the disease did not
14 observe adverse liver findings. In summary, serious
15 and life-threatening hepatotoxicities have been
16 reported in recipients of systemic AAV vector gene
17 therapy products, and variable findings were reported
18 in different public animal studies. Only some animal
19 studies may have been influenced by a number of
20 factors, including the animal species or disease model,

1 dose levels, vector products, and other study design
2 elements.

3 We are asking the committee to please discuss
4 and provide recommendations regarding presenting and
5 mitigating AAV vector-associated hepatotoxicity from
6 CMC, preclinical, and clinical perspectives. Please
7 also discuss additional considerations for vector
8 genome dose determination for subjects for systemic
9 administration. Next, I will discuss thrombotic
10 microangiopathy, or TMA. TMA is a hematologic
11 emergency that requires prompt treatment. It is
12 characterized by damage to the endothelial of arterial
13 and capillaries and microvascular thrombosis.

14 TMA presents clinically as a syndrome of
15 hemolytic anemic, thrombocytopenia, and acute liver
16 injury. There are two primary forms of TMA, thrombotic
17 thrombocytopenic purpura, or TTP, and hemolytic uremic
18 syndrome, or HUS. TTP has been proposed to result from
19 a severe deficiency of the enzyme ADAMTS13 due to
20 acquired autoantibodies or rarely due to genetic

1 mutations. The most common form of HUS, or typical
2 HUS, follow diarrhea caused by Shiga toxin-producing E.
3 coli.

4 In contrast, atypical HUS is thought to be
5 associated with abnormal susceptibility of the host to
6 complement-mediated damage which can be triggered by
7 drugs and toxins as well as by a variety of conditions,
8 such as malignancy, autoimmune disease, hematopoietic
9 stem cell transplant; or infections, such as HIV,
10 influenza, or poxvirus. TMA has been reported with AAV
11 vector-based treatment for spinal muscular atrophy and
12 Duchenne muscular dystrophy.

13 A recent publication described three patients
14 with spinal muscular atrophy who developed TMA
15 approximately one week after treatment with
16 onasemnogene abeparvovec. All three received
17 prophylactics with prednisolone, one milligram per kg
18 per day for 30 days, as recommended in the U.S.
19 Prescribing Information. Two patients had previous
20 exposure to nusinersen treatment for SMA. Two patients

1 experience vomiting at presentation. Two had
2 infections with encapsulated organisms.

3 In these patients, all three had laboratory
4 evidence of complement activation. The three patients
5 all recovered within two to four weeks, following the
6 standard treatment for TMA, but had transfusion of
7 packed RBCs and platelets, glucocorticoids, and plasma.
8 However, one patient developed persistent hypertension.
9 Another patient developed hypertension and nephrotic
10 syndrome, which resolved three months after initial
11 treatment.

12 The onasemnogene abeparvovec U.S. prescribing
13 information had recently been updated to include TMA in
14 the warning and precaution section. The manufacturer
15 also includes a "Dear Doctor" letter to warn healthcare
16 providers and patients of the risks of TMA. Next, we
17 will discuss cases of TMA observed in participants of
18 clinical trials of investigation of AAV vector-based
19 treatments for Duchenne muscular dystrophy. We
20 developed atypical HUS like presentations with

1 complement activation, acute kidney injury, and
2 decreased platelets. They were treated successfully
3 with hemodialysis, platelet transfusion, and eculizumab.

4 TMA following systemic administration of high
5 doses of AAV vectors has been linked to complement
6 activation. The exact method of them and predisposing
7 practice for complement activation, however, have not
8 been clearly identified. Proposed risk mitigation
9 plans for TMA have included prophylactic, the drug
10 eculizumab, and complement C1 esterase inhibitors,
11 increased dose of the prednisolone in the month after
12 administration of the AAV vector, and modification of
13 the manufacturing process to increase product purity by
14 reducing the percentage of empty capsids in the final
15 product.

16 Likewise, in healthy NHPs, acute
17 thrombocytopenia, coagulopathy, complement activation,
18 and hepatotoxicity were reported following IV
19 administration of high-dose levels of AAV vectors.
20 However, in contrast to the TMA observed in humans,

1 adverse kidney histopathology was not reported in these
2 animals. We would like to ask the committee to please
3 discuss how to prevent or mitigate TMA risk from
4 clinical and product perspectives. For example, please
5 discuss the role of predisposing factors, product
6 quality, total dose of vector and capsids, and the
7 timing and choice of treatment for TMA.

8 Next, I will discuss AAV vector-associated
9 Dorsal root ganglion toxicity. Primary sensory neurons
10 in the DRG transmits sensory stimuli from the periphery
11 to the central nervous system. Several studies
12 evaluating different AAV vector-based treatments in
13 nonhuman primates have noted histopathology findings in
14 the primary sensory neurons in of the DRG. These
15 findings consist of minimal-to-moderate cell body
16 degeneration within the DRG as well as minimal-to
17 moderate axonal degeneration along the extending dorsal
18 tracks of the spinal cord and in peripheral nerves.

19 Several factors have been associated to
20 decrease the severity of damage to peripheral sensory

1 neurons, including direct administration of high doses
2 of the product into the cerebrospinal fluid. However,
3 the minimal-to-moderate DRG toxicity observed in
4 nonhuman primates have not been associated with
5 clinical findings. It is also important to note that
6 the studies in other animals, such as mice and young
7 pigs, have reported similar pathology-affected
8 peripheral sensory neurons.

9 In clinical trials, there have been two case
10 reports of neuronal loss within the DRG following
11 intrathecal administration of an AAV vector-based
12 product. One case occurred in subjects enrolled in a
13 trial for giant axonal neuropathy, or GAN. Autopsies
14 performed eight months later revealed severe neuronal
15 loss, but it had not been accompanied by inflammation
16 or by clinical finds or symptoms of DRG toxicity. The
17 other case involved a participant enrolled in a
18 clinical trial for familial amyotrophic lateral
19 sclerosis, or ALS.

20 The participant experienced tingling sensation

1 and pain in the hand and one foot three to four weeks
2 after receiving an AAV vector-based product carrying
3 the gene encoding superoxide dismutase 1, or SOD1.
4 Sural sensory nerve action potential and less median
5 nerve sensory potential had been normal before vector
6 administration but were absent 10 weeks afterward.
7 Autopsy performed 15 and a half months later revealed
8 neuronal loss in the DRG.

9 We would like to ask the committee to please
10 discuss the clinical relevance of DRG and peripheral
11 nerve toxicity findings in NHP to human,
12 recommendations on preclinical study design to help
13 characterize the significance of animal studies humans,
14 and any additional risk mitigation plans for
15 participants of clinical trials. Next, I'm going to
16 discuss brain MRI findings following AAV vector
17 administration. Direct intraparenchymal administration
18 enabled targeted delivery of AAV vectors to different
19 regions of the brain and decreased systemic toxicity
20 associated with high IV doses of phosphorous.

1 This fact is used in several clinical trials
2 for treatment of various neurodegenerative disorders.
3 One trial involved direct parenchymal administration of
4 an AAVrh10 vector expressing tripeptidyl peptidase-1
5 for the treatment of late infantile Batten disease.
6 Brain MRIs within 48 hours after vector administration
7 showed T2 hyperintensities localized to the sites of
8 AAV vector administration. During the course of the
9 trail, all 13 participants developed new T2
10 hyperintensities shortly after product administration.

11 Among these 13 participants, these localized
12 abnormalities persisted in 7 subjects at 18 months
13 after therapy, while in two other participants,
14 abnormalities dissolved. Of note, 18 month MRIs was
15 not performed in 4 participants. In the acute period,
16 serious adverse events, including seizures, abnormal
17 movements, and MNCs occurred in 6 of the 13 children.
18 The cause of these findings is poorly understood.

19 However, because this trial was conducted in
20 participants with a progressive neurodegenerative

1 disorder and the trial used an unrandomized design, it
2 is it difficult to discern whether clinical changes and
3 MRI findings were caused by the AAV vector or by the
4 delivery system and procedure. Findings in healthy
5 NHPs have reported MRI abnormalities and
6 histopathological findings in the brains of NHPs
7 following direct intraparenchymal administration of
8 AAVrh10 vectors. Histopathological findings included
9 mild to moderate immune cell infiltrates and gliosis
10 that corresponded with MRI abnormalities at the
11 injection tracks.

12 Long-term studies reveal similar findings that
13 were dose dependent and not reversible after a year of
14 follow up. However, no neurobehavioral deficits were
15 recorded. Histopathological findings at the injection
16 site were also reported in healthy rodents following
17 intraparenchymal AAVrh10 vector administration. We are
18 seeking committee's recommendations regarding general
19 design of preclinical studies to further discern the
20 neurotoxicity risks of intraparenchymal administration

1 of AAV vectors and how to discern distribution of the
2 clinical findings in recipients of gene therapy
3 products.

4 We are also asking for recommendations on
5 implementation of risk mitigation plans before and
6 after AAV vector administration as well as the
7 appropriate duration of monitoring of these trial
8 participants. Next, I'm going to discuss potential
9 oncogenicity of AAV vector gene therapy. AAV vectors
10 can integrate into genomic DNA. The AAV vector genome
11 tend to integrate near active genes. Although most
12 vector genomes do not integrate, vector integration has
13 been reported in both animals and humans. Intentional
14 oncogenesis by AAV vector genomes and development of
15 hepatocellular carcinoma has been reported in several
16 mouse models.

17 Further analysis in the model mice reveals
18 that AAV aided oncogenesis that's associated with
19 vector integration into the mouse Rian locus, including
20 regions and encoding numerous regulatory RNAs. One AAV

1 in the tumor formation has only been observed in mice.
2 A long-term study valid in IV administration of AAV
3 vectors expressing factor 8 in a canine model of
4 juvenile hemophilia A reveals hepatic clonal expansion
5 up to 10 years post dose. AAV vector integration
6 events were detected in genes associated with cell
7 growth and/or transformation. However, no sign of
8 nodule formation or oncogenesis were observed.

9 Data from animal studies suggest several
10 factors may influence the incidence of HCC following
11 AAV vector administration. So far, there is only one
12 case report of HCC in a participant of an AAV vector
13 clinical trial. That trial used AAV5 vector for
14 treatment of hemophilia B. After careful
15 investigation, it was thought unlikely that the AAV
16 vector contributed to development of HCC. Instead, the
17 HCC was thought likely due to the participant's prior
18 history of hepatitis C.

19 The recently updated FDA guidance for *Long*
20 *Term Follow-up After Administration of Human Gene*

1 *Therapy*, in general, recommends a risk-based approach
2 for determining the duration of the long-term follow-up
3 protocol. The recommended follow-up duration for AAV-
4 based gene therapy products is up to 5 years due to the
5 lower risk of integration by the AAV vector genome, as
6 opposed to the risk with products using the retroviral
7 vectors or genome edited components. AAV vectors
8 integration and oncogenicity will be the first
9 discussion topic following this presentation.

10 We have the following questions for the
11 committee. Please discuss the limitations and
12 advantages of conducting animal studies to identify the
13 risks of AAV vector-mediated oncogenicity in human
14 subjects in terms of animal species, healthy versus
15 disease models, and animal age, in-life and terminal
16 assessment, and the eventual follow up. Please discuss
17 oncogenicity benefit-risk considerations in human
18 participants such as the patient's age, preexisting
19 conditions, and vector dose.

20 DR. LISA BUTTERFIELD: Rosa, I'm sorry to

1 interrupt. We only have three minutes left for
2 questions. Perhaps reading the questions to the
3 committee, we can pass on that. Did you have any other
4 remarks before we have a moment for a couple of
5 questions?

6 DR. ROSA SHERAFAT-KAZEMZADEH: I apologize for
7 going over time. I'm so sorry, but I'm almost at the
8 end of the presentation. I think just a summary, and I
9 would like to thank the FDA members that helped us and
10 supported us for this presentation and the Advisory
11 Committee Planning Working Group and also thank the FDA
12 and committee members for your time and attention. I
13 apologize for going over time. I'm very grateful.

14

15 **FDA PRESENTATION Q&A**

16

17 DR. LISA BUTTERFIELD: Thank you very much.
18 We have two minutes for questions for the speaker. I'm
19 watching for your raised hands. Then you can turn on
20 your camera and microphone when I call on you. I'll

1 ask the first question. Is there any evidence for a
2 role for BMI? When you were talking about the
3 toxicities with XLMTM, you mentioned larger and heavier
4 for the patients with toxicities. I'm wondering if
5 there's any evidence for a relationship to BMI.

6 DR. ROSA SHERAFAT-KAZEMZADEH: Thank you for
7 that question. Yes, very important point. Generally,
8 I'm advised to use the lean body mass for calculations.
9 However, a lot of these children, as mentioned, were in
10 the heavier side. That is actually one of the
11 questions that we have for the committee, in terms of
12 the build cap based on the weight. I usually use lean
13 body mass -- sorry, lean body weight, instead of the
14 measured state.

15 DR. LISA BUTTERFIELD: Thank you very much.
16 If there's one other short question, we can do that
17 now, or we can move on. Okay. Well, we only have 30
18 seconds left. We'll have a lot of discussion time
19 after the other two presentations from the guest
20 speakers. Thank you, again, and let me now introduce

1 our first guest speaker focusing on the topic of
2 session one. Session one is on vector integration and
3 oncogenicity risks. Our first invited speaker is Dr.
4 Mark Sands, a professor of the Department of Medicine,
5 Oncology, Stem Cell Biology at Washington University
6 School of Medicine. Welcome Dr. Sands to speak about
7 AAV integration in vitro and mice. Thank you.

8

9 **SESSION 1: VECTOR INTEGRATION AND ONCOGENICITY RISKS**

10 **INVITED SPEAKER PRESENTATION: "rAAV INTEGRATION: In**
11 **Vitro & MICE"**

12

13 DR. MARK SANDS: Thank you, Dr. Butterfield,
14 and good morning to everyone. I've been asked to give
15 a very brief summary, or overview, of recombinant AAV
16 integration in cells cultures and in small animals,
17 typically in mice. Here's my disclosure slide, and you
18 can see I consult for several gene therapy companies.

19

20 The way I wanted to handle the discussion this
morning was to present a number of papers, essentially

1 in chronological order and basically break them up into
2 three different periods. The first period from 1997 to
3 2001, what did we know about AAV integration prior to
4 the first observation of the hepatocellular carcinoma?
5 Then from 2001 to 2016. Once hepatocellular carcinoma
6 had been reported, what were we able to learn after
7 that? Then finally, I'd like to discuss two very
8 recent papers that discuss various conditions or
9 situations that may affect the rate of hepatocellular
10 carcinoma following AAV (audio skip). The first paper
11 I'd like to discuss is from 1997—

12 DR. LISA BUTTERFIELD: I apologize, Dr. Sands,
13 I have to interrupt, there's an AV issue. I have to
14 ask you start again for the FDAs transcription. If you
15 could begin again with your first slide, apologies.

16 DR. MARK SANDS: Was it my screwup? That's
17 unusual. Okay, all right. For the AV people, I've
18 been asked to provide a brief overview of what we know
19 about recumbent AAV integration in culture cells and in
20 small animal models, specifically mice. Here's my

1 disclosure slide. The way I wanted to approach this
2 talk was to present a number of papers and briefly
3 summarize those papers in chronological order, break it
4 up into three different periods.

5 The first period from 1997 to 2001, which was
6 prior to the first report of hepatocellular carcinoma.
7 Then, from 2001 to 2016, what have we been able to
8 learn since that first report? Finally, I want to talk
9 about two very recent papers discussing the various
10 situations or conditions that might affect the
11 development of hepatocellular carcinoma following AAV.

12 The first paper I'd like to talk about was
13 from 1997, this is David Russell's group. They were
14 trying to determine the frequency structure of
15 recombinant (audio skip). They performed these
16 experiments *in vitro*, or actually in cell cultures.
17 The two main takeaway points from this early study was
18 that the AAV vectors, the integrated AAV vectors that
19 they were able to recover, there didn't seem to be any
20 specific pattern of integration. In other words, no

1 hot spots. They appeared to be randomly dispersed
2 throughout the genome.

3 What was interesting though is that none of
4 the integrated vectors were fully intact. There were
5 lots of deletions arranged (audio skip), particularly
6 in the (audio skip), but also throughout the
7 (inaudible).

8 Now, at the same time, Dr. Samulski's group
9 are also looking at the structure of the integrated AAV
10 vectors. They did this also in cultured cells and also
11 in a cell-free system. Very much like David Russell's
12 group, they didn't see any obvious site preference, or
13 no hot spots in (audio skip). They also -- again,
14 similar to Dr. Russell's data -- none of the ITRs that
15 they were (audio skip) integrated vectors were intact.

16 What they did show, though, that was of a
17 little bit of concern at the time, was that it appeared
18 that these common AAV vectors favored actively
19 transcribed regions (audio skip). Of course, this
20 brings up the possible scenario of (audio skip) and

1 subsequent dysregulation.

2 Then, in 1999, Hiroyuki Nakai, who's a post-
3 doc in Mark Kay's lab. He published a paper trying to
4 interrogate the structure of the integrated AAV vector
5 in vivo (audio skip) mouse liver. Similar to what they
6 discovered in (audio skip) cells, was that the
7 integrated AAV vectors were almost invariably
8 rearranged. The ITRs and some (audio skip) vector.
9 They also identified two independent integration events
10 in gene (audio skip) ,one was in alpha 1 collagen. The
11 other integration event was in a ribosomal RNA.

12 Again in 2001, Dr. Nakai, in Mark Kay's lab, I
13 think for the first time really demonstrated that the
14 extrachromosomal DNA -- in other words these stable
15 episomes that occur following a (audio skip). These
16 stable episomes were the primary source of expression
17 from AAV vectors. At this time the field knew that
18 there were episomal forms and integrated forms, but it
19 wasn't clear where the expression was coming from.
20 Again, Dr. Kay's group (audio skip) from sources.

1 Also, they showed that the rate of integration was
2 relatively low, anywhere in the range from about 5 to
3 10 percent of the AAV vectors were actually integrated.
4 Now, of course, this was a good thing because that
5 would minimize the (audio skip).

6 Up to 2001, what we knew at that stage was
7 that level of integration, the frequency of integration
8 of AAV vectors, was relatively low. Again, on the
9 range between 1 and 10 percent. Chromosome vector
10 junctions are typically near ITRs, but the ITRs are
11 almost invariably rearranged. Some minor changes
12 (audio skip) .

13 Also it was shown that there was minimal or no
14 homology between the area of the genome where the AAV
15 vectors integrated and the vector sequence, and no hot
16 spots or no distant integration sites identified.
17 Unfortunately, the structure of the integrated genome's
18 that were (audio skip) didn't provide any real insight
19 into the mechanism of (audio skip). Up to 2001, the
20 good news was that at that stage no one had really

1 observed any serious acute or chronic toxicity being
2 demonstrated.

3 Then, in 2001, we published the results of a
4 long term study that we had done that showed the
5 presence of hepatocellular (audio skip) following AAV
6 (audio skip). The way this experiment was performed
7 was that we identified animals during the neonatal
8 period, and this was an animal model of a lysosomal
9 storage disease called mucopolysaccharidoses type VII.
10 Once we identified the affected animals, we gave those
11 animals an intravenous injection of an AAV vector --
12 again, between post-natal Day 1 and post-natal Day 3.
13 We then analyzed those animals and left some on the
14 shelf for a longevity study.

15 The initial results were very positive. We
16 saw persistent expression and very dramatic clinical
17 and behavioral improvement. In fact it was very
18 difficult to distinguish the treated animals from
19 normal animals. However, the animals that were still
20 alive at 18 months of age, three out of five of those

1 animals had developed hepatocellular carcinoma. When
2 we went back and looked through the records from the
3 experiment, there were several other animals between 12
4 and 18 months of age that had either died
5 spontaneously, or were sacrificed for analysis, that
6 also had hepatocellular carcinoma.

7 Now, unfortunately, the way the experiment was
8 designed, we designed an efficacy study not a toxicity
9 study. Given that fact, and the fact that the tools to
10 identify the integration events were not that
11 sophisticated at the time, it was impossible to
12 determine whether the AAV vectors were the causative
13 factor for the hepatocellular carcinoma.

14 Then what we set out to do at that point was
15 to try to corroborate these data and replicate this
16 study. In the interim, a couple of other studies were
17 published, another one by David Russell's group. And
18 what they showed was that the simple administration of
19 an AAV vector did not increase the mutation rate at
20 all. So that was a very positive finding. What they

1 also showed, though, that was that the recumbent AAV
2 seems to integrate preferentially in either areas of
3 spontaneous or reduced double strand breaks. In other
4 words, if there's a genotoxic event or insult it causes
5 double strand breaks, it appears as though the AAV
6 vectors will preferentially integrate in those regions.

7 In another study in 2005 -- again by Hiroyuki
8 Nakai in Dr. Mark Kay's lab -- they analyzed about 350
9 AAV integration sites in mouse livers. Those of you
10 that remember 2005, this was really a Herculean feat.
11 The techniques were not as sophisticated as they are
12 today. What they did discover is they actually did
13 discover an integration hot spot in the mouse genome.
14 This was where a number of AAV integrants were
15 identified within ribosomal RNA gene systems. Now,
16 this didn't raise a lot of red flags at the time,
17 because there's lots of ribosomal RNA gene repeats and
18 if you disrupt a small number of them there may not be
19 any clinical consequences.

20 However, what they also showed in this study

1 was that more than 50 percent of the integration events
2 occurred near transcription start sites or in CpG
3 islands. This sort of echo's back to the data that Dr.
4 Samulski showed, where they liked (audio skip) AAV
5 first to integrate near transcribed genes, so this
6 could be problematic.

7 Then, in 2007, we were actually able to
8 replicate the initial findings of hepatocellular
9 carcinoma following AAV reduction. The experimental
10 design was exactly the same as what we published in the
11 initial observation. Intravenous injection of the
12 first generation AAV vector during the neonatal period,
13 between postnatal Day 1 and 3. Then follow those
14 animals for a long period of time. In this particular
15 study we also included a wild type group to try and
16 distinguish between whether the hepatocellular
17 carcinoma was associated with (audio skip) or not.

18 What we showed is that nearly 50 percent of
19 the AAV-treated mice developed hepatocellular
20 carcinoma. And the frequency of hepatocellular

1 carcinoma between MPSVII mice and wild type mice was
2 essentially the same. It didn't appear as though
3 MPSVII exacerbated this (audio skip) .

4 Now, then in collaboration with David Russell
5 and Dan Miller at the University of Washington, they
6 were instrumental in helping us identify integration
7 sites within those tumors. The data, frankly, was
8 shocking. We were able to isolate four independent
9 integration events from tumors from four different
10 animals. In each case -- I don't know if you can see
11 my pointer there or not, if you look at the figure on
12 the right. All four of these integration events
13 occurred within a very small region of the genome,
14 about a 6,000 base pair region. Again, a very small
15 region of the genome.

16 All four of these vectors integrated within,
17 in what's called the *Rian* locus. There's two important
18 things that we discovered, not a single one of these
19 integrated vectors were intact. In fact, the only
20 thing that was there was the inverter terminal repeats,

1 motor sequences and a little bit of the expression
2 because that's -- beyond that. A little bit of the
3 (audio skip).

4 Then when we looked at the effects of these
5 integration events, when we looked at the
6 transcription, most of the genes downstream of these
7 integration events -- and that included microRNA, snRNA
8 and several genes, several of which are associated with
9 cancer -- all of those genes downstream from these
10 integration events were dysregulated.

11 Then, quite a bit of time went by -- about
12 seven or eight years went by. And then almost at the
13 same time, two papers were published: one from Barbara
14 Triggs-Raine's group and the other from Chuck
15 Venditti's group. What they showed, in this particular
16 study, they were trying to treat the mouse with
17 Sandhoff disease, which is another liposomal storage
18 disease. They did the same thing, intravenous
19 injection in newborn animals and let them live for a
20 long time. Eight of the 10 animals developed

1 hepatocellular carcinoma. When they analyzed these
2 tumors, several of the tumors had integration events
3 within the *Rian* locus, basically confirming what we had
4 seen previously.

5 The second paper that came out almost at the
6 same time was from Chuck Venditti's lab, and Randy
7 Chandler was the first author. In this particular
8 study, they injected a relatively large number of
9 animals with a number of different vectors, different
10 serotypes, or different capsid proteins, different
11 promoters, different transgenes. At the end of the
12 day, what they showed was that greater than 50 percent
13 of the mice that received recombinant AAV at birth,
14 intravenously, developed hepatocellular carcinoma.
15 When they analyzed the integration sites, they
16 confirmed that the *Rian* locus was a hot spot in those
17 tumors. Interestingly, they also were able to generate
18 enough data to suggest that the hepatocellular
19 carcinoma looked to be dose dependent. They also
20 showed that strong promoter enhancer combinations

1 increased the incidences of hepatocellular carcinoma.

2 Then, when you compile all the data, when you
3 look at all the available data at this time, it would
4 appear as though the hepatocellular carcinoma, the
5 development of hepatocellular carcinoma, was
6 independent of transgene, it was also independent of
7 genotype because there's now multiple different feed
8 bottle and wild type animals that develop
9 hepatocellular carcinoma at about the same rate. Then
10 the next thing that Randy and Chuck did was to compile
11 all of the known AAV integration sites that have been -
12 - in most studies -- that had been identified at that
13 time. And mapped each of those independent
14 integrations onto to the *Rian* locus in multiple
15 species, mouse, rat, rabbit, human, dog, and even in
16 elephant.

17 What was interesting is that about 50 percent
18 of those integrations in the *Rian* locus, all occurred
19 within a very small, approximately 60 base pair region,
20 of the mouse *Rian* locus. What's interesting about that

1 is this 60 base pair region here does not exist in
2 other species. For example, in rabbit, human, dog or
3 elephant, this sequence is not there. It looks like at
4 least half of the AAV integrants in the *Rian* locus all
5 occur within this rodent-specific region. I will point
6 out at this point, about 50 percent of the integrants
7 also integrate in the *Rian* locus in regions of that
8 locus that have essentially identical sequence to the
9 human genome.

10 What did we know up to 2016? We know that
11 systemic delivery of AAV can cause hepatocellular
12 carcinoma in mice. AAV integration in those tumors is
13 primarily in the *Rian* locus, and many of the integrants
14 within the *Rian* locus are located within that unique
15 sequence of 60 base pairs that seems to be unique to
16 rodents. There's also the highest frequency of
17 hepatocellular carcinoma that's seen if AAV is
18 administered during the newborn period. Strong
19 promoter enhancer combinations increase the frequency.
20 Also, it appears as though the hepatocellular carcinoma

1 is dependent on dose and also, again, the strong
2 promoter enhancers.

3 Then these last two points, I want to point
4 out that up to this point there's a been very low
5 frequency of hepatocellular carcinoma observed if AAV
6 is administered to adult mice. Also, to my knowledge,
7 there's never been hepatocellular carcinoma observed
8 following CNS-directed AAV-mediated gene therapy.
9 These two points are important for the next two papers.

10 In the same issue of *Molecular Therapy*, in
11 February of this year, a collaborative group from David
12 Russell's group, Markus Grompe and Willscott Naugler,
13 they asked the question of whether liver injury --
14 chronic liver injury -- would affect the rate of
15 hepatocellular carcinoma, and in particular, in adult
16 animals. Basically what they did is they induced non-
17 alcoholic fatty liver in these animals and then
18 delivered adeno-associated viral vectors. They
19 delivered two vectors, one vector was a *Riantargeted*
20 construct. Basically this AAV vector had two arms that

1 were analogous to the *Rian*-locus, and they
2 preferentially integrated into that site.

3 In adult animals, with non-alcoholic fatty
4 liver, there was 100 percent penetrance of
5 hepatocellular carcinoma. But perhaps more relevantly,
6 they also used what would be considered a more
7 conventional AAV vector. It did not have homologous
8 arms, so it did not preferentially integrate within the
9 *Rian* locus. Following induction of non-alcoholic fatty
10 liver and then injection of a conventional vector, in
11 adult animals there was now a 50 percent penetrance of
12 hepatocellular carcinoma.

13 In the same issue of *Molecular Therapy*, back
14 in February, we published a study where we were trying
15 to treat the murine model of Krabbe. Krabbe disease is
16 a profoundly demyelinating disease and it's very
17 serious. And it's been refractory to most therapies,
18 including AAV-mediated therapy.

19 We determined earlier that if we combined
20 multiple therapies, targeting multiple different

1 (inaudible) mechanisms, we could dramatically increase
2 efficacy. So we performed a CNS-directed, AAV-mediated
3 gene therapy experiment in combination with bone marrow
4 transplant and small molecule substrate reduction drug.

5 Now, the interesting part is these animals
6 responded better than we had ever seen before. It was
7 really remarkable. Unfortunately, there was a nearly
8 100 percent penetrance of hepatocellular carcinoma in
9 combination-treated prep A mice and in wild type mice.
10 We did a cohort of wild type animals to determine
11 whether the supports were the same. Now all the tumors
12 had recombinant integration in the *Rian* locus, and some
13 of them had tumors in tumor suppressors.

14 Well, in my talk I originally had the table of
15 the integration sites, so I'll just review it here.
16 Again, every single animal that had hepatocellular
17 carcinoma had integrations in the *Rian* locus. In fact,
18 one animal had two independent integrations within that
19 locus in the tumors. Again, 4 out of 10 of the tumors
20 had AAV integrations in either known tumor suppressors

1 or candidate tumor suppressors, genes that affect
2 different types of cancer -- there was an integration
3 in the gene that was associated with breast cancer.
4 And in genes that regulate cell growth or cell death.
5 This was actually quite troubling to us.

6 In conclusion, up to this point what we know
7 about AAV integration in small animal models is that
8 AAV can stably integrate with the mouse genome. The
9 vast majority of integrated vectors appear to be either
10 mildly or grossly rearranged. Unfortunately, we still
11 don't understand the mechanism of integration in the
12 mouse. The integration of AAV in the *Rian* locus in the
13 mouse is associated with hepatocellular carcinoma.

14 Hepatocellular carcinoma appears to be dose
15 dependent. It's also dependent on promoter enhancer
16 strength. There's a low frequency of hepatocellular
17 carcinoma in adult mice, also a low frequency following
18 CNS-directed gene therapy. However, if you induce
19 liver injury, for example non-alcoholic fatty liver,
20 that can exacerbate the hepatocellular carcinoma

1 phenotype in adult animals. And also if you perform an
2 experiment with adjunct therapies, it might have a mild
3 oncogenic potential that greatly exacerbate
4 hepatocellular carcinoma.

5 With that I will close. I had an
6 acknowledgement slide in there, but apparently it got
7 removed. I'd like to acknowledge several people in my
8 lab, Anthony Donsante made the first observation of
9 hepatocellular carcinoma. Yedda Li, another graduate
10 students, showed the increase in hepatocellular
11 carcinoma following combination therapy. And of course
12 I'd like to thank David Russell's group, University of
13 Washington, for help with the initial integration
14 event. We've been collaborating with Chuck Venditti
15 for several years now on this particular project.
16 Finally, there was a roundtable discussion that was
17 recently sponsored by the ASGCT on this exact topic.
18 If you want more information, the link was on that last
19 slide. With that I'll stop and take any questions.

20

1 **INVITED SPEAKER PRESENTATION Q&A**

2

3 DR. LISA BUTTERFIELD: Terrific. Thank you
4 very much, Dr. Sands. We now have 10 minutes for
5 questions. I see we have the first question and
6 remember to turn on your microphone and your camera,
7 please. Dr. Vite.

8 DR. CHARLES VITE: Hi, Mark, nice talk. I
9 have a question. I think it's a question of knowledge.
10 If you found a similar incidence, or a similar
11 occurrence in people -- I don't know enough about
12 hepatocellular carcinoma. Ultrasound, CTs in mice or
13 model, how early can you predict the onset of disease
14 if you have therapy that might induce this occurrence
15 in terms of thinking about colonic cancers? What are
16 the kind of tests that can be done, or have been done
17 in animal models or in people, that could help predict
18 an onset of hepatocellular carcinomas in a potentially
19 treated group?

20 DR. MARK SANDS: Yeah, that's a great

1 question, Charles. First of all, in mice, we
2 approximately know the rate of formation of
3 hepatocellular carcinoma. First of all, we don't know
4 if it's a problem in humans. Second of all, if it's a
5 problem, we don't know the rate of progression. I
6 would think the standard ultrasound, MRI, CT would be
7 probably the first attempt you would make at screening
8 these patients or following these patients.

9 There's one experiment that I've always wanted
10 to do, but I've never been able to get funding for, was
11 to actually try to look for circulating tumor DNA.
12 That technology is advancing pretty quickly. And that
13 may be an effective method to determine whether a
14 patient is going to develop a tumor down the road, or
15 it's in the early stages of hepatocellular carcinoma
16 development. I'm not a clinician either, so, again,
17 what comes to mind, ultrasound, CT, perhaps circulating
18 tumor DNA analysis.

19 DR. LISA BUTTERFIELD: Thank you very much.
20 Our next question is from Dr. Joseph Wu.

1 DR. JOSEPH WU: One is that the
2 hepatocellular carcinoma that you saw in the mice are
3 quite concerning. But are they consistent across
4 different strains of mice or do you see some particular
5 strain that are more prone to hepatocellular carcinoma
6 and some strains that are more resistant? My second
7 question is, do you see a difference between sex
8 differences, male versus female mice? Yeah.

9 DR. MARK SANDS: Right, right. Both great
10 questions. All the work that we've done, all of our
11 mouse models are on a congenic lactic background. If
12 you go to the Jackson Lab tumor website, they've got
13 the relative susceptibility of various strains to
14 hepatocellular carcinoma. Interestingly, black six
15 mice seem to be actually resistant to hepatocellular
16 carcinoma, for whatever that means for the studies that
17 we've done.

18 Are there mouse models where they're more
19 susceptible? Certainly I think there was a study
20 published by Doug McCarty's (phonetic) group, where he

1 did it in a C3H mouse, which are more prone to tumors
2 anyway. He saw a high frequency of hepatocellular
3 carcinoma. There's also different disease states. For
4 example, (inaudible), those animals are more prone to
5 hepatocellular carcinoma. They have a higher frequency
6 of hepatocellular carcinoma following AAV. What was
7 your second question? I'm sorry.

8 DR. JOSEPH WU: The second question was do you
9 see a sex difference in terms of the mice?

10 DR. MARK SANDS: Right, right. We haven't
11 looked at that carefully. But it's well-known in the
12 field that the transduction rate in female mice is less
13 than in male mice. There appears to be as an effect of
14 estrogen. In the second to last study that I showed,
15 the group that I'll call it fatty liver, they did get
16 higher frequency in male mice. In fact, when they
17 treated male mice with estrogen, the frequency of
18 hepatocellular carcinoma decreased. So there is a sex
19 difference, and it appears to be estrogen-related.

20 DR. JOSEPH WU: Thank you.

1 DR. LISA BUTTERFIELD: Thank you. We've got
2 about five more questions. Let's see how many we can
3 get through. Dr. Roos.

4 DR. RAYMOND ROOS: Yeah, why liver cancer? Is
5 that clear? Second, do different serotypes have
6 different effects, different frequency? Third, I
7 realize AAV vectors lack grip, but are there
8 engineering methods that you could make human vector
9 AAVs less oncogenic?

10 DR. MARK SANDS: Right, right. Both great
11 questions. Why the liver? I'm just a dumb Ph.D. and I
12 don't study liver physiology, so I don't know. Except
13 to say that during liver development or liver
14 regeneration, if you will, the *Rian* locus is
15 transcriptionally a very active locus. Based on the
16 data where it appears that AAV likes to jump into
17 actively transcribed regions, that's one potential
18 explanation why AAV jumps into that locus in the liver.
19 Okay.

20 The other thing of course is that virtually

1 all of these vectors do an intravenous injection, the
2 liver is really the primary target. Even capsid
3 proteins that can direct more of the vector to the
4 brain or to the heart, for example, still the majority
5 of the virus goes to the liver in each case. It's
6 going to take the biggest hit.

7 Then, your second question, can you
8 incorporate the normal AAV mechanism for integration
9 into chromosome 19, where wild type AAV integrates?
10 It's a great question. I think that would be a good
11 place for a lot of research. Trouble is, it's a small
12 vector and you start putting in extra genes that may
13 target the integration to a safe harbor, now you have
14 less and less space for your expression (audio skip).
15 So it's difficult.

16 DR. RAYMOND ROOS: Thank you.

17 DR. LISA BUTTERFIELD: Thank you. Dr. Lindsey
18 George please. You're muted.

19 MR. MICHAEL KAWCZYNSKI: Lindsey, make sure
20 you're not muted on your own phone.

1 DR. LINDSEY GEORGE: Okay, there we go. Sorry
2 about that. Two questions, the first is, you mentioned
3 that the integration was primarily the ITRs. Did you
4 see this independent? If you tried different ITRs, did
5 you still see integration events in the *Rian* locus?
6 Then the second -- that were associated with
7 hepatocellular carcinoma. And then the second is --
8 and you may have mentioned this, and I missed it -- the
9 *Rian* locus is not a human ortholog, what are your
10 general thoughts on the translation of your work and
11 several others related to -- you know, in human
12 studies?

13 DR. MARK SANDS: Sure. The first question,
14 have we tried different inverter terminal repeats? We
15 have not and most of the field has used the original
16 AAV2 ITRs. It would be interesting to use ITRs from
17 other serotypes of AAV and see if that makes any
18 difference. Then, with respect to the *Rian* locus, so
19 the *Rian* locus in the mouse, there is a very highly
20 conserved *Rian* locus in humans as well. The overall

1 locus is very similar between mouse and human, but
2 there is that one unique region where many of the AAV
3 vectors integrate into there.

4 Again, we and others have pulled out
5 integrations across a large area of the *Rian* locus, not
6 just this unique region. What affect will that have on
7 humans? We don't know at this point. Are the
8 hepatocellular carcinomas in the mouse dependent on
9 that unique 60 base pair region? We don't know yet,
10 there's not been enough research yet. So we really
11 don't know how to translate this or extrapolate this to
12 the human situation at this point.

13 DR. LISA BUTTERFIELD: All right, thanks
14 again. We are at time. We're going to move now to the
15 second invited speaker on this topic and then we'll
16 have to save additional questions for the full
17 committee discussion in a couple of hours. It's my
18 pleasure to introduce Dr. Denise Sabatino, Research
19 Assistant Professor of Pediatrics at the Perelman
20 School of Medicine, to also tackle the topic of vector

1 integration and oncogenicity risks now in large animal
2 models. Dr. Sabatino please.

3

4 **INVITED SPEAKER PRESENTATION: "AAV INTEGRATION STUDIES**
5 **IN LARGE ANIMAL MODELS: NON-HUMAN PRIMATES AND DOGS"**

6

7 DR. DENISE SABATINO: Thank you, Dr.
8 Butterfield, and thank you to CBER for inviting me to
9 present today. I was tasked with presenting studies on
10 AAV integration in large animal models, focusing on
11 non-human primates and dog models. These are my
12 disclosures. While preclinical studies in large animal
13 models are commonly performed to support translation of
14 AAV gene therapy approaches, only a small number of
15 these studies actually perform AAV integration studies.
16 Shown here are non-human primates and dog studies on
17 AAV integration that have been published or presented
18 publicly.

19 The non-human primate studies use wild type
20 animals, so these are not a disease model, while the

1 dog studies, thus far, have used the hemophilia A dog
2 model. These different studies use different AAV
3 serotypes, different transgenes, but most of these --
4 and different routes of administration. But most of
5 these use IV approaches and most of these use
6 transgenes that are targeting the liver.

7 A range of vector doses have been used,
8 between 5 times 10 to the 12th and 9 times 10 to the
9 13th, which are clinically relevant doses. An
10 important point is that there are differences in the
11 duration of follow-up in these studies. The non-human
12 primates studies are usually followed for up to six
13 months. In contrast, the dog studies allow long-term
14 follow-up for -- in these studies -- up to 12 years
15 after vector administration.

16 The methods used in these types of studies
17 center around the use of next generation sequencing,
18 which provides the technology to produce an extensive
19 amount of sequencing data. Three methods have been
20 developed to perform these types of studies, ligation-

1 mediated PCR, linear amplification-mediated PCR and
2 target enrichment sequencing. These methods are
3 similar in that they fragment the genomic DNA and
4 attach linkers or adapters to these fragments. Then
5 PCR steps are used with primers in the vector genome
6 and then in these linker sequences to selectively
7 amplify integration sites. The test approach also
8 includes some additional enrichment steps. These
9 methods all currently use the alumina sequencing
10 platform and then the analysis is performed using
11 custom bio-informatic analysis Shopware.

12 There are several limitations to these types
13 of studies that include the primer selection, which can
14 influence the recovery. Also, the use of restriction
15 enzymes in some of these methods does not permit
16 identification of expanded clones, and this is a point
17 I'll elaborate on later in my talk. These methods can
18 provide data on the genomic positions of integration as
19 well as hormonal abundance, but it is challenging to
20 use these methods to estimate integration frequency.

1 Shown here are the five studies in non-human
2 primates, and I've tried to summarize the findings from
3 this work. These studies found that the majority of
4 sequences were vector-vector sequences. These could be
5 episomal or integrated forms, but most of them are
6 vector-vector rather than into the genomic sequence.
7 The vector sequences were very complex and rearranged,
8 and the studies showed, in some studies, no preference
9 for gene coding regions. However, other studies have
10 found recurrent integrations around transcription
11 units. Overall, no clustering or hot spots of
12 integration sites were reported.

13 Now, while the integration frequency is
14 reported to be low in these studies, there are some
15 methodological concerns about how that analysis is
16 typically performed. The majority of integration
17 events were detected as single events, so one or two
18 cells but in one of these studies there were what
19 appeared to be a small clone of up to seven cells
20 detected. Importantly, there's been no evidence of

1 nodules or tumors or malignancies in any of these non-
2 human primate studies.

3 Two dog studies have been performed to look at
4 AAV integration and both of these studies have been
5 performed in the hemophilia A dog model. One study has
6 been performed at the University of North Carolina in
7 Chapel Hill by my group in collaboration with Dr. Tim
8 Nichols. And the other studies been performed by Dr.
9 David Lillicrap's group at Queens University. These
10 dogs have less than one percent saturated activity and
11 have a bleeding phenotype that mimics the phenotype in
12 humans, and this really allows the opportunity to
13 evaluate efficacy and safety in a long-term setting.
14 Importantly, both of these studies were initiated in
15 the early 2000s with early generation AAV vectors.
16 These approaches use different promoter elements and
17 different AAV serotypes, and the doses used in these
18 studies were clinically relevant. The methods for the
19 AAV integration analysis were different between these
20 two studies.

1 Now I'm going to go into more detail on the
2 results of our study, which really illustrates some
3 common themes, but also found some unique findings. In
4 our study we used -- I apologize, the figure seems to
5 be disrupted. We used two different AAV vector
6 delivery approaches. The two chain delivery approach
7 delivered the canine factor eight transgene in two AAV
8 vectors, while the single chain delivery approach
9 delivered the canine factor eight B domain deleted form
10 in a single AAV vector.

11 If we look at the image on the right panel,
12 you can see that we observed dose dependent long-term
13 factor eight expression after AAV delivery with no
14 decline in factor eight expression in these animals.
15 The level of expression at the end of the study were
16 between 2 and 11 percent of normal levels, so
17 demonstrating durability of expression. Interestingly,
18 two of the animals in the study had a gradual rise in
19 factor eight expression. One of these dogs was treated
20 with the two-chain delivery approach, the second was

1 treated with the single-chain delivery approach. This
2 began about four years after vector administration, and
3 then continued for the duration of the study.

4 We have accessed liver function by analyzing
5 ALT, AST, and alpha-fetoprotein levels throughout the
6 study. There were some mild elevations in ALT, but
7 these were not consistent with any specific liver
8 pathology. The AFP levels were not elevated, and so
9 the increase of factor eight levels in these two dogs
10 were not associated with any abnormal liver biomarkers.
11 Clinically, there was no evidence of tumors or
12 malignancy in any of the dogs in this study.

13 The DNA analysis was performed on liver
14 samples that were collected at the end of the study.
15 The vector copy number analysis was performed by
16 quantitative PCR methods that then allow estimate of
17 the copy number, which represents both episomal and
18 integrated forms. The AAV integration site analysis
19 was performed in collaboration with my colleague Rick
20 Bushman at Penn. This is performed by ligation-

1 mediated PCR followed by Illumina sequencing, and they
2 analyzed the data using their custom software pipeline
3 they termed AAVenger.

4 The method that we used to perform the
5 integration analysis allowed us not only to identify
6 unique integration sites, but also estimate clonal
7 abundance. For these analysis, the DNA samples were
8 sheared using sonication, which then randomly fragments
9 the DNA and then adapter sequences were attached to
10 these fragments. The primers that were used resided in
11 the ITR and then the adapter sequence, which allowed us
12 then to selectively amplify and sequence these
13 integration events. This also allowed us to assess
14 clonal abundance by counting the number of unique
15 genome break positions that were associated with each
16 integration. This example here shows five different
17 genomic positions. From this example, we would
18 estimate the clonal abundance to be in at least five
19 cells.

20 I want to point out that studies that use

1 restriction enzymes, rather than random shearing of the
2 DNA, cause DNA fragmentation that is associated with
3 specific integration events who all have the same
4 genomic break point. So all of the events would have
5 the same genomic break point, and this would not allow
6 an estimate of clonal abundance.

7 Shown here is a summary of our DNA analysis.
8 We analyzed between 5 and 29 liver samples from each of
9 these dogs. The vector copy numbers were generally
10 less than one vector copy per diploid genome. We
11 selected six of the dogs, including two of the dogs
12 that had this rise in factor eight expression, for DNA
13 integration type analysis. We selected three liver
14 samples from each of these dogs, and the samples
15 collected, or used for the analysis, represented
16 samples that had low and a middle range and a high
17 vector copy number for that particular dog. This
18 analysis resulted in identification of about 1700
19 unique integration events.

20 I apologize for this slide, but the panel on

1 the right shows that there was a positive correlation
2 pertaining to vector copy number and the number of
3 integration events that we detected. We also looked at
4 the distribution of AAV integration events, which,
5 shown in this upper right panel, shows that they were
6 distributed throughout the canine genome. We found
7 that integration was favored in transcription units in
8 all the genes when we compared them to randomly
9 simulated events.

10 In these figures, the gray bars represent a
11 million random simulations, and the blue arrows
12 represent the observed frequency observed in the dogs.
13 The integration sites were also in genomic features
14 associated with active transcription, such as CpG
15 island, as well as GC-rich regions. As I mentioned,
16 the way that we performed these studies allowed us to
17 identify unique integration events, but also to look at
18 the abundance of the cell clones. The analysis that we
19 performed identified 54 unique or abundant clonal
20 populations. We defined abundant clones as those that

1 were identified in a unique site in at least five
2 cells.

3 This represents 54 out of the 1700 integration
4 events that we detected. Shown here -- again, I
5 apologize for this figure. But what is shown here is
6 the 15 most abundant clones for each of these dogs, and
7 each color represents a different gene, and each bar
8 represents a different clone. What we found was that
9 about half of these clonal expansions were in genes
10 that are known to be involved in cell growth in cancer.
11 Five of these genes were identified in more than one
12 dog, and these are highlighted by these asterisks.
13 These five genes have been associated with
14 transformation in humans. Two of the clones had a
15 clonal abundance of greater than 100, and that was a
16 clone in DLEU2. This is a clone or a gene that has
17 been commonly deleted in leukemia and has been shown to
18 play a role in HCC. And PEBP4 is also a gene that has
19 been implicated in multiple cancer cell types.
20 Overall, these data then supported the hypothesis that

1 integration of these genes was the mechanism
2 responsible for the outgrowth of the cells that we
3 observed.

4 We also performed a cluster analysis by
5 scanned statistics and identified five low sites where
6 we observed clustering of these integration events.
7 Illustrated here are EGR2, CCND1, and EGR3. All three
8 of these genes were also found in integration events in
9 expanded clones. Again, this provides some additional
10 evidence that integration within these genes might be
11 associated with cell persistence and proliferation.

12 I apologize for this slide. These are not
13 illustrating the method I want to share, but this is
14 supposed to be showing the full length vectors and then
15 the integrated forms of the vectors. While we did
16 identify a full length coding sequence of a vector, in
17 one of the expanded clones it continued to impact
18 coding sequence and an impact promoter, but most of
19 what we observed was very rearranged vectors that were
20 rearranged or truncated. These studies do support a

1 hypothesis, and perhaps a candid explanation for the
2 increase in factor eight expression could be the clonal
3 expansion of cells harboring integrated vectors in the
4 two dogs that had this rise in factor eight expression.

5 We also observed extensive rearrangements of
6 the AAV vector, so more than 80 percent of the
7 integration sites showed apparent integration of AAV
8 into itself. How might we know this? Well, we knew
9 that there was no integration observed in the genomic
10 factor eight locus, so there was no integration -- the
11 factor eight C DNA was actually where the
12 integration events resided. We know this because there
13 was no integration in intron and the sequence reads
14 went across exon-exon boundaries. There was no
15 integration within the Bdomain, which is excluded from
16 the actual vector sequence.

17 There was extensive vector rearrangement, and
18 we don't know if these were actually integrated or
19 episomal forms. And we don't know if these
20 rearrangements occurred during vector production,

1 although I think there's evidence in the field now that
2 there may be some rearrangements occurring during
3 vector production, but it could also occur after
4 transection of the target cells.

5 I just want to summarize our findings from the
6 dog study. We did observe stable and sustained
7 expression in these dogs for up to 10 years, but two of
8 these dogs did have a gradual rise in factor eight
9 activity. We believe these studies support a
10 hypothesis that this observation may be due to clonal
11 expansion of cells with integrated vectors. The AAV
12 integration was favored in transcription units and
13 oncogenes. The structures of these vectors were very
14 truncated and rearranged. Most of the forms we
15 detected were vector into vector forms. We did observe
16 some clustering of integration sites at five loci.

17 The clonal expansion we observed in five of
18 the six dogs that we analyzed in this study, we did see
19 mild asymptomatic elevations of liver enzymes, but
20 these were not consistent with any specific liver

1 pathologies, they were not consistent with the
2 elevation in factor eight expression. At the end of the
3 study, the liver pathology was consistent with age-
4 related findings that we also observed in naïve dogs
5 that were analyzed alongside. Importantly, while AAV
6 integration and clonal expansion were observed, the
7 dogs had no evidence for tumorigenesis.

8 I wanted to compare the two dog studies from
9 our group and from David Lillicrap's group. They do
10 have some similarities but they also have some
11 differences in what they have observed. Both studies
12 found in most of the forms are these vector-vector
13 forms, these are the most abundant forms detected. And
14 there are some differences in the distribution of the
15 integration events that we observed. It's important to
16 note that the canine genome itself is not well
17 annotated and so this can impact the analysis.

18 We found transcription units were factored
19 when we annotated the canine genome by transferring on
20 annotation from human genomes, but this was not

1 observed if you only use the canine annotation only.
2 Clustering was observed at some integration sites in
3 both studies, but the common sites were not identified.
4 The majority of the integration events we detected were
5 detected at single sites. But in our study we did
6 observe clonal expansions in some dogs, and this has
7 not been reported in the study from David Lillicrap's
8 group. While integration events were observed in all
9 these animals, no tumors were detected in any of the
10 dogs in these studies.

11 I wanted to touch on some of the challenges of
12 these AAV integration studies that this work
13 highlights. We know that AAV primarily remains
14 episomal and the assays we performed yield mixtures of
15 the episomal and integrated forms. The vector-vector
16 sequences, while they suggest concatemeric forms, we
17 don't know if these are actually integrated or
18 episomal.

19 There are secondary structures in the ITR that
20 can impact recovery of integration events. We

1 observed, and others have observed -- as Dr. Sands
2 mentioned -- that these vector genomes are often very
3 rearranged or truncated. This certainly can impact the
4 recovery of the integrated vector genomes.

5 Importantly, some of these vector genomes then result
6 in non-functional protein -- or would not result in
7 functional protein.

8 The tissue sampling in these studies really
9 represents a small population of the cells in the
10 tissue, especially here in the setting of solid
11 tissues, in the setting of the liver in particular.
12 Serial sampling of the same cell population is not
13 possible in these solid tissues; and furthermore, the
14 population size of the integrated vectors can be very
15 challenging to estimate because the analysis only
16 yields a subsample of what is actually the full
17 population in the tissue. Our studies illustrate, and
18 raise the point, that comparative genomics with
19 different model organisms and the quality of annotation
20 can lead to some apparent differences between the

1 studies. Vector design may influence and affect AAV
2 integration, and certainly the duration of follow-up
3 may impact the findings. While it's thought that AAV
4 integration may occur early after vector
5 administration, this is not well understood, and it is
6 not clear what occurs longitudinally.

7 While no malignancy has been detected in any
8 of these large animal studies, clonal expansions that
9 we observed in our dogs certainly raised the
10 possibility that these events could have genotoxic
11 consequences. Certainly studies of wild type viruses
12 have linked integration events to transformation, and
13 in the setting of retroviral gene therapy, potential
14 mechanisms for insertional mutagenesis and clonal
15 expansion have been documented.

16 Four of these mechanisms are illustrated here.
17 Each of these types of examples of mechanisms have been
18 found in animal models and, as shown here, they've now
19 been also observed in the clinical setting. These
20 include enhancer insertion. Enhancer insertion can

1 certainly upregulate genes near a site of integration
2 events. Promoter insertion that can promote
3 transcription of nearby genes, and this has been
4 proposed as a mechanism in work by Chuck Venditti's
5 group, after an AAV integration event that appeared to
6 drive HCC formation in mouse models.

7 Insertional activation can be another
8 mechanism. And an activation by a three prime end
9 truncation can occur by disrupting, for example, three
10 prime untranslated regions involved in regulation of
11 the gene. In this example it affected a microRNA
12 binding site. In the setting of AAV gene therapy,
13 these are also potential mechanisms for activation of
14 genes involved in cell growth and cancer. I will say
15 that the truncations and rearrangements we see in the
16 AAV genomes may make it difficult to predict the
17 consequences of these events in these types of
18 settings.

19 DR. LISA BUTTERFIELD: Sorry to interrupt, I
20 want to make sure we still have some time for questions

1 at the end.

2 DR. DENISE SABATINO: This is my final slide.
3 I'll just conclude by saying that as we've observed
4 that the AAV genomes are a mixture of episomal and
5 integrated forms. These genomes are very complex and
6 highly rearranged and lead to complexity of the
7 molecular structures we can identify. Clonal
8 expansions have been detected, but the mechanisms and
9 the biological significance of these are really not
10 known.

11 I also want to point out that the technology
12 and analysis tools are inherently limited and
13 alternative technology, such as long read sequencing,
14 are under evaluation. At this point it's not clear
15 whether these will provide any additional advantage for
16 these kinds of studies. Thank you very much for your
17 attention.

18

19 **INVITED SPEAKER PRESENTATION Q&A**

20

1 DR. LISA BUTTERFIELD: Terrific, thank you.
2 We have several questions already, so first, Dr.
3 Kenneth Berns please.

4 DR. KENNETH BERNS: Thank you for a very nice
5 presentation. The main question I have, and it's
6 really not for you specifically, is do you have any
7 sense of to what extent the actual base composition at
8 a junction sequence effects the ability of the
9 technology that you're using to actually detect that
10 junction?

11 DR. DENISE SABATINO: Perhaps what you're
12 asking is really addressing one of the limitations of
13 the technology, right? For example, in our work, we
14 use primers and interior part of the ITR, but a lot of
15 these ITRs were truncated. The placement of the
16 primers in these sort of strategies will impact
17 recovery of events that you will detect in the
18 analysis. Is that answering your question? As best as
19 I can?

20 DR. KENNETH BERNS: I'll tell you, you

1 confirmed the uncertainty that's inherent in all of
2 this stuff and what you do find is obviously
3 interesting. The question is, what happens if
4 (inaudible), maybe. Thank you.

5 DR. DENISE SABATINO: Thank you.

6 DR. LISA BUTTERFIELD: Next, Dr. Heller
7 please.

8 DR. THEO HELLER: Thank you. That was an
9 excellent talk and what I really appreciated was that
10 it was accessible to even a dumb clinician, not just
11 dumb Ph.D.s as the previous speaker noted. My question
12 is, are the dogs euthanized and if so -- or even on
13 liver biopsy. In humans we often seen clonal expansion
14 on biopsy and then we can delay the capture
15 microdissection of those specific areas and that allows
16 you to scan a far larger area of liver, volume of
17 liver. Has that been done?

18 DR. DENISE SABATINO: The dogs have been
19 euthanized. I want to emphasize that one of the dogs,
20 Linus, for example, the dog that had this increase in

1 factor eight expression, an ultrasound was performed
2 and a liver biopsy during the course of the study. But
3 (inaudible) found there were no concerns about nodules
4 or any areas of concern in the liver.

5 At the time of necropsy for all of these dogs,
6 there was no evidence for nodules or tumors, and there
7 were no clinical concerns as well, so that correlates
8 with that finding. We did carefully perform the
9 necropsy by looking at very small pieces of liver to
10 try to ensure that we try to address this question of
11 whether there are any nodules that we could investigate
12 further, and that was not the case.

13 DR. THEO HELLER: Let me just clarify, I'm not
14 talking about nodules that would be seen on crops or on
15 ultrasound. I'm talking about nodules that would be
16 seen on 40x magnification, you'd see a cluster of five
17 or seven cells that seems to be expanded. This is not
18 something that would be seen by any imaging or naked
19 eye?

20 DR. DENISE SABATINO: Sure. We did perform

1 some studies to look at the immunohistochemistry of
2 factor eight expression, and in some of the dog tissue
3 you do see small populations of maybe less than 10
4 cells, those little clusters.

5 DR. THEO HELLER: Yes.

6 DR. DENISE SABATINO: But this was not found
7 in all the dogs. It was not -- but it may suggest that
8 there might be some expansion.

9 DR. THEO HELLER: Yeah, so my question is,
10 would laser capture microdissection of those eight
11 cells be more helpful than looking for the proverbial
12 needle in the haystack? For example, in hepatitis B,
13 that sort of small cell dysplasia, the number of terms
14 for it is far more useful than green with imaging on
15 modularity.

16 DR. DENISE SABATINO: I think that's an
17 excellent point, and we have not performed those
18 studies. I think that's an excellent point. In some
19 cases, in the other dog study, in Dr. Lillicrap's
20 group, they do find nodular hyperplasia. And it may be

1 of interest also to do some more careful analysis of
2 these regions within the liver. I think that raises an
3 excellent point.

4 DR. THEO HELLER: Thank you.

5 DR. LISA BUTTERFIELD: Thank you. I'll see if
6 there are any other questions from the committee, and
7 also ask you when you were looking, you showed us the
8 timeframe for the analyses in the animals. It was a
9 pretty wide range of the number of years. Is there
10 anything driving that or is that just when the study
11 ended for that particular group of animals? Is there
12 anything that you've learned to date that suggests a
13 minimum time before it's worthwhile looking at these
14 integration sites and amplification events?

15 DR. DENISE SABATINO: I think this is an
16 interesting question, of the dogs we performed the
17 integration site analysis on, some were tissues
18 harvested more early on and some were tissues harvested
19 at 10 years after vector administration. Of the six
20 dogs we looked at we did find clonal expansions in five

1 of them. One of those was only after a few years after
2 vector administration.

3 As for why we terminated the study at these
4 different time points, the dogs were not all treated at
5 the same time and so we had selected a time point to
6 terminate the study. I should point out a few of the
7 dogs were terminated earlier due to some concerns about
8 bleeding events because these are hemophilic dogs. So
9 they were events not to be thought related to AAV
10 delivery, but there were some decisions made to
11 terminate those animals because there was some
12 challenges in maintaining them.

13 DR. LISA BUTTERFIELD: Thank you. Dr. Roos.

14 DR. RAYMOND ROOS: In these rearranged AAV
15 genetic sequences that are integrated, is this random
16 or do you see some preference for particular genetic
17 areas?

18 DR. DENISE SABATINO: Are you asking about the
19 sites of integration or the structures of the
20 integrated forms? Or both?

1 DR. RAYMOND ROOS: The integrated material
2 from the AAV.

3 DR. DENISE SABATINO: I want to point out that
4 it's quite technically challenging to validate and pull
5 out these structures, and all of the ones that we
6 characterized were found in expanded clones, so it
7 allowed us to further investigate them. There were no
8 common themes in the structures that we identified,
9 but, again, we only looked at a handful of structures.

10 DR. RAYMOND ROOS: Thank you.

11 DR. DENISE SABATINO: Because we could only
12 evaluate those in some of the -- what looked like were
13 expanded clones, where we had enough material to
14 analyze.

15 DR. RAYMOND ROOS: Thank you.

16 DR. DENISE SABATINO: Thank you.

17 DR. LISA BUTTERFIELD: All right, I think we
18 have time for one more question. Dr. Barry Byrne
19 please.

20 DR. BARRY BYRNE: My question really pertains

1 to both presenters. Your studies may be, in some
2 cases, decades ago and there are significant changes in
3 vector manufacturing technology and methods of
4 characterization, like in Mark's case. Do you know if
5 there are any retains of these preparations used in
6 neonatal mice that would allow for contemporary
7 characterization of the dose or advantageous proteins
8 in the preparations? Is that known?

9 DR. DENISE SABATINO: Well, I could comment.
10 In our studies we have vector sequences, or we have the
11 vectors still available in the freezer. We've realized
12 they are valuable and that we are interested in trying
13 to understand better what the vector genomes look like
14 in those AAV vector preparations. To your point, the
15 vectors used in the dog study -- our dog study -- were
16 really first generation AAV vectors. There are
17 differences between those vectors and the current
18 vectors in terms of vector design, for example. To
19 your point, I think it is instructive to consider
20 evaluating this in newer vectors and newer vector

1 preparation.

2 DR. LISA BUTTERFIELD: Dr. Byrne, you're muted
3 again.

4 DR. BARRY BYRNE: There we go, thanks. I'm
5 not controlling that. Yeah, the observed differences
6 between neonatal and adult animals is obviously
7 striking here. I wonder whether either of you could
8 speculate on the influences of neonatal extramedullary
9 hematopoiesis that might, in effect, make the effective
10 dose per hepatocyte to be significantly higher in
11 neonatal animals than in adult animals when the liver's
12 fully populated by hepatocytes. Any thoughts about
13 that, influence of that, have there been any neonatal
14 dog studies that you're aware of?

15 DR. DENISE SABATINO: There haven't been any
16 neonatal dog studies that I'm aware of. I think it's a
17 really interesting point. It also raises the point
18 that in a study where the livers are undergoing
19 significant hepatic proliferation, how does that
20 influence AAV integration? I'm not aware of any dog

1 studies that are ongoing to look at that more
2 carefully.

3 DR. BARRY BYRNE: Okay. Thanks.

4 DR. LISA BUTTERFIELD: All right. Thanks very
5 much. Thanks, Dr. Sabatino. Now we are ready for a
6 lunch break. This is going to be just under 30
7 minutes, so we will back at 45 minutes after the hour,
8 sharp. So that's 9:45 here in San Francisco, and 12:45
9 p.m. for all of you on the east coast. Thank you very
10 much. When we come back, we will have the OPH session.

11

12

[LUNCH BREAK]

13

14

OPEN PUBLIC HEARING SESSION

15

16 MR. MICHAEL KAWCZYNSKI: Welcome back to the
17 70th meeting of the Cellular Tissue and Gene Therapy
18 Advisory Committee Meeting. We'd now like to hand it
19 back to Dr. Butterfield.

20

DR. LISA BUTTERFIELD: Welcome back, and

1 welcome to the open public hearing session. Please
2 note that both the Food and Drug Administration (FDA),
3 and the public believe in a transparent process for
4 information gathering and decision making. To ensure
5 such transparency at the open public hearing session of
6 the advisory committee meeting, FDA believes it's
7 important to understand the context of an individual's
8 presentation.

9 For this reason, FDA encourages you -- the
10 open public hearing speaker -- at the beginning of your
11 written or oral statement to advise the committee of
12 any financial relationship you may have with the
13 sponsor, its product and, if known, its direct
14 competitors. For example, this financial information
15 may include the sponsor's payment of expenses in
16 connection with your participation in the meeting.
17 Likewise, FDA encourages you at the beginning of your
18 statement to advise the committee if you do not have
19 any such financial relationships.

20 If you choose not to address this issue of

1 financial relationships at the beginning of your
2 statement, it will not preclude you from speaking.
3 With that, I'd love to turn over this session to
4 Jarrod. Jarrod.

5 MR. JERROD COLLIER: Thank you, Dr.
6 Butterfield. Okay, for OPH Session, day one session
7 one, we have four OPH speakers and we will start with
8 Michael Themis. Michael Themis if you could please
9 introduce yourself and start your presentation, you
10 have five minutes.

11 DR. MICHAEL THEMIS: Thank you very much for
12 the committee for allowing me to speak today. I'm
13 Michael Themis from Brunel University, London, and
14 representing TestAVec. The title of my talk is
15 InGetox, a human in-vitro assay to evaluate the
16 genotoxicity of gene therapy vectors. We have been
17 commissioned to set up the study and to develop a model
18 for gene therapy vectors, including lentivirus and AAV
19 by the SBRI CRACK IT challenge, InMutaGene challenge,
20 sponsored by Novartis and GSK.

1 Essentially, our model is to address the
2 problems that we have with oversensitivity of animals
3 and sampling problems that we've already heard about
4 earlier on today, transfer of data from mouse to app to
5 human, and getting enough material to work with.

6 Second slide, please. The model is in two parts, the
7 first is an eligibility test which basically tests the
8 patients ability to repair their own DNA. If you look
9 at the graph on the right-hand side, you can see when
10 you infect cells -- and each of these is a T-cell line
11 -- you get DNA damage. Well, antivirus this curve out
12 five hours in our assay where we can see DNA damage and
13 measuring it, we see this happening at 10 hours, and
14 repair normally takes place after 24 hours. If a
15 patient can repair their DNA, then obviously they can
16 go towards gene therapies. If they can't repair their
17 DNA, as you can see by the blue line, then they would
18 be suggested, we would consider whether they'd be
19 eligible for gene therapy. The next slide, please.

20 The title of the slide is A Human Model. Here

1 is the overview of our study. We apply InGeTox or
2 lenti to our AAV vectors. We infect either the stem
3 cell or liver cell stage and we basically do
4 genotoxicity tests which include insertion sites,
5 clonal tracking, insertion site profiling integrity of
6 the vector, truncations between the vector and the
7 host, and epigenetic changes after infection.

8 This is some data simply showing that we can
9 convert IPS Cells to liver-like cells and
10 organocultures 3D. We can infect them very well with
11 the vectors. And for the purpose of today's
12 presentation, we compared a thin vector with a LTR
13 vector for antiviruses or a strong APOE -- oh sorry CB7
14 promotor -- with a weak APOE promotor in AEB2. Next
15 slide, please.

16 Integration amounts. Overall, this slide
17 shows that you get much fewer integrations with AAV
18 than you do with an lentiviral vectors. However, you
19 do see quite a few insertions and a lot of these
20 insertions are indeed cancelled genes where there would

1 be proton or gene suppressors.

2 We can carry on these studies over time. And
3 we can actually measure the insertions site by clonally
4 tracking these insertion sites by sequence counts. And
5 therefore, we can look at clonal evolution in the IPS
6 to liver model. The next slide please.

7 When we look at gene expression and we do our
8 NASX studies, we both see the both lenti and AAV strong
9 promoters as opposed to SIN vector or weak promotor AAV
10 over a global increase in gene expression.

11 Next slide, please. For fusion transcripts in
12 our model for all five tests, we see lentiviral vectors
13 do have a number of fusions between the genome and the
14 vector. And we see also for the vector in AAV strong
15 promotor, but not the weak promotor, an increase in
16 fusion transcripts. We also see -- on the next slide
17 please -- for the methylome changes. In other words,
18 when we infect them, if we do it three days or 30-day
19 time points for the lenti, or for at the three day time
20 points for AAV, we saw overall genome metalation

1 changes in the methylome of the cells.

2 But our model in (inaudible) I've been given
3 provides a tolerance of eligibility tests, plenty of
4 material to work with. We can actually generate a
5 liver model. We have also a neuronal model, a cardio
6 (inaudible) model, a developing T-cell model. We can
7 molecular genetically profile with our five tests. We
8 provide control vectors, it's robust, reproducible,
9 rapid and reliable. It's a human test so we've avoided
10 animal studies which are oversensitive.

11 We can provide information on the outcome of
12 these out-reads and offer vector redesign and retesting
13 and, of course, I say we have a ton of tissue types to
14 work from and our platform essentially is being
15 designed for lenti and AAV vectors as a replacement for
16 all the confusing models that are out there that so far
17 haven't been accepted by any of the regulators.

18 I thank you very much for your time. The
19 final slide is just the acknowledgements. We worked
20 closely with gene Werk well known in this field for

1 insertion site work and many of the other collaborators
2 were involved from various centers worldwide. Thank you
3 very much.

4 MR. JERROD COLLIER: All right, thank you very
5 much, Dr. Themis. Next, we'll move to Dr. David
6 Lillicrap of the Queens University. Dr. Lillicrap you
7 have five minutes, you can introduce yourself.

8 DR. DAVID LILLICRAP: Thank you very much.
9 Thank you for giving me the opportunity to present this
10 study. I'm from Queens University in Kingston in
11 Canada. The work I'm going to present has been
12 partially supported by BioMarin and we work in
13 collaboration with a group at gene Werk in Heidelberg.

14 So, the first slide you should be looking at
15 shows you Chromosomal Distribution of AAV Vector
16 Integration events. This occurred in a study that was
17 carried out now over a decade ago in eight hemophilia A
18 dogs that were treated with three different serotypes
19 of AAV, AAV 2, 6, and 8. Throughout the follow-up
20 between 8 and 12 years, they persistently presented

1 with vector eight levels between 5 and 10 percent in
2 six of the eight dogs. The information that you see on
3 this slide here was generated in collaboration with
4 gene Werk. The results were obtained with LAM-PCR and
5 target-enriched NGS sequencing. Sequencing was carried
6 out on an Illumina platform and customized
7 bioinformatic analysis was carried out. And the
8 sequences that were obtained were mapped to CANFAM 3
9 and CANFAM 4 reference canine genomes.

10 You've already seen some of this information
11 presented by Dr. Sabatino nicely just before the break.
12 And you can see that there's integration here in
13 autopsy liver samples throughout the canine genome.
14 So, if we could go to the next slide, please. What you
15 see on the next slide is that while the vast majority
16 of the integrations are either single copy or low-
17 frequency integrations, there are certain regions of
18 the genome whether it's preferential integration of the
19 vector sequences. And on this slide highlighted on
20 chromosomes 14, 28, and the X chromosome, you can see

1 many integration events that have occurred in regions
2 of these chromosomes of about 10 to 15 mega bases.

3 Whether these are clonal integrations or
4 whether they're just repetitive integrations into open
5 chromatin domains I think still remains to be realized.
6 We did not see dominant clonal expansions in the
7 analysis we have carried out in our studies.

8 So, on the next slide, please. So, the next
9 slide shows the results of the post-mortem liver
10 appearances. Three pathologists independently
11 evaluated the livers of these animals. And when they
12 did that, they showed that on both gross and
13 microscopic analysis that there was no evidence of
14 adenoma or carcinoma. No significant parenchymal
15 inflammation, no evidence of cirrhosis, hepatitis, or
16 fibrosis in the livers. There was microscopic
17 multifocal parenchymal nodularity. So nodular
18 hypoplasia was seen in six of the eight animals but we
19 recognize that this is an age-related change that's
20 seen in dogs. We see this in dogs that have not been

1 treated with any gene therapy. Then lastly there was
2 evidence of canine vacuolar hepatopathy, which is a
3 form of disease in the liver that can be produced by
4 adrenal hypoplasia or exposure to corticosteroids or
5 even in dogs with chronic illnesses.

6 So, if we could go to the last and final
7 slide. So, this is the slide where I'm just going to
8 summarize information. When I do this, I'm going to
9 highlight areas of similarity and differences with Dr.
10 Sabatino's presentation that you heard earlier this
11 morning. So, first of all, after more than 10 years of
12 follow-up, the majority of the information -- the
13 majority of vector forms we see -- are likely non-
14 integrated episomal forms where you see a vector/vector
15 junctions on the sequencing analysis that we did. So
16 that's in agreement with Dr. Sabatino's work.

17 The integration frequency that we were seeing
18 was about 1 in 1,000 to 1 in 10,000 cells -- see
19 integration events. And there are some common non-
20 random sites of integration that I highlighted in my

1 presentation. The integrated vector sequences are
2 highly heterogeneous with rearrangements and truncated
3 forms of the vectors. The integration sites were
4 predominantly intergenic in intergenic regions and
5 target analysis showed no evidence of dysregulated
6 adjacent loci. And we saw no evidence of liver
7 adenomas or carcinomas postmortem.

8 We did not see clonal expansions which were
9 dominant in the studies that we did. And we did not
10 see preferential clustering around adjacent growth
11 regulatory genes. So, thank you very much indeed for
12 allowing me to give this presentation.

13 MR. JERROD COLLIER: Thank you very much Dr.
14 Lillicrap. Next, we will move to Dr. Jing Yuan
15 representing Pfizer.

16 DR. JING YUAN: Hi, can you hear me?

17 MR. JERROD COLLIER: Yes.

18 DR. JING YUAN: Okay, good. My name's Jing
19 Yuan, I'm a gene therapy toxicologist at Pfizer Drug
20 Safety R&D. Thanks for the opportunity here to present

1 Pfizer's view on AAV vector genome integration and the
2 safety risk. So, I will first talk briefly about
3 existing evidence on AAV vector integration from both
4 preclinical and clinical data. Then I will provide our
5 core messages derived from those evidence. In the end,
6 I will provide our recommendations.

7 So, as presented by Dr. Sands earlier this
8 morning, preclinical evidence for AAV insertional
9 mutagenesis was mainly from the neonatal mice. Next
10 slide, please. The integration happened in the Rian
11 locus that lead to hepatocellular carcinoma
12 development. However, the same Rian locus does not
13 exist in humans. Will you please move to my first
14 slide? Another major evidence came from hemophilia A
15 dogs treated with AAV vector expressed in canine factor
16 eight as Dr. Sabatino presented this morning. This dog
17 had AAV integration and clonal expansion, but no sign
18 of tumor after more than 10 years follow-up.

19 So, so far AAV vector integration and the
20 tumor development have been analyzed in different

1 animal models. Other than neonatal mice, tumor has not
2 been shown in other animal species. For the other
3 animal models, AAV vector integration has been detected
4 in a very low frequency and the integration seems
5 random and across the genome. So preclinically, AAV
6 vector insertional mutagenesis is species-specific to
7 mice and age-specific to neonates.

8 Also, AAV vector integration rate is estimated
9 several orders of magnitude lower than nature mutation
10 rate in humans. Clinically, there is also no evidence
11 of cancer caused by AAV vector integrations from over
12 20 years clinical development, including FDA-approved
13 therapies with many years follow-up in children. Last
14 year this one hepatocellular carcinoma incident from
15 Eureka (inaudible) B trial was later determined
16 unrelated to AAV integration. In addition, we know
17 that a variety of AAV serotypes are endemic to humans
18 and wild-type AAV integrates. Still, no cancer has
19 been definitively associated with wild-type AAV
20 infection.

1 Next slide, please. So, what we have learned.

2 First of all, it is important to note that, unlike
3 lentiviral vectors, AAV vector by design is non-
4 integrative. Tumor risk identified in the neonatal
5 mice so far has not been proven in large animals and
6 humans. And sufficient integration data has been
7 generated in animal models and those data have been
8 repeating the existing findings, which suggest the
9 integration is low in frequency and the random. And
10 integration data in animals has not demonstrated
11 translatability to human tumor risk.

12 Currently, no evidence suggests that AAV
13 therapy poses additional cancer risk in humans, and
14 liver organ health, including tumor development, is
15 closely monitored in clinical trial participants. So
16 based on this core messages, we recommend that until
17 there is clear evidence linking AAV vector integration
18 to cancer in humans and the reference of non-clinical
19 data to clinical outcome has been determined,
20 preclinical integration study in animal models should

1 not be required due to limited value to support human
2 tumor risk assessment. And preclinical integration
3 data should not be a limiting step to R&D submission.

4 Integration data may be generated in animals
5 when higher integration risk is expected. For example,
6 with new vector design carrying certain risk factors.
7 Clinically, we think that the routine clinical
8 monitoring is a reasonable approach, and the current
9 guidance on the long-term follow up should continue the
10 adequate. Follow up liver tumor biopsy may be analyzed
11 for linkage to AAV vector integration if tumor is
12 identified.

13 Additional long-term observation study needs
14 to be specific to the patient population driven by
15 benefit and risk assessment. Overall, we appreciate
16 the committee to balance the benefit and risk for AAV
17 gene therapy and carefully inform a regulatory policy
18 to support this innovative drug development. Thank you
19 for your time.

20 MR. JERROD COLLIER: Okay, thank you very

1 much, Dr. Yuan. Lastly, we have Dr. Radoslaw Kaczmarek
2 representing Indiana University School of Medicine.
3 Dr. Kaczmarek if you can introduce yourself, you have
4 five minutes.

5 DR. RADOSLAW KACZMAREK: Thank you. Can you
6 please move to next slide(audio skip).

7 MR. MICHAEL KAWCZYNSKI: Hold on one second.

8 DR. RADOSLAW KACZMAREK: Yes?

9 MR. MICHAEL KAWCZYNSKI: Just hold on. We're
10 going to start you over just because we lost your audio
11 for a second here, and I just want to make sure the
12 studio has it back. Just hold on one second, sir.

13 DR. RADOSLAW KACZMAREK: Mm-hmm.

14 MR. MICHAEL KAWCZYNSKI: All right, I will
15 restart your time. Okay.

16 DR. RADOSLAW KACZMAREK: Is this my slide?

17 MR. MICHAEL KAWCZYNSKI: All right, go ahead
18 sir.

19 DR. RADOSLAW KACZMAREK: Is this my slide?

20 MR. MICHAEL KAWCZYNSKI: Yes, sir.

1 DR. RADOSLAW KACZMAREK: Thank you. Good
2 afternoon, my name is Rado Kaczmarek, I am honored to
3 present this perspective on vector integration and
4 homogenesis risk on behalf of the National Hemophilia
5 Foundation. Hemophilia Federation of America, World
6 Federation of Hemophilia, and the European Hemophilia
7 Consortium. We are grateful to the FDA for this
8 opportunity to present our position. Next slide,
9 please.

10 Hemophilia is a deficiency of coagulation
11 factor 8 or factor 9 which results in prolonged
12 bleeding. Recurring bleeding episodes mostly affect
13 the joints and lead to joint damage and disability over
14 time. Some bleeds occur in vital organs, including the
15 brain, and these are life-threatening. AAV vector used
16 for gene therapy for hemophilia are administered by an
17 intravenous bolus injection and target the liver. Next
18 slide, please. AAV gene therapy for hemophilia will
19 likely be licensed in the coming months and years.

20 Several phase three clinical programs are well

1 underway and have demonstrated the potential to provide
2 curative factor levels for years. The hemophilia
3 community, for whom this therapy has been the holy
4 grail for decades, now welcomes this progress and
5 remains optimistic as the field moves forward. But
6 many questions still remain about the short-term and
7 long-term safety. Next slide, please.

8 One aspect of long-term safety is vector
9 integration and potential oncogenicity. Its reliably
10 low rate of integration has led the field to
11 misperceive AAV as non-integrating vectors. But they
12 do integrate and with trillions to quadrillions of
13 vector particles injected that still means tens of
14 millions of potential integration events. So, in
15 absolute terms, this number is not small by any
16 measure. Next slide, please.

17 Although no confirmed cases of insertional
18 neurogenesis have been confirmed in the clinic, new
19 clinical studies have shown that it is possible, and
20 the risk may be affected by multiple vector quality

1 attributes, the importance of which we have yet to
2 fully understand. History of liver disease, which is
3 common in the general population, due to prevalent
4 known alcoholics having liver disease and yet more
5 common in people with hemophilia due to gasterogenic
6 viral hepatitis, may also affect the risk as the study
7 suggests. Even though all together this risk remains
8 theoretical in humans it's (audio skip) to be aware of
9 it and to watch out for it. All these uncertainties
10 merit a long-term follow-up and we believe it should be
11 life-long.

12 This can only be achieved by registries, and
13 we believe that there is a need for more aggressive FDA
14 policy mandating establishment of workable registries.
15 In 2018 the hemophilia community developed a core
16 outcome set to facilitate the life-long follow-up with
17 patients who have received gene therapy and this core
18 outcome set was used to develop the gene therapy
19 registry by the World Federation of Hemophilia in
20 collaboration with multiple stakeholders.

1 The FDA have been an active discussant on the
2 development of the registry. Global data collection on
3 all hemophilia patients who receive gene therapy is
4 essential in keeping patients safe and maximizes the
5 chances that rare adverse events in a small patient
6 population over a large geographical area will be
7 detected. Next slide. Thank you.

8 MR. JERROD COLLIER: Thank you very much Dr.
9 Kaczmarek and thank all the OP speakers for session
10 one. At this time, that concludes the session for OPA
11 speakers for session one, I will now turn it over to
12 Dr. Butterfield.

13

14 **COMMITTEE DISCUSSION OF QUESTIONS**

15

16 DR. LISA BUTTERFIELD: Great, thank you very
17 much Jarrod, and thank you to all of the speakers in
18 that session for sharing your information and
19 commentary. So, what happens now is the committee
20 discussion and this is on session number one, vector

1 integration and oncogenicity risks. So, we have a lot
2 to discuss today and tomorrow, so this is how we're
3 going to have the flow of the committee discussion.
4 This session has four different questions, and so to
5 maintain focus I will read them in turn.

6 First, we will focus on the first question
7 that I'll read, we'll have that discussion, we'll have
8 roughly 20-ish minutes to talk that through. I will
9 briefly summarize some of the key points, and any
10 recommendations from the committee at the end of that,
11 and then we will move sequentially to question two with
12 the same format. I hope that sounds clear and we'll
13 start then with the first question.

14 The first question to the committee for
15 session one, vector integration and oncogenicity
16 inherative. Please discuss the merits and limitations
17 of the animal studies to characterize the risk of AAV
18 vector-mediate oncogenicity and provide recommendations
19 for a specific preclinical study design elements to
20 include the animal species, healthy versus disease

1 models, and animal age, in life and postmortem
2 assessments including methods for integration analysis,
3 and duration of follow-up post-dose. This is our first
4 section and so I'm going to be watching for raised
5 hands, but first let me turn this over to -- initially
6 our discussant -- Dr. Charles Venditti to help lead
7 this discussion. Don't hear you yet.

8 MR. MICHAEL KAWCZYNSKI: Okay, Charles, just
9 make sure we got you connected here.

10 DR. CHARLES VITE: That work? Okay, so I have
11 a question regarding one of the topics we were talking
12 on in terms of integrative administration.

13 DR. LISA BUTTERFIELD: So before we go to the
14 group discussion, Dr. Vite, we're going to have Dr.
15 Venditti start the discussion, as the discussion
16 leader, and then I will call on everyone in turn based
17 on the raised hands on the list. Thank you.

18 DR. CHARLES VENDITTI: I wanted to thank the
19 committee for inviting me to participate and stress
20 that my opinions that I roll off here are mine and they

1 do not reflect an official NIH or National Human Genome
2 Research Institute opinion on this position. I want to
3 acknowledge the excellent presentations that came
4 earlier today, because I'm going to be highlighting
5 some of the messages that I think were transmitted
6 quite clearly in the early talks on the animal species
7 and some of the things that I'm going to try to address
8 in question A.

9 With respect to background, I would also like
10 to mention something for everybody in the audience and
11 to the committee as well, which is that there was a
12 paper that appeared yesterday from Dr. Marcus Krampe's
13 group that's quite relevant to today's discussion. But
14 I don't think we're going to be able to cover in
15 detail. But for those that are interested, it's
16 entitled AAV Integration in Human Hepatocytes And it
17 was just released last night in Molecular Therapy.
18 It's quite relevant to the discussions today so I might
19 make some comments related to this paper. For those
20 who want the detailed information, the reference is

1 YMTHE5602 and it can be found online.

2 I think one of the messages that was quite
3 clear from Dr. Sands' presentation and the discussants,
4 thereafter, are that the majority of the observations
5 to date that have seen HCC formation after therapeutic
6 AAV administration have been in neonatal mice. I think
7 we have to acknowledge that the neonatal mouse -- for
8 those who don't know about mice -- the neonatal mouse
9 is the equivalent of a very premature infant. It has
10 fused eyes. And one could even wonder whether or not
11 how relevant that is for the human neonate.

12 With that as an exception, or a limitation, I
13 think we all recognize. Also the major limitation that
14 I think that everybody would recognize immediately is
15 do humans predict mice? Some people would say no, some
16 people would say maybe. I think we can learn a lot
17 from mice. And the fact is what we're looking at here
18 as a theoretical risk, a signal. And in the early
19 animal model studies, that were detailed by Dr. Sands -
20 - some of them were done in my group here at the NHRI -

1 - we were not set up to do a study to a specific query
2 oncogenicity and HCC risk in genotoxicity. The studies
3 were designed to look at durability therapy. And in
4 those studies, a long-term complication that was seen
5 in the mice, most mice after the age of one year to
6 more typically after 18, or 16 to 18 months, developed
7 an HCC. So, when we think about the animal species
8 we're going to use, and how long we're going to monitor
9 the animals, I think this is a very important
10 consideration.

11 Someone does a study in a neonatal mouse model
12 with an AAV vector, and they sacrifice the mice at six
13 months, eight months, nine months, and they conclude
14 that they didn't see any pathology. Well, they may not
15 have waited long enough to see whether there was a
16 signal from that AAV that could be something to be
17 concerned about. But I think we have to remember that
18 when we look at the aggregate data and also keep in
19 mind in perspective, as I mentioned, that the studies
20 were not designed initially to look specifically at

1 genotoxicity, they looked at the durability therapy.

2 Nevertheless, what emerged from the studies is
3 that it really didn't matter what the genotype of the
4 animal was, so the disease state I think in some of the
5 models doesn't matter. Now there's been studies that
6 looked at disease-prone mice for other reasons that
7 mice develop HCCs and then tried to look at
8 accentuation of that risk by chemical methods and by
9 AAV to those mice. And those studies we didn't
10 discuss, but they are important studies and ask a
11 different question as we think about human translation,
12 which is to a patient that has a very low risk of HCC.
13 And there are genetic syndromes predisposed to this
14 that people can discuss later, what would be the risk?

15 Related to this, again, is also the age of
16 administration. We talked a bit about this. In the
17 human translation, one area of translation and one that
18 I and others have an interest in are to target inborn
19 (audio skip) metabolism and metabolic disorders that
20 oftentimes arise early in infancy and childhood. In

1 those patients, we will have to go in at a younger age.
2 So when one creates the animal models to recapitulate
3 the enabling data for a therapy, these are
4 considerations I think that are very important.

5 If you're going to try to treat a neonatal
6 condition, you probably don't want to use an adult
7 mouse and study that mouse. You might want to use the
8 neonatal model. And now you're going to be getting
9 into the considerations that can (audio skip). The
10 dose, as we heard, is critical. And one of the things
11 that we also didn't really focus on in great detail
12 that I think we're going to come up on other question
13 for, are the real details of the vector design and
14 genotoxicity imparted by the vector itself.

15 We have learned great lessons from the
16 integrating vector field that this is critical to
17 mitigate risk. In AAV vectors, the emerging data is
18 that -- at least from studies we have done that are in
19 the process of being prepared for publication based on
20 genome sequencing -- it looks like an enhancer

1 insertion event with transactivation. That's the
2 mechanism. So, if that's the mechanism, that
3 immediately makes a prediction about what studies could
4 be done and what types of strategies could be
5 undertaken to mitigate risk.

6 So, I don't know that I'm going to make more
7 comments on A. On section B we also heard some really
8 nice presentations about the integration studies. Dr.
9 Sabatino -- that's a landmark study, very, very
10 important -- points out in her presentation there's
11 different methods to detect AAV integrations. Some
12 depend on the inverted terminal repeat detection, and
13 some depend on a sharing with a less biased approach.
14 And I think if you were to look at the paper I
15 referenced in the beginning -- that paper from Dr.
16 Grampe's group -- there's a very important advance in
17 using next-generation long-reach sequencing to get
18 around the short read techniques that might limit the
19 interpretation of AAV integrations. So, one of the
20 things that I think that comes from the studies we're

1 presented, is that AAV integration studies there might
2 be a need to standardize such studies.

3 And how to do that, and whether there should
4 be centralized labs that are certified for this,
5 whether they should be reference standards for this,
6 those are questions I think would be good for the
7 committee.

8 The last question regarding the durability or
9 duration of follow-up, I'll allude to the comments I
10 made to section A. Which is conditions that I think we
11 would treat in the pediatric period where we're looking
12 for long-term durability. We probably need to do mouse
13 studies that match, that are longer in term and that
14 have longer-term follow-up. That have different doses,
15 including doses that might be near the toxic range, and
16 follow the animals long-term.

17 So, with that I will just make one other
18 comment, which is something that also didn't come up
19 today in discussion. And it's a controversial topic/
20 It's whether or not, in some rare cases, wild type AAV

1 can be associated with integrations and HCC. There's
2 certainly suggestive data for this, and that in turn
3 has led to mechanistic studies on what is it that's
4 driving that from the AAV's, and that, in turn, informs
5 vector design. So, with that, I'll end my comments and
6 pass the baton to the next speaker.

7 DR. LISA BUTTERFIELD: Terrific. Thank you
8 very much for setting the stage so well for our
9 discussion and for reading the literature yesterday.
10 So, we've got a series of participants, and let's go
11 now to Dr. Vite and hear from you please.

12 DR. CHARLES VITE: Yes, thank you. So, I
13 would also bring up that it's wonderful to hear from
14 Dr. Venditti as a review. I'd also bring up that the
15 route of administration has got to be one of the
16 important topics that we go on. We've been talking
17 primarily about intravenous administration, even
18 targeting of the liver. I think on Dr. Sands' comment
19 about how the CNS-directed delivery did not result in
20 any hepatocellular carcinomas in early studies but now

1 there is some evidence of that. I think the question I
2 would have there still would be exploration of the
3 mechanisms since very little AAV could typically get
4 out of the CNS into the liver that high incidence of
5 hepatocellular carcinomas in those become very, very
6 interesting. But I did want to stress that if we're
7 talking about primarily intravenous and the
8 administration to the liver, other methods of
9 administration (audio skip) whether they be intraocular
10 or intra-CSF delivery, all may require different types
11 of monitoring for an integration to be focused on the
12 liver because of hepatocellular carcinomas.

13 But I think the idea of studying integration
14 in all different tissues can become an overwhelming
15 episode, especially if there is no evidence of any
16 neoplasia's as of yet in those tissues. And I do agree
17 with Dr. Venditti that setting them larger for a longer
18 period of time is going to become an essential thing.

19 DR. LISA BUTTERFIELD: Great, thank you very
20 much. Next up we have Dr. Bushman, please. We can't

1 hear you yet.

2 MR. MICHAEL KAWCZYNSKI: You're still muted,
3 you have your own phone muted.

4 DR. FREDERIC BUSHMAN: Can you hear me okay?

5 MR. MICHAEL KAWCZYNSKI: Yes.

6 DR. FREDERIC BUSHMAN: So, I wanted to just
7 comment following up on the integration study analysis
8 and some of the technical points that Dr. Venditti
9 raised. So, we did the genomic work with Denise on the
10 dogs, and just commenting on some of the differences
11 with the Lillicrap study. I think they found much less
12 integration in transcription units, but I think we used
13 different annotation of what were the transcription
14 units on the dog genome. So, we transported human
15 annotation over onto dog, and so had much more of the
16 genome annotated as transcription units.

17 So, I think there's probably a relatively
18 simple explanation for that. The question about,
19 again, comparing to the Lillicrap work, no dominant
20 clonal expansion. I think, again, the methods used --

1 the details in the methods -- could have a big effect
2 on whether or not you detect a clonal expansion. So,
3 the way we detect it, again, repeating what Denise
4 said, shearing the DNA, ligating on adapters, and then
5 you have a unique integration site. If you get a lot
6 of adapters in different positions, that meant there
7 were a lot of DNA chains in the initial mix. And
8 that's how we know if we see clonal expansion.

9 So, things like your sampling effort, how much
10 sequencing did you do? How efficiently did your linker
11 ligation go? All those kind of details influence your
12 recovery and your interpretation of whether you saw
13 clonal expansion or not. So, the details of that, I
14 think, are quite important.

15 Then lastly, we've heard several speakers
16 mention the numbers of integration sites per cell, the
17 population of integration sites per cell. And I think
18 that is a very difficult thing to estimate. Denise and
19 I, and some collaborators wrote a preprint, and it
20 should be out on Med Archive in a couple of days. It's

1 submitted to a journal. Just briefly, if you carry out
2 an integration site sequencing study on a sample -- say
3 for dogs or humans or whatever -- and sequence and
4 analyze, you'll find some integration sites. Now, it's
5 only a sample of what's there, that's not all of what's
6 there. You know that because if you go back to the
7 tube and do it again, you get overlapping sites but a
8 lot of different ones as well. And do it again you get
9 still new ones, again, still new ones. So, you're
10 getting a sample of the population, not the whole
11 population. So, figuring out what's really there
12 involves mathematical methods to reconstruct the size
13 of the population from a subsample of that population.

14 So, we would not been hearing about that in
15 the integration site field. We use (inaudible) one
16 which gives a lower estimate. That doesn't estimate
17 the population, it gives a lower bound. And I think
18 the whole issue is really fraught with technical
19 challenges. And so, we tried to lay that out some in
20 this recent paper. So, I think the field could really

1 use a hard look at how we're estimating these numbers
2 and what the uncertainties are. I'll stop there.

3 DR. LISA BUTTERFIELD: Thank you very much,
4 Dr. Bushman. Next, we have Dr. Zeiss.

5 DR. CAROLINE ZEISS: Yes, thank you. My
6 question pertains to the dog studies. There is
7 integration but it's unclear to me if that actually
8 confers a proliferative advantage to those cells.
9 There is a native rate of plume expansion in normal
10 livers, and you can see that with cytochrome C oxidase
11 staining. And it goes (inaudible) tract to a DNA
12 methylation patterns and mitochondrial DNA patterns.

13 The question is if you were to do, for
14 example, serial liver biopsies and it would come down
15 to laser capture microdissection and look at the extent
16 of those clones that are expanded that have integration
17 sites -- AAV integration -- compared to those that
18 don't, it may not be the case that AAV actually drives
19 a preferential clonal proliferation. So, I don't think
20 we actually know that yet.

1 The other point I'd like to make is that the
2 dog pathology is completely unremarkable for dogs of
3 that age. I think that relying on tumors as being an
4 indicator and humans are exposed to some oncogenic
5 drivers in the liver that dogs have less access to, so
6 I do think we need something that is more sensitive
7 that actually hooks the integration events to
8 proliferative potential of those cells, And I will
9 stop there. Thank you.

10 DR. LISA BUTTERFIELD: Thank you very much.
11 Dr. Herzog, please.

12 DR. ROLAND HERZOG: So, the studies in the
13 mice, they're quite elegant but I just want to point
14 out, that since the relevant human study is already
15 being done in form of gene therapy for SMA, we should
16 pay close attention to what happens in those patients
17 over time. Because if you think about that, these are
18 patients that are treated very young, they receive a
19 very high dose of vector systemically. It is a capsid
20 that I believe transduces human hepatocytes quite well

1 and they're using a (audio skip) promotor, so enhancer
2 promotor so there was strong cell-specific
3 enhancer/promotor combination.

4 As far as I'm aware, there hasn't been cancer
5 formation observed in those patients, but I don't know
6 how many patients were treated in total and how long
7 they've been followed up. But I think we need to pay
8 close attention to that to see how relevant and
9 predictive these neonatal mouse studies really are in
10 that regard.

11 DR. LISA BUTTERFIELD: Thank you very much.
12 Anything else Dr. Herzog?

13 DR. ROLAND HERZOG: No. I just wanted to make
14 sure that we're thinking along the lines of what would
15 happen in human gene therapy.

16 DR. LISA BUTTERFIELD: Thank you. Do put your
17 hand down after you've spoken and then you can put it
18 back up and go back to the line of speakers if you have
19 an additional comment, please. So, Dr. Heller.

20 DR. THEO HELLER: Thank you. I'd like to

1 strongly support what Dr. Zeiss said. I think that
2 those suggestions were very pertinent. I also want to
3 step back and instead of talking about animal models,
4 wanted to think about human models. In humans we have
5 viruses that integrate, and we have a sense of what
6 happens to those humans. And we know that it takes
7 decades to develop cancer, for example, in hepatitis B
8 or in HIV -- HIV not always decades. But based on
9 these models, I think long-term follow-up. And the
10 fact that Dr. Venditti said, that the smaller children
11 would be treated, makes it even more important that we
12 take the timeline in humans and what we already know
13 about human biology into account when talking about
14 these models. Thank you.

15 DR. LISA BUTTERFIELD: Thank you very much.
16 Dr. Breuer.

17 DR. CHRISTOPHER BREUER: I don't believe there
18 is one answer to this question. And I think that the
19 answers to this question would need to be tailored to
20 one, the diseases being treated with regard to its

1 severity and the currently available treatment options.
2 Then, two, I'd like to point out that any animal study
3 could potentially be irrelevant, and you will only find
4 that out after the fact when you compare your
5 preclinical and your clinical data. And so far based
6 on the data presented today, while there's strong
7 animal evidence pointed to the possibility of formation
8 of liver tumors -- and this is something that will need
9 to be monitored very carefully clinically -- so far,
10 the signal in the clinic doesn't seem to be very
11 strong. I wonder if this is the wrong question.

12 DR. LISA BUTTERFIELD: Thank you very much.
13 Dr. Venditti, please.

14 DR. CHARLES VENDITTI: I wanted to rekindle a
15 comment that was made by Dr. Bushman. I think it's
16 worth discussion. Which is as we think about
17 integration studies for AAV and what we can learn from
18 them, it's important to put it in perspective from the
19 integrating vector field. And just think about the
20 limitations that we have and what we might learn.

1 First of all, I did hear some comments made by
2 one of the speakers that AAV did not have integration
3 preferences and it's random. I think there's been a
4 number of citations in the earlier presentations today
5 that show that AAV does have a tendency to integrate
6 towards the five prominent genes and actively
7 transcribe genes. It's not truly random. But I would
8 also caveat that by saying we have very low numbers of
9 integration events compared to some of the studies that
10 have been done, with integrating vectors, where there
11 are hundreds of thousands of events that could be
12 captured.

13 And by doing this with more (audio skip)
14 events that in some cases are (audio skip) you can
15 learn about preferences much (audio skip) than you can
16 with AAV integrations require, I think, at some level
17 simulation studies as is mentioned. But I think it's
18 worth mentioning this and thinking about how can you
19 (audio skip) datasets and what types of (audio skip) we
20 could use to get more and more (audio skip) saturate in

1 some ways the number of integrations we could get in
2 hepatocytes, and other tissues as was raised by Dr. V.
3 That's very important and hasn't been studied much.

4 So, I think it's worthwhile rekindling this
5 because it is important. And I would also suggest and
6 mention as well that going back to the fundamental
7 question of animal species and monitoring, to my
8 knowledge and the studies of SMA, very long studies
9 that were year of age and treaded mice as neonates, I
10 don't know that I ever saw any of those papers. I
11 didn't specifically look for the studies as recently as
12 yesterday. But unless one watches the mice for very
13 long times -- and again, this is what Dr. Sands
14 observed originally in his studies. You have to watch
15 the mice of more than a year, sometimes more than 16
16 months, to see the events come on. So, if you end your
17 tox studies at a year, you might not see an event that
18 occurs at 16 months of age with a higher frequency.

19 The other thing that I think's worth
20 mentioning on the SMA vector that's being used in

1 humans -- again, I'm not an expert in this field -- but
2 that's a double-stranded (audio skip). The
3 configuration of that AAV is different than the single-
4 stranded AAVs, but all the studies we heard today with
5 the hemophilic animals and the mice studies, use
6 single strand AAVs. Is there a difference in the
7 integration patterns between an SC and an SSAAV, I
8 don't know that anyone's really precisely determined
9 that.

10 I think it's something that could be important
11 because it could drive integration preference. And
12 also whether or not the exact sequences in the promotor
13 enhancers, and in the small sequences that sometimes
14 carry into AAVs from the original (audio skip). I'm
15 talking now about AAV (audio skip) element that Ian
16 Alexander and Grant Logan described in nature genetics
17 a few years ago. Whether those are sequences
18 approaches. Earlier today, in many of the studies
19 we've heard about, it is my understanding that those
20 sequences carried over.

1 So, I'm wondering if we could have more of a
2 discussion of density of integrations required to get a
3 confident idea about do we see a (inaudible)
4 preference? I would say that the dog study, it didn't
5 need a lot to see that integration preference in some
6 of the genes that Dr. Sabatino discussed. There was a
7 clear integration preference, there were clear CISs.
8 But I think it's worth now to have more confidence
9 about that as we think about should we be doing routine
10 integration studies in another species and what can it
11 tell us?

12 So, I would turn the floor back to my
13 colleagues and I look forward to their comments.

14 DR. LISA BUTTERFIELD: Thank you very much,
15 and so let's then pause with the line of questioners
16 here and perhaps, Dr. Bushman, if you could come back
17 on and comment on specific question to you that was
18 just raised. Also, what you've learned comparing AAV
19 and lenti and more about the integration. Thank you.

20 DR. FREDERIC BUSHMAN: Thank you. Can you

1 hear me okay? For the retroviruses, gamma
2 retroviruses, and lenti viruses, they each have -- as
3 you all know -- they encode their own integration
4 enzymes. And those enzymes find host factors. HIV
5 integrates finds LEDGF/p75, which tethers and targets
6 to integration reactive transcription mutes. Gamma
7 retroviral bacteria bind to the BRD proteins, gene five
8 prime Ms and integrate there.

9 With AAV it seems to be totally different as
10 most of you all know. It seems like there's
11 preexisting double-strand breaks in cells. And then
12 there's a DNA repair pathway. When there's a break,
13 you can copy from the chromosome across AAV and onto
14 the F chromosome on the other side and pick up a piece
15 of AAV that way. So, there's sloppy junctions. DNAs
16 may break more commonly at the ITRs because they form
17 hairpins which are substrates for nucleases. So, it's
18 a much more complex and heterogenous event.

19 So, I think it's going to be largely a
20 question of where are double strand breaks enriched,

1 and where do you repair efficiently going through that
2 whole process? It does seem like many studies have
3 seen active transcription units are a little more
4 favored for integration by AAV, and so why is that? I
5 think there may be some evidence that double strand
6 breaks may happen a little more often in active
7 transcription units because of (inaudible) formation
8 and stuff like that, but kind of an interesting
9 research area going forward.

10 But really much, much different mechanisms for
11 the retrovirus, which have tethering mechanisms versus
12 AAV, which is integrating at double strand breaks in
13 cells. So, did that answer your question?

14 DR. LISA BUTTERFIELD: I think that certainly
15 helped. Yes, Dr. Venditti?

16 DR. CHARLES VENDITTI: Yeah, no, those are
17 fascinating comments. I'm just wondering if you can
18 comment. What I'm wondering about specifically is, how
19 many sites do we need for AAV? The studies that we
20 have, we usually don't get, we get thousands of sites.

1 Whereas the integrating vectors can get (inaudible) of
2 magnitudes more, and then you can learn more about
3 specific patterns. It just seems to me like -- is that
4 going to be a fundamental limitation of the AAV
5 integration studies. In addition, the biology's very
6 different, but do we have enough sites? How can we get
7 more sites without limits?

8 DR. FREDERIC BUSHMAN: Yeah. It's probably a
9 little less efficient isolating AAV integration sites.
10 Because you have this sort of heterogenous structure
11 and the ITR can fold and so that suppresses PCR, at
12 least some of the time. I guess how many you need
13 depends on exactly what your question is. If you're
14 interested in very rough -- whereas integration favored
15 relative to transcription units you may not need that
16 many. But is there something special about certain
17 rare regions of the chromosome?

18 I guess another question would be initial
19 integration versus accumulation of sites after
20 selection, after long-term growth because those are two

1 separate things. Integration happens initially, then
2 some integrants can promote or oppose subsequent growth
3 of cells inside an organism. And so distinguishing
4 between those two, particularly after long-term growth.
5 Often, it's hard to get cells because there aren't that
6 many cells left or something after a long outgrowth.
7 So that can take a lot of sampling effort.

8 So, I think much of it comes down to exactly
9 what your question is. And most statistician would say
10 that then dictates what you need for a sample size.

11 DR. LISA BUTTERFIELD: Thank you both very
12 much for more details that really, I think, inch on a
13 lot of the different questions where we're trying to
14 discuss and make recommendations on. So, let me go
15 back to the folks who wanted to make additional
16 comments on this first question and the sub-questions
17 before we round it out then move to the next. Dr.
18 Barry Byrne, please.

19 DR. BARRY BYRNE: Thanks, Lisa. So, I just
20 wanted to reiterate one of the points that was made

1 earlier because I think there's some agreement, general
2 agreement, that the toxicity are truly dose-related.
3 And also the exposure to any (audio skip) proteins and
4 vectors (inaudible) are increased proportionally with
5 the new dose. So, these are complex biologics and
6 really there needs to be a more centralized and
7 coordinated effort to standardize dose considerations
8 because this is, I think, a first principle and
9 (inaudible) study.

10 So that's something I think that the field can
11 work towards. It's a precompetitive issue that we work
12 towards in our center years ago to develop a national
13 reference standard. But this is not frequently
14 referred to in quantifying or making a ratio of a given
15 test article in an animal study, just something that
16 can be considered.

17 And then, regarding the other parts of the
18 question related to species, these models and age. So,
19 since it's clear that these are not acute events, long-
20 term studies are warranted. So, other rodent species

1 are going to be considered. And there was a
2 consideration of a platform study to be done in rats
3 through the MIST program of toxicology. That's
4 consideration on how to get the largest number possible
5 longer-term follow-up in those animals because they
6 have a very comprehensive tumorigenicity assessment for
7 small molecules and that could be applied to AAV
8 vectors as well. Or in canines who have been used in
9 the hemophilia studies.

10 But again, they're purpose-bred animals that
11 might give a more uniform response than the outbred
12 species that would be -- primates, for example, would
13 not probably be very informative. And the in-life
14 assessments in rodents probably do need to be for their
15 lifespan. And biopsies are not really practical for
16 in-life assessment, but the total follow-up and
17 monitoring should be life-long. That concludes my
18 comments about this first question.

19 DR. LISA BUTTERFIELD: Great, thank you very
20 much. Dr. Kenneth Berns, please. Can't hear you yet.

1 DR. KENNETH BERNS: Can you hear me now? I
2 think a couple of basic points. One is remember that
3 the ITR is the equivalent of a holiday junction, which
4 was long described intermediates in DNA per
5 combination. Secondly, the question to me is if you
6 think about the different kinds of AAV vectors which
7 sequences -- AAV sequences let's say in the vector --
8 are we worried about? Should we really be worried
9 about any AAV sequences beyond the ITR in terms of
10 promoting insertion?

11 I don't know if that's been well studied up to
12 this point. I would say that in the French studies
13 with HCC, where they found evidence for wild-type AAV
14 2, it was important not only to have the right side ITR
15 but also 100 or 150 unique sequences from the right end
16 of the genome. And that recapitulated studies that
17 have been known 40 years ago looking at the combination
18 between AAV and SV40 in cell culture. But most
19 vectors, the way they're made, don't contain that
20 unique sequence from the right end of AAV.

1 So, I think that's something that we really
2 have to think about is what is it that's important in
3 terms of the AAV sequence that we should be thinking
4 about in terms of insertion. And then what's
5 important? So, yeah, I think that the thing that was
6 pointed out earlier in this morning's discussion is
7 that although the capsid vary in various vectors, in
8 fact, the ITR almost inevitably, as far as I know, is
9 from AAV 2. And the question is, is that important?
10 Do we have any way of knowing that? I don't think
11 that's been looked at in great detail. But these are
12 things it seems to me that we're really going to think
13 about before we can really get serious about this
14 question of AAV-inducing oncogenicity.

15 DR. LISA BUTTERFIELD: Thank you very much,
16 Dr. Berns. So, let's have one more comment here, and
17 then let me try to sum up. I've let this go a little
18 long because I think a lot of these comments touch on
19 some of the later questions that we won't probably need
20 as much time for. But let's go to Dr. Ahsan, please.

1 DR. TABASSUM AHSAN: Great, thank you. This
2 has been a great technical and scientific conversation
3 and I think that it's been really robust and dynamic.
4 But if I think about the context of the question
5 there's a few things here. It does seem that it is --
6 and I think another member mentioned this -- it's a bit
7 premature, it seems like, to provide really general
8 recommendations on preclinical studies this time.

9 There are questions related to species
10 relevance and how to tease that apart and how and which
11 parameters should be set. So, I do think that B is a
12 little bit more interesting in terms of the integration
13 analysis. It does seem that there are indications that
14 there's preferential insertion sites. Really digging
15 into that and how that might really relate to the
16 oncogenicity is, I think, pretty important. I think
17 we've got a couple things happening simultaneously, the
18 technology is still evolving. But we do need to start
19 to drive towards standards, otherwise we're really not
20 going to be able to generate the database of

1 information that we need to make informed decisions as
2 we move forward. Especially because some of these
3 technologies have unintentional data bias based on the
4 method. I think we're starting to get a sense of the
5 scientific issues that are out there, but we need to
6 start to drive towards some types of standardizations
7 and understanding that'll help us give solid
8 recommendations for preclinical study design as we move
9 forward.

10 So, I just wanted to reiterate in terms of the
11 question, how the science and the technology relates,
12 and that maybe this question is definitely a bit
13 premature to set specific recommendations.

14 DR. LISA BUTTERFIELD: Terrific, thank you
15 very much. So, as we think about the first question
16 about vector integration and oncogenicity, let me
17 summarize a few key points that I think I've heard from
18 this initial discussion. And then you can let me know
19 if there's something else that should be included or
20 changed. In terms of the merits and limitations of

1 animal studies, I heard general agreement that animal
2 studies are, in general, problematic with their
3 relationship to humans. Sometimes they show us what is
4 possible, and other times they are not sufficiently
5 representative of the human situation. And that the
6 studies in neonatal mice have shown us what a
7 particularly sensitive animal model can show us is
8 possible. But the neonatal mice might not be a good
9 model even for newborn humans. Although, a newborn
10 human model is critical when dealing with inborn eras
11 of metabolism and gene therapy in that realm.

12 So, in terms of animal species, there's
13 relevance certainly also to dogs and there's also
14 consideration of other animal models that are less
15 studied, including rats. So, in healthy versus
16 diseased models, we didn't really discuss that much but
17 animal age, I think I've covered neonates being
18 particularly sensitive. Another consideration, though,
19 is the route of delivery. We've spoken mostly about
20 intravenous delivery that does preferentially go to the

1 liver, and that is the model and approach that is most
2 likely to induce liver disease if at all compared to
3 other routes of the delivery where the assessments
4 might need to be organ-specific based on route of
5 delivery.

6 In terms of assessments, and methods for
7 integration analysis. We had a great discussion on
8 this and a paper that came online just yesterday was
9 brought to our attention that's also highly relevant
10 about new technologies. So, there are very complex
11 multiple types of sequence analysis, multiple types of
12 interpretation of that sequence analysis, that can give
13 us different levels of samples and different levels of
14 confidence, that we're looking at all of the important
15 sites of integration and learning everything there is
16 to learn.

17 Long reads is a newer technology that will
18 need to be assessed before we know what the best
19 approaches are and those also may be model-dependent.
20 There are certainly preferential sites, but AAV

1 integration is also sufficiently nonspecific in that
2 double-stranded breaks and open enhancer regions
3 depending on the cell state and type can all be
4 targeted making this a very complex analysis, but very
5 important.

6 And, so, there has been a call from several
7 members of the committee for standards. Standards
8 around assay controls, standards around dose because
9 all of these effects increase with increasing dose. In
10 terms of duration of follow-up post-dose, there seem to
11 be a strong call for longer assessment that in mice 12
12 to 16 months, or for the life of the mouse, might be
13 more appropriate. For dogs, these assessments have
14 been, while they can be informative starting at two
15 years out past a decade, also gives additional
16 information. And we need to know the difference
17 between early integration but also late effects which
18 include clonal expansion. But also, a critical need to
19 differentiate between vector-induced clonal expansion
20 and age-induced clonal expansion, particularly

1 mentioned in hepatocytes.

2 So, I think these were the key points that I
3 heard so I've got one hand raised. Dr. Barry Byrne, if
4 you want to add to that summary, please.

5 DR. BARRY BYRNE: Yes, thanks, Lisa. There is
6 another point worth raising because, obviously, they
7 must attempt interest in understanding the frequency of
8 these events in neonates. Since June 2019, when
9 Zolgensma was approved for the treatment of SMA,
10 there's now been over 1,000 children who have received
11 it. And now in the past year the majority of states,
12 doing newborn screening, lead to exposure to the
13 treatment in the first few weeks of life. So, there's
14 an opportunity there for establishing a cohort of long-
15 term follow-up in these patients, where they don't have
16 an underlying liver disease but they'd received a
17 systemic exposure to an AAV9 vector, (inaudible).

18 So, if there's something to be learned then
19 that experiment, in fact, is already underway. I think
20 that warrants consideration of how those patients will

1 be followed long-term.

2 DR. LISA BUTTERFIELD: Perfect, thank you.
3 So, with that, I'd like to move us to the second
4 question for the next period of discussion. And that
5 second question is that current literature suggests
6 that various factors may affect AAV-mediated vector
7 genome persistence, vector integration, and risk of
8 oncogenesis. Please discuss benefit/risk
9 considerations for AAV vector-mediated oncogenesis such
10 as patients age at the time of treatment, preexisting
11 liver conditions-- for example, infection with
12 hepatitis B or C virus -- and high vector dose.

13 So great. So, there is the next question that
14 we need to discuss. And I think we touched on some of
15 this certainly in the animal models about the animal's
16 age. And we've just touched on some underlying liver
17 conditions in that setting. So, the first hand up, Dr.
18 Barry Byrne, please.

19 DR. BARRY BYRNE: I didn't have my hand up,
20 but I can comment if you like.

1 DR. LISA BUTTERFIELD: Okay, great, and then
2 we'll move down. We've got additional two other
3 questions after your initial comment.

4 DR. BARRY BYRNE: Yeah, so, essentially, we
5 always have to consider risk/benefit relevant to the
6 deduced target. So underlying liver disease that could
7 predispose someone to further immune, maybe, injury or
8 genotoxicity would unfortunately probably be excluded
9 from these therapies. And, as well as the prior
10 exposure to viral pathogens that predispose to
11 oncogenicity. So we just have to consider the severity
12 of the underlying illness and, since they'll take some
13 time to get definitive answer to these questions, we
14 would have to start with the population that's the
15 least at risk.

16 But understanding that those that are the most
17 vulnerable and the most severity still have access to
18 the therapy. Because they're really transformative and
19 in many cases lifesaving so we don't want to leave
20 those patients out of the equation of that for having

1 access.

2 DR. LISA BUTTERFIELD: Thank you. Dr.
3 Venditti.

4 DR. CHARLES VENDITTI: So, the comment I would
5 have, I think there is an important lesson about the
6 existing liver conditions, and other people can speak
7 in greater detail than myself about this. If someone
8 has hep B or hep C, hopefully, we're going to check
9 every patient before they receive AAV, see if they have
10 that. Those have a significant risk of incurring an
11 HCC formation later in life. So that's something we
12 have to be careful about and I realize some patients
13 get -- the example would be hemophilia. Or some
14 patients that have this and they really don't have
15 other options, they've enter the trials, they're known
16 to have hep B and hep C. And then there's the recent
17 patient that was mentioned in the briefing book but has
18 been discussed a bit, who developed an HCC and was said
19 not to be related to AAV, although I don't think
20 there's a publication of that data's been made public

1 for examination at this point to my knowledge.

2 But the other comment I would make as we think
3 about risk/benefit, again, from the perspective of
4 pediatric (audio skip) is that we do have patients that
5 the treatment for those patients is an elective liver
6 transplant. These patients will not develop liver
7 failure and jaundice and perish from the liver that
8 doesn't work. They basically need that hepatic enzyme
9 where their lives are in jeopardy and their quality of
10 lives are really, really diminished.

11 So, as we think about these patients -- again,
12 it would be an interesting discussion to have further
13 with the committee, whether we should reverse the
14 position of saying let's find the oldest patient or the
15 mildest patient and treat them. Again, this beckons
16 back to the days of OTC deficiency in severe inborn
17 areas of the renal cycle, whose patients are stabilized
18 in the neonatal period and then just right away getting
19 them ready for liver transplant because that's the only
20 treatment we have. So, in such a patient I think the

1 risk/benefit equation is really shifted down. And with
2 aggressive monitoring in such a patient, even though
3 those livers might be perhaps more prone to malignant
4 transformation that remains as an unknown. We would
5 just have to have aggressive monitoring of those
6 patients to try to use (audio skip) stripped away of
7 elements that we've learned from the preclinical
8 studies that can confer genotoxicity. Such is very
9 strong enhancers if they're not needed in the
10 (inaudible), maybe they can be removed.

11 So, I do think as Dr. Berns points out this
12 is, it can be a patient-centric decision about
13 risk/benefit. But, again, I think looking at certain
14 populations -- the example of SMA, for example.
15 There's not going to be older patients, per se, with
16 severe infantile SMA, they don't survive.

17 That paradigm can, I think, be extended to
18 other patients that have inborn errors in metabolism
19 where we're currently detecting these patients at birth
20 by newborn screening, and then telling them your best

1 treatment is going to be an elective liver transplant
2 someday when you're really, really severely affected.
3 I'm just hyperbolizing that comment because not that
4 that is what people say in the clinics, but that is an
5 approach to some patients.

6 Which, again, in my mind, as a clinician
7 shifts our risk/benefit about what patient we should
8 offer transplant to versus an AAV treatment that may be
9 theoretical and have risk with it. At the end of the
10 day, if that patient did have a genotoxic event related
11 to a liver problem, they would revert to the natural
12 therapy which would be an elective liver transplant.
13 So, as we think about the patient populations that
14 matters to the individual diseases that we consider for
15 AAV, especially as we move down in age. Thank you.

16 DR. LISA BUTTERFIELD: Thank you for those
17 considerations. Dr. Ruth, risk/benefit.

18 DR. RAYMOND ROOS: Yeah, I just wanted to be
19 more specific about SMA since it's been mentioned a
20 couple of times and that is the SMA1. These babies

1 that are born with SMA1, 95 percent will be deceased by
2 age two. Now there may be more than one treatment
3 available, but perhaps using more than one treatment is
4 important here. Also, Duchenne dystrophy where AAVs
5 are being used in clinical trials. Patients survive
6 longer but nevertheless we don't really have
7 alternative treatments for Duchenne dystrophy.

8 So, benefit/risk is certainly important and
9 has to be considered here. Not that we shouldn't pay
10 attention to AAV integration and oncogenesis, but we
11 need to clarify what we're treating, what the
12 alternative treatments are. Thanks.

13 DR. LISA BUTTERFIELD: Great, thank you.
14 Okay, I'm not seeing other hands up. Like to hear from
15 other members of the committee. And we also want to
16 make sure over the next couple of questions that we're
17 able to hear from pretty much all of the committee
18 members. Professor Fox, please.

19 DR. BERNARD FOX: Yes, it was a really
20 enjoyable program and great talks today. One of the

1 questions that I have deal with the double-stranded AAV
2 that was used in the SMA trial, if I'm understanding
3 this correctly. Where 500 patients have been -- or
4 greater than 500 patients have been treated in the
5 single strand AAV vectors. And the first question
6 (audio skip) that they're combined, but did the double-
7 stranded AAV vectors show the same sort of HCC
8 expression in the mouse models? And I think was Dr.
9 Bushman who was talking about where they perceived
10 potentially a difference between the single-stranded
11 and the double-stranded AAV vectors in terms of being
12 able to integrate when you have double-stranded breaks.
13 And is anything known about differences in those two
14 areas?

15 DR. LISA BUTTERFIELD: Thank you. Dr.
16 Bushman?

17 DR. FREDERIC BUSHMAN: Real quick, I don't
18 think we've ever compared. I'd have to go back and
19 look over all the published studies to see if there
20 were double-stranded, single-stranded, and whether it

1 was stated they could be compared. We've never
2 compared them to each other.

3 DR. BERNARD FOX: So, it seems like if there's
4 500 patients' data with the double-stranded AAV vector,
5 and there's other AAV things beyond a single strand
6 that that would be an important thing to note or be
7 referencing back to the data from the double-stranded
8 AAV vector. That's my only point.

9 DR. FREDERIC BUSHMAN: It would be interesting
10 to study the SMA subjects if samples are available and
11 ask if there are systematic differences. That would be
12 a good study.

13 DR. LISA BUTTERFIELD: Thank you both. Dr.
14 Herzog.

15 DR. ROLAND HERZOG: Just a quick comment about
16 the hepatitis virus (inaudible). I think that may
17 require the input of hepatitis/hepatology experts to
18 see. Because it may be that the answer is more
19 differentiated depending on how severe the disease
20 pathology is, and also what kind of treatments that the

1 patients have (audio skip) more complex. I think
2 there's something in that most definitely.

3 DR. LISA BUTTERFIELD: Thank you, and I will
4 remind the committee that session two is exclusively
5 devoted to hepatotoxicity, so we will have much more
6 detail about that soon. Dr. DeFilippi, please.

7 MR. JAMES DeFILIPPI: Thank you and I've quite
8 enjoyed what I've seen so far. First of all, I'm not a
9 physician, so take my comments with a grain of salt. I
10 am a hemophilia patient and have had hepatitis B and C.
11 And so, I guess what I am myself wondering is if
12 there's a way to design some of these studies to
13 understand if there's a way to simulate that in the
14 dogs, for example, that are being studied. Can they
15 have hepatitis and then have it be resolved, and then
16 be treated with AAV therapy to see if that then creates
17 more of a predisposition for oncogenesis or not? So
18 that's one question that's on my mind.

19 And then maybe the other comment that I would
20 make that's not directly related, is when looking at

1 the risk/benefit for this -- I mean, I think certainly
2 for some of the other conditions that have been
3 discussed where mortality without this type of therapy
4 is quite high at a young age, the risk/benefit is a lot
5 different than it would be for somebody in the
6 hemophilia community, where there's a lot of other
7 therapies outside of AAV therapies that certainly can
8 provide a higher quality of life. So, I think the
9 risk/benefit needs to be considered, not as a general
10 consideration, but more on a case by case or disease by
11 disease basis.

12 DR. LISA BUTTERFIELD: Thank you. Then let's
13 have one final comment for this section from Ms.
14 DiCapua. And then I'll do a quick summary and then
15 we'll move to the third question. We can't hear you
16 yet.

17 MS. PEGGY DiCAPUA: Okay, sorry. I'm not a
18 doctor either but I just wanted to mention something
19 about the risk/benefit as a parent of a child who
20 passed away from a rare disease at age eight. And

1 there were no treatments or anything back in the '90s
2 when he was diagnosed. I think there's one treatment
3 now. But when it comes to risk/benefit, and when
4 you're looking at a life expectancy that's not long, it
5 plays a huge factor in it. And there's times I don't
6 know how I feel on this issue because quality versus
7 quantity was always on our mind.

8 But if it's anything that could give a little
9 bit of quality for a little bit longer, I think it's
10 important to weigh that out versus having the perfect
11 science down as to what it is and isn't going to do.
12 When there's no other alternative, the risk/benefits
13 huge.

14 DR. LISA BUTTERFIELD: All right, thank you
15 very much. So, I'm going to quickly sum this up and so
16 we'll move to the third question after this. So, what
17 I'm hearing about risk/benefit is really this is a
18 disease-by-disease situation. And that there certainly
19 is risk of illness, for example, with IV-delivered AAV,
20 in patients with underlying liver conditions including

1 hepatitis B and C viruses. That this does increase the
2 risk. But there's also the consideration that in some
3 cases, for certain diseases, liver transplant is
4 standard of care and could be performed after the gene
5 therapy instead of being overly concerned about that
6 risk on the front end before the gene therapy.

7 So, there's also, depending on a number of
8 these diseases, very little time to decide whether to
9 go forward or not. So, my hearing of the discussion is
10 that the benefit/risk considerations are disease by
11 disease and patient setting by patient setting.

12 Anything to add to that before we move onto
13 the third question? Great. Well thank you very much.
14 I do see one hand up from Dr. Crombez. Did you want to
15 have a quick word on question two before we move on?

16 DR. ERIC CROMBEZ: Yes, thank you. I
17 appreciate the discussion and particularly the focus on
18 benefit/risk, particularly when it comes to younger
19 children with these disorders with really high unmet
20 medical need. The one thing I was wondering about as

1 you read the summary was, with some of these
2 preexisting liver conditions and with the focus on
3 hepatitis -- to me there's also an important difference
4 between patients with active disease. And now that we
5 have viable treatments for a lot of these
6 comorbidities, you also have the potential to go
7 through treatment, wait for that liver to come in much
8 more of a healthy state, and then have the gene
9 therapies once these comorbidities have resolved.

10 DR. LISA BUTTERFIELD: Great, thank you for
11 that additional perspective. So, with that, we'll move
12 to the third question and that's considering the risk
13 of oncogenesis that we've been discussing. One, please
14 provide recommendations on safety monitoring measures
15 that should be included in clinical trials. And please
16 provide recommendations on the duration, frequency and
17 method of long-term follow-up for patients receiving
18 AAV vectors.

19 So, for this do we want to start with Dr.
20 Venditti? Did you have an introductory discussant

1 statement to make?

2 DR. CHARLES VENDITTI: Yeah. I would say that
3 in terms of providing specific recommendations, I think
4 we've heard a lot about -- and again it's worth
5 emphasizing and maybe calling other people. We have a
6 hepatologist on our committee. If we're concerned
7 about HCC, which is really what the focus has been on
8 in the discussion at this point in time. HCC is a very
9 slow-growing malignancy to my experience. Again, it's
10 very limited from what I do.

11 So, I think if we look into the pediatric
12 population where some patients that for other reasons
13 can be at risk for (audio skip) that we could look at
14 as a starting point. But I think a very fundamental
15 place would be, again, obtaining what sort of imaging
16 studies we can obtain. I think knowing something about
17 a patient, if they have a fibrotic liver or fatty
18 liver, could be an important starting point and
19 something you can get a little bit of an opinion about
20 by an ultrasound or FibroScan. (Audio skip) whether

1 you could do a CT. And then if you do the CT, you can
2 miss a lot of things that are small so why would you do
3 it? But sort of a baseline imaging study at some level
4 monitoring patients.

5 And then, routine monitoring I think for HCC
6 and whatever the best biomarkers are that came up as a
7 (inaudible) point that (inaudible) to the vector, would
8 it be something to the possible HCC (audio skip)
9 example of the classic marker. Then I think some
10 people think of them as (audio skip). And then follow
11 those patients would be (audio skip) to look for any
12 changes? And if such a change was observed then we
13 would escalate to (inaudible) where you might (audio
14 skip) judging whether you might do a (audio skip).

15 But I do think there's going to be patients
16 who have a baseline increased risk due to their
17 underlying shifting focus -- because this is my focus.
18 We do know that certain inborn errors of metabolism
19 happen. So, should we be more aggressive about those
20 patients who potentially should be getting higher

1 dosing? The answer is, yes. Like the studies that
2 were mentioned earlier about NASH (phonetic) in the
3 mouse models, when there's NASH and the mice are given
4 AAV, even if they're adults, some of them developed
5 HCC. Now whether or not that would -- is that going to
6 translate to the human, it's an unknown? But these are
7 the things I think would dictate fundamental
8 discussions about how frequently we should do it.

9 I would defer to our other experts on the
10 panel about the details of what's the best method,
11 what's the most contemporary method, because certainly
12 this is not something new for this field. This is
13 something that's done in the clinic. And so, I guess,
14 with that I would invite comments from others and thank
15 everybody for listening. I'm going to turn my camera
16 off.

17 DR. LISA BUTTERFIELD: Thank you. So, let's
18 go to Dr. Hawkins, please.

19 DR. RANDY HAWKINS: Thank you very much.
20 Actually, my question referred to the last area and it

1 revolves around the human subject. And my question is
2 whether genetic analysis of parents and the pedigree
3 had given any insight into complications in the vector?
4 That is how -- is this actually something that's been
5 looked at?

6 DR. LISA BUTTERFIELD: Thank you, okay. So,
7 if there is someone who can weigh in on the background
8 genetics question please do so, and then let's also
9 move to Dr. Heller, next.

10 DR. THEO HELLER: I have a hepatologist on the
11 panel. The approach to HCCs were worked on. The
12 screenings were worked on once we do the
13 recommendations and the guidelines are clear. We do
14 the ultrasounds every six months with or without AFP.
15 AFP has fallen in and out of favor in the guidelines.
16 Liver enzymes on how (audio skip) it's a standard
17 approach. The problem with the approach is that if you
18 talk about different diseases the risk profile is
19 different.

20 So, for NASH somebody said you can treat

1 hepatitis B or C, but for example obesity or NASH is a
2 risk factor. Cirrhosis or underlying fibrosis is a
3 risk factor. It's not as simple as treating the
4 underlying disease. It would really mean looking at
5 the patients and their particular risk file. With
6 NASH, for example, we see HCC occurring prior to
7 cirrhosis. If you look at a disease like hepatitis C,
8 it's more strongly associated with fibrosis and less so
9 with the hepatitis C.

10 But I think the angle -- going back to the
11 last question, which Dr. Butterfield summarized so
12 well, it would be going back to the individual disease
13 and the risk profile for that disease that's been
14 treated that would really determine the difference.
15 But screening (inaudible) CT is standardized. We try
16 to avoid CTs because their radiation, particularly in
17 younger people. MRIs are fantastic and superb, but
18 incredibly expensive. And the consensus is to do
19 ultrasounds every six months.

20 DR. LISA BUTTERFIELD: Thank you for that

1 summary. Mr. DeFilippi, please.

2 MR. JAMES DeFILIPPI: I wanted to make a quick
3 comment on the question number three, particularly with
4 respect to the follow-up. I mean, I think certainly my
5 entire life I've been seen at hemophilia treatment
6 centers. I think that proves to be a pretty effective
7 model for patient follow-up long-term.

8 My physicians at the various HTCs that I've
9 worked with certainly know everything there is to know
10 about my health and how it's evolved over the years.
11 And so, I think when you think about how patient
12 monitoring and long-term follow-up for patients of gene
13 therapy, I don't know why you wouldn't follow the same
14 model.

15 It would make sense that given the risk of
16 oncogenesis, that even if it's slight, it's there,
17 right. So, following the patients for the rest of
18 their lives makes a lot of sense. And having a routine
19 model where patients check in either at a center like
20 an HTC or something similar, either in person or

1 virtually, makes a lot of sense. I think registry data
2 certainly seems to be one of the solutions to
3 understanding how effective this can be over the long
4 haul.

5 DR. LISA BUTTERFIELD: Thank you. Dr.
6 Bushman.

7 DR. FREDERIC BUSHMAN: I just wanted to
8 comment on a new technology that's coming along that
9 may be relevant in the context of safety monitoring
10 measures and that's analyzing cell-free DNA. There's
11 recently a paper showing that you can detect at least
12 some subset of integration sites with integrating
13 vectors in cell-free DNA. And so, it remains to be
14 seen how well that pans out. But if it were to work
15 really well maybe that's a safe way to access like
16 solid tissues, liver, stuff like that.

17 And so, it'll be interesting to see how far
18 people can go with that and how/whether you can
19 forecast on the future with the kind of data you would
20 get analyzing cell-free DNA.

1 DR. LISA BUTTERFIELD: Terrific, thank you.
2 Barry Byrne, please.

3 DR. BARRY BYRNE: Hi. On the question 3B,
4 this has been very well considered in the January 2020
5 guidance from the FDA for long-term follow-up after
6 administration of InMutaGene therapy products. And it
7 really broke it down into differentiating integrating
8 vectors from those episomal vectors like AAV.

9 But given the evidence that there's potential
10 for AAV integrations in some tissue, maybe a blend of
11 the recommendations we could say to recommend 15-year
12 follow-up for those with integrating vectors and a
13 five-year follow-up in the case of AAV vectors. But
14 one consideration would be that there would be a long-
15 term follow-up self-reported database. After that, in-
16 person observations which would be part of --as I just
17 pointed out in specialized care centers, patients often
18 get continuity, care life long and any long-term
19 follow-up registry that's integrated with electronic
20 medical record would capture those events. So that's a

1 consideration on how to achieve that multiyear follow-
2 up across a variety of providers and sites of care.

3 DR. LISA BUTTERFIELD: Thank you. Okay, I
4 still have some hands up from folks who've already
5 spoken. Do you want to keep those hands up for new
6 things or have we discussed this section? Dr. Hawkins,
7 did you have something to add? Your hand is still up.

8 DR. RANDY HAWKINS: No, I did not.

9 DR. LISA BUTTERFIELD: Okay. And Dr. Bushman
10 did you have anything additional? Your hand is -- no,
11 okay. So, Dr. Ruth?

12 DR. RAYMOND ROOS: Sorry, my camera fell off
13 but perhaps you hear me anyway. A few people had
14 mentioned repository. I just wanted to stress that I
15 thought it would be a good idea for FDA or some similar
16 group to have a catalog of AAV gene therapies with
17 different serotypes and trying to put things all
18 together if possible. So that perhaps would answer
19 questions regarding hepatotoxicity, oncogenesis, et
20 cetera. Thanks.

1 DR. LISA BUTTERFIELD: So, your suggestion is
2 data sharing and pooling perhaps medical record data
3 across different serotypes? If I understand correctly.

4 DR. RAYMOND ROOS: Yes. Which is potentially
5 complicated because ones dealing with different private
6 companies. But on the other hand I think it would be
7 very informative to do this. It's complicated and also
8 labor-intensive. But at the end of the day maybe it's
9 also informative.

10 DR. LISA BUTTERFIELD: Thank you. Okay, a
11 final comment on question three from Dr. Crombez,
12 please.

13 DR. ERIC CROMBEZ: Thank you, again. And do
14 appreciate the discussion and I'm in agreement with the
15 conversation around long-term treatment and importance
16 of that. And while I do also agree with the potential
17 risk here, just also wanted to remind everyone that at
18 this point in humans, at least, it is still a
19 theoretical risk. And I think my concern really
20 focuses on the method part of question B. And

1 particularly with the discussion on children and with
2 gene therapies really moving in to younger and younger
3 patients, that the method can become very challenging.
4 And even with things like MRI can require sedation
5 there. So just weighing the burden of this
6 surveillance with the fact that, to this point, with
7 quite a bit of human data collected is still a
8 theoretical risk.

9 DR. LISA BUTTERFIELD: Thank you, I appreciate
10 that. Okay, so as we think about safety monitoring
11 measures in clinical trials, I think the summary of
12 some of the recommendations and comments we had for the
13 potential risk of HCC, that that monitoring is standard
14 and that every six-month ultrasound may or may not
15 include AFP, that liver function tests are less
16 informative and CT scans are not recommended due to the
17 radiation. And MRI is effective but not as much cost-
18 effective.

19 So that is standardized follow-up for disease
20 correction, for example, in hemophilia also

1 standardized with lifetime follow-up. So, there are a
2 number of standards here that could be followed and
3 there were not recommendations to change those
4 standards, other than to consider for the young
5 children that some of the monitoring would be more
6 challenging in those smaller children.

7 There was some discussion of whether HPV or
8 HCV would have special considerations in that they
9 could perhaps be treated to reduce risk. But things
10 like fibrosis and NASH are much harder to treat and
11 need to be assessed at baseline, and at least
12 considered in terms of their potential risk for AAV-
13 induced HCC should that be something that exists. And
14 that newer, less invasive approaches -- cell-free DNA -
15 - are things that should be explored, and that could be
16 promising ways to have seromonitoring for virus and for
17 integration events.

18 So, if there's nothing to add to that summary
19 we will move to our final question for section one
20 which is posted. And that's on oncogenicity. Please

1 discuss whether some vector designs may enhance the
2 frequency of vector-mediated integration and the risk
3 of oncogenesis. For example, how's the risk affected
4 by promotor-enhancer elements, genome-targeted
5 nucleases or novel AAV vector designs for which there
6 is, to date, limited clinical experience?

7 Because AAV vectors can carry significant
8 levels of co-packaged DNA impurities from the
9 manufacturing process, is the risk of oncogenesis
10 increased due to preferential integration of non-vector
11 DNA? And what types of studies should be performed to
12 assess this risk? So that is our final question for
13 our last 15 to 20 minutes. And so, Dr. Venditti, do
14 you want to start?

15 DR. CHARLES VENDITTI: Thank you, this is a
16 very loaded question. We could spend days on this
17 topic. I will just make some quick comments. To
18 address number one, again, we published a paper on
19 this. There's absolutely no doubt (audio skip) that
20 certain promotor enhancer configurations have a very

1 increased risk of HCC formation in the neonate when
2 they are delivered at higher doses. Many people have
3 observed this. And there's a short list of promotor
4 enhancer combinations that have been published that we
5 are aware of where this risk is elevated.

6 And part of the reason, in my opinion, the
7 field has gone in this direction is because when one
8 does the proof-of-concept studies in an animal model,
9 usually what you would try to do is make something
10 that's very potent to see if you got a signal. And
11 with AAVs being so potent, as they are, and then the
12 promoters and enhancers that people have developed
13 being an additional potency on top of that, the effects
14 can be striking in very, very sick animal models. And
15 that's how, I think, how people got into this in the
16 first place. They were looking for durability of a
17 treatment -- a single treatment -- and finding that
18 mice were literally corrected their whole lives was
19 miraculous.

20 Well, it turns out that maybe it was in some

1 ways too much of a good thing with respect to the
2 expression of those transgenes by the AAV. And the
3 promotor-enhancer configurations in which we've
4 published on, at least, that have been in the
5 literature that seem to have an unquestionable risk of
6 causing HCC in mice, include the enhanced chicken beta
7 actin promoter, DMB promoter and the enhanced TBG
8 promoter.

9 So as a starting point, one of the things one
10 could do is to ask the question in the preclinical
11 studies, is that level of transcription-driven
12 expression needed from my cassette to really give a
13 phenotypic correction? And whether or not the cassette
14 could be modified by removing enhancers, removing
15 heterologous promoters that could activate -- we heard
16 from Dr. Sabatino's talk. From the integrating vector
17 work, enhancer insertion and promotor activation are
18 mechanisms that we know from integrating vectors that
19 cause and promote oncogenesis in integrated vectors.
20 AAVs, again, they can integrate at low rates. The same

1 mechanisms I believe expend.

2 So that's something I think is a basic
3 assessment that can be done early on to de-risk
4 vectors. The other thing that comes up as a
5 theoretical risk, which I wanted to mention because it
6 is important in vector design, comes from a paper that
7 I mentioned earlier but it was brought up a little bit
8 by Dr. Berns in his comments about this element that's
9 at the right hand ITR. And it's a paper from Grant
10 Logan and Ian Alexander entitled "Identification of
11 Liver-Specific Enhancer-Promotor Activity in the 3
12 prime Untranslated Region of the Wild-Type AAV2
13 Genome." And so, unfortunately, this sequence is
14 (audio skip). And it actually is, as Dr. Alexander's
15 group showed in his paper, composed of a set of
16 transcription factor bonding sites; HNF6, HNF4A, GATA6,
17 and HNF1 sequences is packed one site on top of
18 another. And certain AAV serotypes have this near the
19 ITRs. And some of the older vectors derived from
20 pSub201 used this ITR and flipped it to make a

1 cassette, and those sequences have carried over into
2 some of the cassettes. And they're hard to get rid of
3 because they're right adjacent to the ITR. There's no
4 restriction sites that are facile where you can just
5 chop these out.

6 So, if you're using an enhanced vector that
7 has a strong promoter enhancer in it, maybe you want to
8 remove those elements if your vector has them. Some
9 vectors don't have them. Now, this is again
10 (inaudible), we don't know the sequence of every vector
11 that's been given to patients. If we knew we could do
12 an analysis and say, oh, well this vector has these
13 toxic sequences. This vector has the AAV/HCC or the
14 AAV-enhancer element that carried over. And then we
15 could have a rational approach to making a better
16 vector.

17 The reason that I am also emphasizing this is
18 because in Dr. Alexander's paper -- he mentioned it's
19 published *Nature Genetics*. In his analysis he finds
20 remnants of this ITR budding fragment and related

1 pieces of the AAV genome from wild-type AAV's in 10 of
2 the 11 HCCs that were described by the group in France
3 that (audio skip) *Nature Genetics* that suggest that in
4 a rare subset of AAV infections that are associated
5 with HCC -- a very rare subset of HCCs -- this tiny bit
6 of the AAV genome can be found in those HCCs as
7 possibly a contributing factor. So, if we can get rid
8 of that sequence, maybe that's something to do to
9 mitigate.

10 So, with that, I don't want to continue my
11 comments. This is a passionate area for me and an area
12 of research, but I do think this is an important area
13 to consider. And there's other ways to augment
14 expression from cassettes. One can enhance
15 translation, one can enhance RNA stability. You don't
16 always have to go for increased transcriptional
17 activation by an enhancer to get the better transgene
18 expression.

19 So those are the types of things I think
20 should be done, should be thought of early on in the

1 studies so that we don't advance vectors forward that
2 have elements that could have toxicity. Could. In
3 mice they have toxicity. In humans, we're not certain
4 yet, but they could. And so if you can get something
5 that you don't need an answer for, and it also works
6 well, that might be better for the theoretical risk of
7 safety.

8 With respect to double-stranded nucleases and
9 things that -- in these double-stranded breaks, I think
10 that's a separate area. We heard a lot of comments
11 about AAV integrations being stimulated by double-
12 strand breaks and by broken DNA. So that's just a
13 fundamental aspect of some of those approaches. I
14 think there's ways, maybe in the future, that we could
15 use with nucleus engineering to minimize these effects.
16 It's not my area. I don't want to speak a lot about
17 it.

18 But the second area, the quick comment I would
19 make about the AAV, the DNA impurities, and the AAV
20 preps, this is an active area of consideration. And I

1 think the future would be better approaches for MGS and
2 biophysical characterization that is standardized to
3 look at the AAV preps. What is in them? Everyone
4 accepts the fact you can get plasma -- reverse
5 packaging from plasmas. What else is in there?

6 Unless we have better methods -- and there
7 have been some very nice publications that have come
8 out. Which for those who are interested in some of
9 these reports that -- there is not a lot of them. But
10 I think as the field moves, we need to adopt these
11 approaches. QC vectors, so we can get an idea of what
12 is in them. And that could help us determine the purity
13 of the vector and what are the impurities. And for
14 certain, I think if we have reverse package of a
15 truncated genome that carries an enhancer element in
16 it, with some ITR fragment, that could be a toxic
17 contaminate. If you don't know if it's in your prep,
18 how will you figure this out?

19 I would leave other comments for the rest of
20 the panel. Those are my quick comments to start the

1 conversation.

2 DR. LISA BUTTERFIELD: Thank you very much. I
3 think we also heard in one of the talks the notion that
4 needing to know the sequence of the vector that it's
5 informative to know what's going in the patient, but
6 also what is found later, after -- for what might have
7 integrated and what might be existing in clonal
8 expanded cells. So much to discuss, let's start with
9 Professor Fox, please.

10 DR. BERNARD FOX: I guess, okay, can you hear
11 me? I don't see any for my webcam but, okay. I guess
12 it's a naïve question for Dr. Venditti again, I guess,
13 but are not all the vectors sequenced? Don't we know
14 the sequence of the vectors that are used in the
15 clinical trials?

16 DR. CHARLES VENDITTI: I think whether someone
17 knows those sequences, the answer's yes. Whether all
18 the sequences that people have used and the vectors
19 that have gone into patients are better publicly
20 available that are deposited for the community to look

1 at, I think the answer is no. But I would defer to
2 someone else's comments. I don't think they're all
3 available.

4 DR. BERNARD FOX: It seems like that's
5 something that should be available. That's, yeah.

6 DR. LISA BUTTERFIELD: Thank you. So, I'll
7 ask other members of the committee to weigh in on those
8 questions about vector designs and impurities from the
9 manufacturing process, please. Dr. Herzog.

10 MR. MICHAEL KAWCZYNSKI: Dr. Herzog you can go
11 ahead and start speaking. There you go.

12 DR. ROLAND HERZOG: Okay. Yeah, actually a
13 comment related to the question that was just asked
14 about sequencing of vector genomes. I just wanted to
15 point out that in a paper that -- a short communication
16 that just came out in the most recent issue of
17 Molecular Therapy that one of my colleagues, Dr.
18 Weidong Xiao, in his lab found that in AAV vector
19 preparations, that you can have a species of vector
20 genomes that is rearranged in such a way that only the

1 promotor element is left and cDNA Poly(A) sequences are
2 gone. Which extends -- those kinds of molecular forms
3 occurring in your vector preparation depends also on
4 the vector construct. If one of these types of
5 molecules integrates, there is a greater risk of
6 readthrough and activation of adjacent genes has been
7 shown by others about a decade ago. So, even if you
8 have the sequence of your therapeutic vector as it is
9 in, for example, your vector plasmid or whatever
10 production system you're using, your recombinant
11 construct, there's also the question of what does your
12 actual vector genome composition look like after the
13 vector is produced?

14 And that's another area that perhaps (audio
15 skip) pay more attention to that's (audio skip).

16 DR. LISA BUTTERFIELD: And is that tractable
17 given current technologies, or are we in the same boat
18 we are with integration site analysis and it's in a
19 very complex (inaudible)?

20 DR. ROLAND HERZOG: No, I think that's

1 actually easier to analyze with current technology.
2 You can get a number on what your level of
3 quote/unquote contamination would be.

4 DR. LISA BUTTERFIELD: Thank you. Other
5 comments on the novel vector designs and DNA impurities
6 and the types of studies that we should be doing to
7 assess this type of risk? All right, so perhaps all of
8 the points of the committee have already been raised in
9 our first few comments?

10 All right, well let me summarize and see if
11 anyone wants to add anything or ask anything further.
12 So, what a number of the animal models have given us
13 are lessons about known specific promoter-enhancer
14 combinations that can confirm more risk on downstream
15 oncogenesis. We know that higher dose can lead to
16 higher risk and that could be both due to those higher-
17 risk vector elements as well as contamination elements
18 from those repackaged DNA and other contaminants that
19 go hand in hand. So, there are a number of sequences
20 also associated with the ITRs that can perhaps be

1 eliminated to reduce risk. One recommendation is that
2 we can at least analyze and eliminate sequences that
3 have been shown in animal models to confer that risk.

4 There was reference to another brand-new
5 publication of other recombinant vector forms that
6 could be in the prep that we might need to look at.
7 And so plasmid sequence is only part of the aspect that
8 should be looked at. Part of that information is also
9 the full vector sequence. These are certainly things
10 that we can analyze now in the current state of
11 technology, but we do need better analytic approaches
12 to look comprehensively at the vector preparation, as
13 manufactured, to make sure that we're properly
14 assessing the vector sequence, what's in the prep and
15 all of the contaminants, so we can better understand
16 the relative risk of all of those components.

17 So, if there are any other comments, I'm
18 looking for raised hands before I close out this first
19 session. Dr. Wilson Bryan, please.

20 DR. WILSON BRYAN:: I appreciate the

1 discussion by the committee. What I'd like to do is
2 check with my FDA colleagues to see if any of the
3 specific disciplines have questions about what they've
4 heard from the committee. So, let me call on first,
5 Pharm/Tox, Dr. Dan Urban.

6 DR. DAN URBAN: Hello. My name is Dan Urban
7 and I'm a Pharm/Tox reviewer. We really appreciate the
8 informative discussion, and we'll take these
9 recommendations and perspectives back to our FDA
10 colleagues as it relates to the preclinical programs.
11 So no further questions. Thank you.

12 DR. WILSON BRYAN: Thank you, Dan. Dr.
13 Sherafat, Rosa Sherafat.

14 DR. ROSA SHERAFAT-KAZEMZADEH: Thank you.
15 Likewise, I wanted to thank the committee for the
16 informative discussion and also Dr. Venditti, and also
17 the chair, Dr. Butterfield for the nice summary. We
18 take your suggestions and input back to our (inaudible)
19 sponsors (audio skip).

20 DR. WILSON BRYAN: Okay, let me see if anybody

1 has any questions for the committee. Dr. Byrnes,
2 Andrew Byrnes.

3 DR. ANDREW BYRNES: Thank you for the very
4 extensive discussion. No further questions.

5 DR. WILSON BRYAN: Okay, thank you, folks.
6 So, the committee seems to have addressed all of our
7 questions adequately in this first session. Very much
8 appreciated.

9 DR. LISA BUTTERFIELD: Thank you very much Dr.
10 Bryan for confirming that and for making sure that our
11 FDA colleagues have that opportunity. With that, we
12 close out the first question and the first session, and
13 we move to Session 2 which is devoted to the obviously
14 important topic of hepatotoxicity.

15

16

SESSION 2: HEPATOTOXICITY

17

INVITED SPEAKER PRESENTATION: "CLINICAL FINDINGS OF 18 HEPATOTOXICITY OF AAV VECTORS FOR GT"

19

20

DR. LISA BUTTERFIELD: I'd like to introduce

1 Dr. Lindsey George, Assistant Professor of Pediatrics
2 at Children's Hospital of Philadelphia. And this will
3 be discussing toxicity risks of AAV vectors for gene
4 therapy. Thank you very much.

5 DR. LINDSEY GEORGE: Okay. Great. Thank you
6 very much. It's been a really great discussion, so
7 far. And I appreciate the chance to be here.

8 I'm charged with summarizing some of the
9 information that's come out of clinical trial and
10 initial lysis vector as it relates specifically to
11 hepatotoxicity. These are my disclosures.

12 What I'll just briefly review over the next
13 approximately 20 minutes are what we seen come out of,
14 with respect to clinical hepatotoxicity and essentially
15 four disease states, so the hemophilias, Spinal
16 Muscular Atrophy, and X-linked myotubular myopathy.

17 In the absence of having enough time to really
18 talk about liver, I wanted to just emphasize -- Oh the
19 clicks aren't coming up. Okay. Well, I'll just say it
20 and hope that the points are not lost. But I think an

1 important point, which has been made before, is that
2 really if you deliver, certainly a systemic AAV vector,
3 it does ultimately efficiently traffic the liver
4 irrespective of what the target tissue type is. And,
5 so, this is likely related to a couple of factors, not
6 the least of which is hepatic blood flow physiology.
7 So we know that the liver has a dual blood supply to
8 the hepatic portal vein and the hepatic artery system.

9 And similarly, as you can see here, the
10 hepatic sinusoid figure does have a fenestrated
11 endothelium and it allows small particles that are in
12 the systemic circulation to efficiently cross into the
13 sinusoids, and then through receptor-mediated
14 endocytosis be efficiently delivered into hepatic cell
15 types, mainly hepatocytes.

16 So, moving now to the hemophilias. Currently,
17 all AAV trials rely on -- for hemophilias anyway --
18 rely on systemically-administered AAV vectors that use
19 hepatocytes promoters to target hepatocytes expression
20 of either Factor VIII or Factor IX.

1 Hepatocytes are the endogenous site of Factor
2 IX synthesis but are not the endogenous site of Factor
3 VIII synthesis. So, Factor VIII is endogenously made
4 predominantly in a liver sinusoidal endothelial cell.
5 Whether or not that's relevant is not clear with
6 respect to hepatotoxicity.

7 Importantly, and unfortunately, the hemophilic
8 population has underlying comorbidities related to
9 liver disease, and that's largely due to the presence
10 of iatrogenic infection from contaminated blood
11 products in the '70s and '80s. So that's what's
12 depicted in this graph here. Essentially, if you're
13 above the age of 40, nearly all severe hemophilia
14 patients, unfortunately, go typed as Hepatitis C and/or
15 Hepatitis B.

16 And so, there's liver disease as well as
17 underlying risk of hepatocellular carcinoma in the
18 hemophilia population. And taking this into account,
19 most trials -- AAV trials -- exclude patients that have
20 some degree of advanced fibrosis. So anyone with Stage

1 3 or above liver fibrosis.

2 The bulk of hepatotoxicity that's been
3 observed in Hemophilia A trials, at this point,
4 thankfully has really posed more of an efficacy
5 limitation than an actual safety concern. And most,
6 but not all, hepatotoxicity that's been observed in
7 hemophilia seems to generally fall into a bucket of an
8 immune-mediated process. And the initial observation
9 of this process came out of the first systemically-
10 delivered AAV vector which was conducted in patients
11 with hemophilia B and a patient that I think has been
12 discussed quite a bit over the past approximate two
13 decades.

14 This is a man who was enrolled in the highest
15 dose cohort of the trial. You can see the red line
16 here. He expressed Factor IX, and then the Factor IX
17 level fell, and concurrently had a rise in his ALT.
18 And then subsequent translational study, the working
19 hypothesis for what the underlying etiology was -- or
20 the contact was -- as AAV vector particles transduced

1 the hepatocytes, the capsid was broken down inside of
2 the cytosol, and then capsid peptides could be
3 presented on the surface of MHC class I molecules and
4 could be trafficked or recognized by CD8-positive T
5 cells which ultimately would clear these transfused
6 cells. You would therefore have loss of transgene
7 expression and then could have observed a rise in
8 transaminases.

9 This next slide summarizes quite a bit of
10 information with quite a bit of activity in the
11 hemophilia gene therapy space. In short, most of the
12 observations, with respect to what is a presumed
13 capsid-immune response, patients present classically
14 with a rise in transaminases. So, a non-cholestatic
15 picture, typically ALT elevation is most pronounced.
16 And sometimes these elevations are really quite modest,
17 which is what we're monitoring for. Thankfully, have
18 not seen profound liver enzyme elevations. Only a
19 minority are modest and sometimes don't even cross the
20 upper limit of normal.

1 But, classically, the rise in transaminase,
2 and because we have the benefit of being able
3 to equally measure the expression of the transgene,
4 you'll see a decline in Factor VIII or Factor IX
5 activity. And then evaluation of patient peripheral
6 blood mononuclear cells can show reactivity in an
7 ELISPOT assay to capsid peptides.

8 The observation is consequently initially
9 observed within the first three months post vector. And
10 managing the immune response has been traditionally
11 done mostly with glucocorticoids, although there's now
12 some work using other immune-modulating agents. This
13 capsid-immune response has been observed in multiple
14 hemophilia A and B trials, and I have a list of them
15 here.

16 I do want to highlight that abrogating this
17 likely capsid-immune response has not been -- with
18 steroids has not been universally responsive. All
19 vectors have not been universally responsive, so we've
20 seen some participants in clinical trial -- which is

1 what these asterisks mean -- losing all of expression -
2 - and some of the participants, anyways, not all,
3 despite the use of steroids. There continue to be some
4 components of the vector design that may be quite
5 important in the robust and relative steroid-refractory
6 aspect of the immune response. And then, similarly,
7 there are at least a couple of trials now that have
8 reported that patients are put on steroids in the
9 context of a likely capsid-immune response, and then
10 you have recrudescence of that capsid-immune response when
11 the steroids are weaned.

12 But, as I mentioned at the outset of this,
13 importantly, this hepatotoxicity in the hemophilia
14 trials as it relates to hepatic immune response have
15 thus far really not posed any major safety concerns and
16 have really been, at this point, most relevant as it
17 relates to efficacy.

18 The last thing, with respect to hemophilia, I
19 wanted to mention is that most of the transaminase
20 elevations that have been observed fall into this

1 category related to capsid-immune response, but there
2 are some that do not necessarily, and where
3 transaminase elevations have not been consistent with a
4 so-called capsid-immune response. And two of the
5 trials are exemplified here.

6 I'll just highlight an example from one of the
7 trials where investigators had noted multi-month,
8 unclear etiology of mild transaminase elevation that
9 had not been observed in the concurrent setting of a
10 decline in factor activity and have not necessarily
11 been responsive to steroid intervention.

12 So, you could just see here -- in participant
13 6, noted here, you'll see he has transaminase
14 elevations that are above the upper limits of normal.
15 Again, relatively modest on the order of approximately
16 five months. And there's not necessarily an
17 association with factor level, and then steroids are
18 not necessarily associated with the transaminase --
19 it's not necessarily responsive to steroid
20 intervention.

1 So, I move now to observations with Spinal
2 Muscular Atrophy. This group is well aware that this
3 is a progressive neurodegenerative disorder. It has a
4 relatively high frequency for a rare disease among
5 which half of the cases are Type I disease. And
6 unfortunately, this is characterized by mortality or
7 requirement of respiratory support by two years of age.

8 With respect to the natural history of the
9 disease, specific to the liver, up to a third of SMA
10 patients have -- there's been some observations of some
11 degree of underlying liver disease. This includes
12 steatosis that's been observed on autopsy. And then
13 there's some data to support that these patients may
14 have underlying abnormal fatty acid metabolism and/or
15 decreased glutathione stores that may result in some
16 underlying liver disease or vice versa liver disease
17 drives it.

18 The decreased glutathione stores might be a
19 little bit interesting -- and perhaps this is my bias
20 as a pediatric hematologist. But the age at which

1 we're treating the SMA patients corresponds to -- and
2 many of the patients now, particularly with the advent
3 of newborn screening for SMA -- corresponds when these
4 patients have their physiologic nadir and have that
5 switch from fetal to adult hemoglobin in which
6 glutathione would be particularly important for
7 metabolism of red blood cells. And conceivably if you
8 have abnormal glutathione function, you could have a
9 rise in your transaminases, although not to the degree
10 that has been observed here.

11 Anyways, I think this group is well aware
12 of the development of Zolgensma which is the first and
13 currently only licensed systemic AAV vector. It's a
14 AAV9 vector consisting of an ubiquitous promotor,
15 including the SMN1 protein that's delivered at a dose
16 of $1.1E14$ vector genomes per kilogram. And these that
17 were reporting electrotherapy, now at least 800
18 patients that have been treated. There's been really
19 quite remarkable evidence of efficacy. So, this is a
20 little girl who's a toddler and you can see she's

1 running and she has Type 1 SMA. As clearly way outside
2 the anticipated natural history of the disease. And,
3 again, remarkable efficacy. And I'll talk about, in a
4 moment, observations out of clinical trial that
5 ultimately dictated that the drug was approved with a
6 box safety warning for concern around acute liver
7 injury.

8 And then just lastly with respect to the
9 conversation that's been had throughout the day.
10 There's been quite a bit of discussion around the
11 translation of observation with respect to genotoxicity
12 and hepatocellular carcinoma and neonatal mice studies
13 receiving systemic AAV vectors at relatively high doses
14 with an ubiquitous promotor, again, having a high
15 incidence of hepatocellular carcinoma. And perhaps a
16 parallel population that's available thus far, in
17 humans, are these young children with SMA. The median
18 age that patients are receiving vector is three months
19 of age. And that again will likely creep down even
20 further because newborn screening has been quite

1 effective now at identifying these patients.

2 To my knowledge, these young children are
3 receiving a higher-end AAV vector dose that consist of
4 an ubiquitous promoter. And to my knowledge, none of
5 them have developed hepatocellular carcinoma, which is
6 quite optimistic, which may suggest that some of the
7 mouse studies may not necessarily hopefully translate
8 to people.

9 But I think the caveat, obviously, is that
10 some of these newborns -- the earlier patients are five
11 or six years out and many of these patients are really
12 relatively, recently dosed, so we need longer follow-
13 up. And then secondly, that not all these patients,
14 and probably a minority of patients, are having routine
15 liver ultrasounds or surveillance. So, we just might
16 not know yet.

17 Nonetheless, just back to the hepatotoxicity
18 that's been observed with the SMA patients. Among the
19 patients this composite analysis of the clinical trial
20 patients -- and about a hundred of them were included

1 in the analysis -- sixty percent of them had
2 transaminase elevations before vector infusion. And
3 then 90 percent of them had either elevation of ALT or
4 AST, or both, after vector infusion, again with a non-
5 cholestatic pattern. Most of the elevations were
6 relatively modest (audio skip) incidence of abnormal
7 transaminase elevation.

8 Elevations typically occur within the first
9 week post vector and then a month or so after vector
10 when the steroids are weaned. (audio skip) patient.
11 This graph just outlines the number of patients
12 relative to the days on steroids. So, these patients
13 are being treated relatively longer with steroids
14 (audio skip) hepatotoxicity confirmed.

15 I just wanted to highlight two case
16 reports that was published last fall. These two
17 patients received vector with lysis. (Audio skip) who
18 prior to infusion had a history of an elevated
19 transaminase three to four times upper limits of
20 normal. (Audio skip) vector presented with acute liver

1 failure with encephalopathic features. So, it had
2 (audio skip) fold the upper limit of normal. With an
3 elevated expressed bili but relatively modest
4 transaminases and then had significantly reduced
5 function with an INR of 5.3.

6 He underwent an extensive workup, and he was
7 positive only for norovirus. So, there's really no
8 clear etiology for this other than the AAV vector that
9 he received. He was managed -- I have his liver biopsy
10 from presentation. I think it's remarkable here for
11 immuno- (audio skip) chemistry demonstrating how the T
12 cell infiltration (audio skip) essentially brown spots
13 and then evidence of fibrosis centrally throughout the
14 portal system. He was managed through glucocorticoids
15 that (audio skip) over the course of, I believe, about
16 a year. He had a repeat liver biopsy at two months.
17 (Audio skip) showed (audio skip) fibrosis and (audio
18 skip). So, I think it's probably more than two months
19 to weigh the evidence (audio skip) fibrosis.

20 Importantly, I'll continue (audio skip). Again,

1 unclear efficacy.

2 And then the second patient is a 20-month-old
3 female who had a transaminase elevated (audio skip).
4 (Audio skip) eight weeks (audio skip) and acute liver
5 failure with -- examinations were (audio skip) above
6 the upper limits of normal.

7 MR. MICHAEL KAWCZYNSKI: Lindsey?

8 DR. LINDSEY GEORGE: Yeah?

9 MR. MICHAEL KAWCZYNSKI: Can you just pull a
10 little bit away from the microphone a little bit?
11 You're just a little (audio skip). Go ahead again.

12 DR. LINDSEY GEORGE: I hope this is better.
13 (Audio skip) INR. Her workup was negative. (audio
14 skip) showed inflammatory infiltrates (audio skip),
15 positive T cells (audio skip) steroids (audio skip)
16 evidence of efficacy.

17 MR. MICHAEL KAWCZYNSKI: Dr. George --
18 Lindsey? Do you mind? I hate to do this to you, but
19 we are getting a weird -- I know you're going in and
20 out. I'm going to have you -- I hate to have you do

1 this. I'm going have you-- we're just going to take a
2 quick little pause. I want you to disconnect and
3 reconnect your audio quick. Okay? That's all right.
4 We'll just take a quick pause. All you've got to do is
5 hang up your phone and then click on that audio thing
6 and reconnect.

7 In the meantime, thank you. We'll just take a
8 quick moment. I think Lindsey was just having a short
9 little connection issue. And since her talk is so
10 important, we want to make sure that everybody hears it
11 clearly.

12 DR. LINDSEY GEORGE: I'm here.

13 MR. MICHAEL KAWCZYNSKI: All right. Lindsey.
14 There you go.

15 DR. LINDSEY GEORGE: Is this better?

16 MR. MICHAEL KAWCZYNSKI: It does sound a
17 little bit better. Thank you.

18 DR. LINDSEY GEORGE: Okay. I'll try to move
19 quickly so I don't cut into question time.

20 My summary of the SMA clinical trial data with

1 respect to hepatotoxicity is it overall seems to be
2 consistent -- you know, presented with non-cholestatic
3 picture -- it seems to be consistent with immune-
4 mediated pathology. It may be, perhaps, consistent
5 with a capsid immune response but is unclear.

6 And then the last cohort of patients are the
7 X-linked myotubular myopathy patients. I'll just skip
8 anything about the natural history of the data in
9 interest of time, other than to note that this is a
10 devastating disease and roughly half of the patients,
11 you know, mortality within 18 months.

12 The natural history is poorly understood and
13 so there are reports of some -- you know, a small
14 handful of patients reports of having liver pathology
15 but unclear the relationship that this is associated
16 with the disease. I will say, important to the events
17 that were observed in AAV clinical trials, there are --
18 so there's no current therapy -- but there's actual
19 multiple therapeutic strategies and clinical
20 development for this disease. And so, watching these

1 patients actually may in a way serve as a proxy natural
2 history of liver function. You know, patients
3 receiving, for example, enzyme replacement therapy, et
4 cetera. It's important to see what happens with their
5 livers.

6 So, I'll just skip to the trials. The trial,
7 sponsored by Audentes now Astellas. It was an AAV8
8 vector. It was administered in two different dose
9 cohorts. Importantly, the enrolment criteria included
10 patients with a history of pre-existing, clinically
11 significant liver disease. Initially, six patients
12 were enrolled in the lower dose cohorts of $1.3E14$
13 vector genomes per kilogram, and there's a dose
14 explanation. And, ultimately, 17 patients were
15 enrolled in the highest dose cohort of $3.5E14$ vector
16 genomes per kilogram. And then as I outlined, there
17 were, unfortunately, three deaths in this dose cohorts
18 related to presumably complications of liver failure.

19 Just with respect to the doses being used in
20 this trial, they are essentially the highest being used

1 that I'm aware of in any AAV trial. This table
2 outlines a list of trials that are using relatively
3 higher vector doses than used in most trials. And you
4 can see the only parallel is a Duchenne muscular
5 dystrophy trial using these doses.

6 What do we know about these unfortunate three
7 deaths? The boys were five years of age at the time of
8 death. They all had at least one elevated bilirubin
9 before vector infusion. They presented in a picture
10 that's different than the SMA patients and the
11 hemophilia patients. So, they had a markedly elevated
12 bilirubin. So, 35 to 50-fold the upper limit of
13 normal. Four to six weeks after vector administration
14 it progressed to elevated transaminases. These
15 transaminase and bilirubin elevations were unresponsive
16 to immune-modulating therapy. And then, unfortunately,
17 these patients succumbed to complications at anywhere
18 from 20 to 40 weeks post vector. So, two patients died
19 of sepsis and one died of GI bleeding.

20 At autopsy, histological analysis of the liver

1 didn't show any inflammatory infiltrates. Although
2 I'll just note that this is presumably months after the
3 initial insult, and so they may not have been able to
4 catch it. But of course there was evidence of
5 cholestasis, intrahepatic and canicular cholestasis and
6 then evidence of secondary fibrosis. Announcement of
7 these three boys' deaths is now over a year of age and
8 we don't have, to my knowledge, any more information
9 than what I've outlined here.

10 And then lastly as an update of this trial,
11 the trial was put on hold and came off hold in December
12 of 2020. And the decision was made to move forward
13 with the lower dose cohort 1.3E14 vector genomes per
14 kilogram. Unfortunately, as of yesterday, the sponsor
15 announced that they were halting screening and dosing
16 because the subsequent first patient treated,
17 unfortunately, developed abnormal liver function
18 failure a month after dosing. Now, it's unclear if
19 this is a very conservative move and there were modest
20 increases that were potentially more concerning than

1 that. So we just don't know at this point. The
2 patient did have a history of intermittent cholestasis
3 but had a normal liver ultrasound and LFTs at the time
4 of dosing.

5 This is my last slide. And just in
6 conclusion, the clinical presentation on hepatotoxicity
7 that have come out of clinical trials, hemophilia and
8 SMA have generally presented with a non-cholestatic
9 hepatocellular pattern versus the X-linked myotubular
10 myopathy patients have presented with a progressive
11 cholestatic hepatitis. The etiology is not crystal
12 clear, certainly, in hemophilia and SMA but most of the
13 hemophilia observations appear to be immune-mediated
14 and are thought to be a so-called capsid-immune
15 response, but not all. And then in SMA, similarly, the
16 observations appear to be consistent with an immune
17 response. And then X-linked myotubular myopathy, I'm
18 not sure if it's totally clear what that is. I think
19 we need a bit more information.

20 Some of the complications for comparing across

1 these disease cohorts are -- you know, just listed that
2 there's some multiple variables at play here. You
3 know, and conceivably enough the least of which is
4 underlying different co-morbidities, unclear natural
5 history in some of these disease cohorts, different
6 doses used, manufacturing, et cetera.

7 But the objective information out of clinical
8 trials, I think, is important to acknowledge is that
9 morbidity and mortality as it relates to hepatotoxicity
10 has only been observed with delivery of systemic AAV
11 vector doses that are greater than $1E14$ vector genomes
12 per kilogram and actually only in pediatric patients.
13 So that might be an initial place for us to start.
14 So, with all that, I'll close and thank you for your
15 attention.

16

17 **INVITED SPEAKER PRESENTATION Q&A**

18

19 DR. LISA BUTTERFIELD: That was terrific.

20 Thank you very much. So, we now have about a dozen

1 minutes for Q and A with our speaker, Dr. George. And
2 if I can start with one question about the anti-vector
3 immunity: So, if I understood correctly, that's not
4 really a safety signal it's an efficacy signal. And,
5 so, is there anything about that that would be an
6 exclusion criterion from treatment up front or perhaps
7 just redosing exclusions?

8 DR. LINDSEY GEORGE: Do you mean efficacy
9 signal in the hemophilia trial; is that what you're
10 saying?

11 DR. LISA BUTTERFIELD: Yes.

12 DR. LINDSEY GEORGE: So, I think that,
13 thankfully, that the transaminase elevations in the
14 hemophilia trials have been pretty modest. I think,
15 you know, you give a differing opinion. I also give
16 mine. I think that that the observations of
17 hepatotoxicity, thankfully, in hemophiliacs have been
18 modest and have not really posed major safety concerns.
19 And particularly if they're related to a cellular
20 immune response -- a capsid-immune response and they

1 haven't been sustained.

2 It is curious, some of the observations in one
3 or two trials where these patients have had multi-month
4 transaminase elevations with no clear etiology. And
5 although the elevations are modest, the absence of an
6 explanation for what they are then it does raise the
7 question as to whether or not this is a safety signal,
8 simply because we don't have a good explanation for
9 them.

10 DR. LISA BUTTERFIELD: Thank you. Now to
11 questions from the committee, other members, Dr.
12 Heller.

13 DR. THEO HELLER: Thank you. I thought that
14 was an excellent review of a very complex subject with
15 a lot of subtlety and nuance. I have quite a few
16 questions.

17 The first is in SMA. How do we know the
18 enzymes are due to liver damage? Because AST and ALT
19 are found in muscle as well. And they are also found
20 in red cells and you alluded to red cells hemolysis --

1 DR. LINDSEY GEORGE: Yes.

2 DR. THEO HELLER: -- and things like that.

3 What do you find? Should I -- I'll stop. I'll wait.

4 DR. LINDSEY GEORGE: Thank you for giving them
5 to me one-by-one. So, the short answer of what I
6 understand of the SMA data is that there was, in a
7 smaller cohort of patients than was initially observed,
8 quite a bit of effort given to isolating this liver
9 injury versus other sources of AST and ALT elevation.

10 And so, I don't necessarily think this has
11 been broadly applied to the 800 patients to isolate
12 this to liver pathology. But I think in the initial
13 cohort, the initial early phase trial, there was effort
14 given to confirming that this was hepatocyte-specific.

15 DR. THEO HELLER: The second is XLMTM: There
16 was a paper last month which says that "Intrahepatic
17 Cholestasis is a Clinically Significant Feature
18 Associated with Natural History of X-Linked Myotubular
19 Myopathy," *Journal of Neuromuscular Disease*. It seems
20 that it is a common underlying -- they're suggesting

1 that it's a common underlying feature. There are also
2 reports of peliosis hepatitis. And one of the problems
3 with liver disease is that liver enzymes fluctuate in a
4 swivel-tooth pattern.

5 And I saw that you said that patients with
6 pre-existing liver disease were excluded. I wondered
7 how that was determined. You said there was one
8 patient who had normal enzymes at the time of
9 enrollment, but previously had elevations. That
10 doesn't exclude underlying significant liver pathology.
11 Even in cirrhosis, liver enzymes can go up and down.

12 And the one of the deaths had a previously
13 elevated bilirubin, but people with pre-existing liver
14 disease were excluded. And it seems that the liver
15 disease is cholestatic. That doesn't make sense, at
16 all. I really does- -- I'm not criticizing, I'm trying
17 to understand.

18 DR. LINDSEY GEORGE: Yes.

19 DR. THEO HELLER: And then the follow-up
20 question to that, in this cohort was that the liver

1 biopsy reported secondary fibrosis, I don't know what
2 that means. The term, "secondary fibrosis," yes,
3 fibrosis secondary to something, how extensive was the
4 fibrosis that they scored? Was it cirrhotic? Was it
5 not cirrhotic? And what did they mean by secondary in
6 the sense of there was no inflammation? Was this is in
7 a cholestatic-type fibrotic pattern? Or a post-
8 hepatic-type pattern?

9 DR. LINDSEY GEORGE: So, if I may pick away at
10 this a little bit. I don't have an answer -- a direct
11 answer. But I think that two things are true. And I
12 think your point is well taken that, you know, the
13 (inaudible) criteria is to exclude patients with
14 significant underlying liver disease. But to my
15 knowledge that was up to the discretion of the
16 investigator.

17 I mean, again, as we heard earlier, this
18 discussion of balancing risk with efficacy is difficult
19 in this disease which is quite devastating. And the
20 preliminary efficacy, as to my understanding, was quite

1 remarkable coming out of this work.

2 So, conceivably as an investigator, you could
3 be biased to want to enroll a patient in this trial.

4 And I don't know exactly if there are laboratory
5 parameters specified to the investigators. You know,
6 what the criteria were. And as you note, all three of
7 the boys that died as well as this patient that was
8 announced yesterday, they had some kind of history at
9 some point in time of elevated transaminases.

10 And the last point I'll say is -- so, I'm not
11 a neurologist and I haven't been involved in any of the
12 care of these patients. But I think this is a
13 universal issue when we start to think about AAV with
14 hepatotoxicity being the primary AE that's been
15 reported, which is most of the people enrolled in the
16 clinical care of these patients -- or taking care of
17 these patients -- are not hepatologists. So even the
18 finesse that you've mentioned here in one minute is
19 probably way outside my level, and several other
20 people's level of expertise, at understanding liver

1 disease.

2 And so, it is tricky and it probably warrants
3 a little bit of specificity in the protocol if you can.
4 Or, perhaps, some involvement with hepatologists in
5 these patients' care.

6 DR. THEO HELLER: Thinking that it's more
7 of a hepatic picture in SMA and hemophilia, but then
8 XLMTM has more of a cholestatic picture and underlying
9 liver disease is cholestatic.

10 DR. LINDSEY GEORGE: Yeah.

11 DR. THEO HELLER: And so, I don't think that
12 that's random. And there's literature saying that this
13 is more prevalent than you think it is and relying on
14 enzyme in that setting would not be adequate. So, I
15 think if we're talking about toxicity of the vector,
16 it's not fair to talk about toxicity of the vector in
17 the setting of underlying liver disease which changes
18 the picture.

19 DR. LINDSEY GEORGE: Yes.

20 DR. THEO HELLER: Thank you for your

1 answers. Thank you.

2 DR. LISA BUTTERFIELD: Great. We've got
3 two more questions from the committee. The first is
4 from Dr. Roos.

5 DR. RAYMOND ROOS: In the case of SMA, is
6 there evidence of persistence of liver abnormalities?
7 Has that been investigated?

8 DR. LINDSEY GEORGE: Well again, I have not
9 been an investigator or somebody that's been actively
10 involved in the SMA trials so what I understand is from
11 meetings and some literature. But my understanding is
12 the liver enzyme abnormalities, in some cases, have
13 been relatively persistent. And much of the reason why
14 some of these patients are on steroids for multiple
15 months is, I think, clinically what happens is either
16 the investigator or now the treating physician has to
17 take somebody off steroids and then their liver enzymes
18 increase. And so, I think that that's part of the
19 reason why they're on steroids for so long.

20 So, to directly answer your question, yes I

1 think that some of these patients have had prolonged
2 elevated transaminases. You know, some of them the
3 initial elevations have been quite marked, in a
4 minority of roughly three to five percent of patients.
5 But I don't think there's been sustained synthetic
6 dysfunction or whatever. Of course, it would have to
7 be pretty profound liver damage for it to ultimately
8 see those side effects.

9 DR. RAYMOND ROOS: Is there going to be
10 development of cirrhosis or a chronic liver
11 dysfunction?

12 DR. LINDSEY GEORGE: Yeah, I don't know that.

13 DR. RAYMOND ROOS: Thanks.

14 DR. LISA BUTTERFIELD: Thank you. Dr.
15 Hawkins.

16 DR. RANDY HAWKINS: Thank you, Dr. George.
17 Your slide of the young girl spoke to not just quality
18 of life, but life. And so, with SMA, my question is,
19 can all the persons who have this diagnosis receive
20 this treatment?

1 DR. LINDSEY GEORGE: I think it depends on a
2 couple of things. It's my understanding you have to
3 have an AAV (inaudible) antibody status. I believe the
4 cut off is less than 1 to 50,000, or something like
5 that -- 1 to 5,000, I'm sorry. I think two things are
6 -- so with respect to actually what were called
7 exclusions to actually receive the lysis vector,
8 presumably there's two factors at play which is are you
9 eligible based on your (inaudible) antibody status and
10 are you eligible based on insurance status.

11 I think with respect to the immunological
12 gatekeeping, thankfully, I think, with the advent of
13 newborn screening was developed essentially
14 concurrently with the vector -- not necessarily by, but
15 this was concurrently going on. And so newborn
16 screening is not universal in every state in the United
17 States, but in most. And so, I think these patients
18 are being identified much sooner. And so hopefully you
19 can get them at a nadir where they don't have maternal
20 antibodies and they haven't had AAV exposure.

1 And then with respect to the insurance issues,
2 it's probably a little bit beyond my level of
3 expertise. I've been a little bit involved at the
4 Children's Hospital of Philadelphia around this. And
5 let me just say this has not posed a major -- this is -
6 - there are several people that have had to -- there's
7 quite a bit of effort to get insurance approval, but so
8 far every patient was eligible and the families that
9 wanted to move forward with vector received it.

10 Dr. RANDY HAWKINS: Thank you very much.

11 DR. LISA BUTTERFIELD: Thank you. Dr.
12 Venditti?

13 DR. CHARLES VENDITTI: I thank you for the
14 excellent presentation. I'm wondering if there could
15 be, in some of these patients, a transgene-mediated CMI
16 response beyond capsid, especially in the last group of
17 patients you said were the people with withdrawal
18 prednisone, all of a sudden, LFTs shoot up? I realize
19 that there might be limited clinical information, but
20 I'm just wondering if you could comment on that? And

1 if there's something else we should be thinking about
2 with patients that are treated with higher doses of AAV
3 for neurologic indications where the promoters may work
4 in the liver. Are they going to develop a transgene-
5 immune response in the liver?

6 DR. LINDSEY GEORGE: I think that's an
7 excellent point, which I kind of lumped a lot of this
8 into an immune response and was sort of deliberately
9 vague. So, I'll say that, to my knowledge, they still
10 have not demonstrated a correlation with a immune
11 response with a transgene and elevation of
12 transaminases.

13 And in the context of hemophilia, we do
14 look for more of sort of growth measures so to speak.
15 So, we look, for example, at Factor VIII and Factor IX
16 we look for inhibitory antibodies, and some of the
17 trials are looking anti-IGG antibodies. And, then,
18 many of the (inaudible) are perform not only against
19 the capsid but also against the transgene peptides.
20 And there's not been a compelling case for that to be

1 an etiology. But you're right, I think we oftentimes
2 say, you know, this is the hepatocytes- specific
3 promotor or this is a tissue X-specific promotor but
4 it's not confirmed. It's what we have convinced
5 ourselves of but not necessarily confirmed.

6 So, no. It's definitely possible. As I
7 told you, I'm not familiar enough with the X-Linked
8 myotubular myopathy work to know whether or not people
9 have looked at this. And I don't think the information
10 is publicly available. I have heard discussed at
11 meetings, which I think is -- at the risk of -- I just
12 want to make sure that this is the very clearest --
13 it's just discussed at meetings that there was effort
14 to look for expression of the mouse (inaudible) in
15 protein in the biopsies but was not seen. But that's
16 not published, that has not been presented anywhere so
17 I don't know if that's accurate or not.

18 DR. CHARLES VENDITTI: Thank you.

19 Dr. LISA BUTTERFIELD: Thank you all.

20 That was a great discussion. And time is up, and so

1 we're going to move now -- thank you, Dr. George, -- to
2 our second invited speaker presentation in
3 hepatotoxicity. And this is Dr. James Wilson, Director
4 of the Gene Therapy program at the Perelman School of
5 Medicine. Dr. Wilson.

6

7 **INVITED SPEAKER PRESENTATION: "HEPATOTOXICITIES AND**
8 **OTHER RELEVANT FINDINGS IN ANIMAL STUDIES"**

9

10 DR. JAMES WILSON: Great, well we
11 probably should get started and let the clock roll.
12 You can hear me okay?

13 DR. LISA BUTTERFIELD: Yes.

14 DR. JAMES WILSON: Great. Thank you.
15 This is my Conflict of Interest Disclosure statement.
16 I was asked today to talk about nonclinical data as it
17 relates to hepatotoxicity. And I think towards an eye
18 in determining what has been predictive and what has
19 been useful and what should we be considering going
20 forward. I'm going to talk about a story that we put

1 together at Penn, with my colleagues noted here, as it
2 relates to our attempt to try to do this. And this is
3 my wrong talk. This is the DRG talk, my talk for
4 tomorrow.

5 MR. MICHAEL KAWCZYNSKI: Hold on a
6 second. Hold on, sir. Let me see here. You have --
7 this one was -- all right. You have two talks in here?

8 DR. JAMES WILSON: Yeah, two talks.

9 MR. MICHAEL KAWCZYNSKI: All right.
10 Let's see if this is the correct one.

11 DR. JAMES WILSON: I do know a neuron is
12 not a hepatocyte. Leave it at that.

13 MR. MICHAEL KAWCZYNSKI: Okay. Let's see
14 if this the correct one? All right. So, we're just
15 going to take a moment here. We're going to take a
16 quick pause just while I reload Dr. Lindsey's notes.
17 And let me just doublecheck. All right. I'm going to
18 try this one first, sir. Like I said, I'm not quite
19 sure if this is the one. Nope. Not this one. Is this
20 the one yet or --

1 DR. JAMES WILSON: That's the one we just
2 saw.

3 MR. MICHAEL KAWCZYNSKI: Okay. So, which
4 one are we looking for, sir? It's another one for me.
5 It has to do with non-clinical hepatic toxicity.

6 MR. MICHAEL KAWCZYNSKI: All right. Let
7 us take a quick unscheduled break while we pull this
8 in. I'm going to have to go to our file and
9 doublecheck that we have the right one. This is the
10 one that I have, so you'll just bear with me a moment.

11 And, Jarrod, are you there?

12 MR. JARROD COLLIER: Yes, I'm here.

13 MR. MICHAEL KAWCZYNSKI, Jim, do you have
14 your PowerPoint with you?

15 MR. JARROD COLLIER: I'm sorry?

16 DR. JAMES WILSON: No.

17 MR. MICHAEL KAWCZYNSKI: I mean because I
18 can always just have you share your screen.

19 DR. JAMES WILSON: Okay.

20 MR. MICHAEL KAWCZYNSKI: Have you got

1 your PowerPoint? I have two files for you. One's
2 called, James Wilson 1 and one is called 2.

3 MR. JARROD COLLIER: Yes. There should
4 be two.

5 MR. MICHAEL KAWCZYNSKI: All right.
6 That's the one I just had loaded in here.

7 MR. JARROD COLLIER: Okay. Were they not
8 correct?

9 MR. MICHAEL KAWCZYNSKI: Yeah. I think
10 it's the wrong one. So, I'll pull it up again. We
11 don't have the right one, but I'll show you the one I
12 have, sir. I'll load it in real quick. This is the
13 "James Wilson 2," correct?

14 DR. JAMES WILSON: Thank you. Yeah.

15 MR. MICHAEL KAWCZYNSKI: All right. I'm
16 loading it in right now. Hopefully, this is the
17 correct one otherwise we'll just have you share your
18 screen.

19 DR. JAMES WILSON: Okay.

20 MR. MICHAEL KAWCZYNSKI: It'll just take

1 a moment. Although it does have the -- it does show a
2 AAV-mediated (inaudible) dorsal root ganglion.

3 DR. JAMES WILSON: No. That's not it.

4 MR. MICHAEL KAWCZYNSKI: That may not be
5 the right one, sir.

6 DR. JAMES WILSON: Well how would you
7 like me to share my screen?

8 MR. MICHAEL KAWCZYNSKI: There -- I can
9 prompt you -- go ahead right in the center of the
10 screen. Do you see where it says, "Share my screen?"

11 DR. JAMES WILSON: Right.

12 MR. MICHAEL KAWCZYNSKI: Go ahead and
13 click on that.

14 DR. JAMES WILSON: Okay.

15 MR. MICHAEL KAWCZYNSKI, That's all
16 right. In whatever screen you can provide. If you
17 share your desktop, and it will pull it up.

18 DR. JAMES WILSON: All right.

19 MR. MICHAEL KAWCZYNSKI All right, sir.
20 Your screen is coming up. I'll tell you when I see it.

1 DR. JAMES WILSON: Now?

2 MR. MICHAEL KAWCZYNSKI Not yet. There it
3 comes. Now I see your email, sir. So go ahead. You
4 can pull your PowerPoint up.

5 DR. JAMES WILSON: And my PowerPoint is
6 up.

7 MR. MICHAEL KAWCZYNSKI: All right. Drag
8 it up over -- there you go. We see it, sir. Go ahead
9 and take it away.

10 DR. JAMES WILSON: But do you see it
11 animated?

12 MR. MICHAEL KAWCZYNSKI: Adeno-Associated
13 Virus -- Yep. Adeno-Associated Virus-Related
14 Toxicities PowerPoint.

15 DR. JAMES WILSON: And do you see the
16 animation here?

17 MR. MICHAEL KAWCZYNSKI: Not yet. Now
18 you're on Disclosure Statement.

19 DR. JAMES WILSON: Okay. Let's see. You
20 can see Disclose Statement/Acknowledgments?

1 MR. MICHAEL KAWCZYNSKI: Yep. Now we see
2 acknowledgments.

3 DR. JAMES WILSON: Okay. And then you
4 see "Spectrum of Toxicities?"

5 MR. MICHAEL KAWCZYNSKI: Yeah. We see
6 the "Potential Mechanisms Leading to Organ Damage."
7 Yes.

8 DR. JAMES WILSON: How about, "Spectrum
9 of Toxicities?"

10 MR. MICHAEL KAWCZYNSKI: Spectrum of
11 Toxicities associated with Systemic AAV.

12 DR. JAMES WILSON: And then we go to
13 potential mechanisms.

14 MR. MICHAEL KAWCZYNSKI: All right. So,
15 we have your PowerPoint up. If you want to continue as
16 you want to go.

17 DR. JAMES WILSON: Okay. Yeah. I think
18 I'll leave it here so we don't try to animate this.
19 Okay. Well, let's get back to where we started which
20 was what we attempted to do here was develop non-

1 clinical data that would help predict the spectrum of
2 clinical adverse events related to liver toxicity. And
3 suffice it to say that the sponsors, the investigators
4 in the field, had generated pre-clinical data to
5 support the risks and benefits in these programs.
6 Since they were unexpected results, those pre-clinical
7 models were really not that helpful.

8 So, in preparation for the talk today, what we
9 tried to do is reflect on the data that we generated
10 and that had been generated that may be useful in
11 identifying common mechanisms that lead to this diverse
12 array of clinical sequelae. And what we found is a
13 possible initial insult following high dose AAV. It is
14 a reduction in platelets and variably and increase in
15 LFTs.

16 Next slide. This says, "Potential
17 Mechanisms Leading to Organ Damage." As we generated
18 our non-clinical data in non-human primates, there was
19 a common theme that was thrombocytopenia and also some
20 evidence for dysregulation of coagulation. And what

1 we're going to hear about later is this syndrome of TMA
2 that may be associated with some of these toxicities in
3 clinic.

4 But also, in our non-clinical studies,
5 there was evidence for a different kind of coagulation
6 defect which is DIC. And as we present the non-
7 clinical data, we did want to share with you -- or just
8 talk about now what may be some common features which
9 may be some ways to differentiate these two, with DIC
10 being stimulated by tissue necrosis and inflammation
11 and leads to microvascular thrombosis. But there is a
12 secondary activation of fibrinolysis. Whereas TMA
13 possibly is initiated by endothelial cell damage and
14 activation and consumption of platelets leading to
15 microvascular thrombosis and organ damage.

16 And as we talk about our studies in non-human
17 primates, we're going to point out some differences and
18 some similarities between these two, both leading to
19 organ damage, but in the setting of TMA affecting the
20 kidney and also hemolysis. Whereas, with severe DIC, a

1 sort of a multi-organ damage.

2 There were two case reports I will present and
3 then two studies that we developed to try to address
4 some of these hypotheses. The first is shown here
5 which is really our first experience at injecting high
6 dose AAV into non-human primates.

7 DR. LISA BUTTERFIELD: Sorry. Dr.
8 Wilson? We're still on Spectrum of Toxicities. Is
9 that the slide you're still on?

10 Dr. WILSON: No. I'm supposed to be on
11 slide six. Do you see six? Case report 1.

12 MR. MICHAEL KAWCZYNSKI: So just move the
13 slides on your computer.

14 DR. JAMES WILSON: Yeah. I am.

15 MR. JARROD COLLIER: Dr. Wilson, are you
16 able to see the slides that you're presenting, at this
17 time?

18 DR. JAMES WILSON: Yeah. Do you guys see
19 these slides?

20 MR. MICHAEL KAWCZYNSKI: Yes, we see

1 "Spectrum of Toxicities.," We're looking at your
2 email.

3 DR. JAMES WILSON: How about this?

4 MR. MICHAEL KAWCZYNSKI You'd have to
5 drag it. There you go. Yep. Now open it there.

6 DR. JAMES WILSON: And we're at
7 "Potential Mechanisms?"

8 MR. MICHAEL KAWCZYNSKI: Yes, but make
9 that full screen, sir.

10 DR. JAMES WILSON: Yeah. I think it's
11 going to -- here, Let's see. Do you see that?

12 MR. MICHAEL KAWCZYNSKI Yes, and then hit
13 play. There you go. Now you're at the first slide.
14 Yeah. Now you're on the first slide, so now you've got
15 to advance.

16 DR. JAMES WILSON: I am advancing. So
17 second slide, "Disclosure?"

18 MR. MICHAEL KAWCZYNSKI: You're on slide
19 4, "Spectrum of Toxicity," again. "Disclosure
20 Statement." You're going backwards.

1 DR. JAMES WILSON: I think it's just
2 delayed. "Acknowledgements?"

3 MR. MICHAEL KAWCZYNSKI: Yes.

4 DR. JAMES WILSON: "Spectrum of
5 Toxicities?"

6 MR. MICHAEL KAWCZYNSKI: Yes.

7 DR. JAMES WILSON: "Potential
8 Mechanisms."

9 MR. MICHAEL KAWCZYNSKI: I'll tell you
10 when that one pops up. Yes. "Potential Mechanisms."
11 Yes.

12 DR. JAMES WILSON: I think I talked about
13 that. Case Report.

14 MR. MICHAEL KAWCZYNSKI There you go.

15 DR. JAMES WILSON: Well it's animated, so
16 I just have to go slow so --

17 MR. MICHAEL KAWCZYNSKI: Okay. You're in
18 charge of the slide, so we'll follow along. All right?
19 Take it away.

20 DR. JAMES WILSON: All right. So, the

1 first case report was our first experience with
2 infusing high dose AAV into non-human primates. These
3 were juvenile macaques in which the vector was an AV9-
4 like vector delivered at a dose of 2×10^{14} .

5 I'm just going to pull up the data down below.
6 You guys should see ALT at this point. So, what we
7 found are three animals that were dosed. One of all of
8 -- all of three developed a syndrome of bleeding. Two
9 of which then recovered and one of which went on to
10 develop hemorrhage, shock, and the animal had to be
11 euthanized. When we evaluated these three animals with
12 respect to transaminases, all three had an increase
13 within the first week. And one was much more severe --
14 the one that had to be euthanized.

15 I now have total bilirubin that is up.
16 And, again, there was an increase in the first week of
17 bilirubin. The two animals that survived, this
18 resolved. The animal that was severely ill-developed
19 an increase in creatinine. But all three of the
20 animals, coincidence with the increase in

1 transaminases, developed a drop in platelets. In the
2 two animals that recovered, this resolved. And the
3 hemoglobin was stable except for the animal that
4 developed a severe hemorrhage and died.

5 The necropsy performed on the animal that was
6 euthanized showed severe liver necrosis. And there was
7 an absence of cellular infiltrate. But importantly,
8 deposition of fibrin and intravascular coagulation.

9 I'm on Case Report 2. So, with those
10 data available to us, we began to consider other
11 experiments to address what factors would potentially
12 influence this fatal systemic toxicity. And this study
13 was actually meant to compare AAV9 to a variant of AAV9
14 called PHP.B that presumably had better delivery to the
15 CNS. But we selected doses for this experiment that
16 were lower than $1e14$ because we did not expect to see
17 systemic toxicity.

18 So, I'm just going to show you the data for
19 ALT, total bilirubin, and platelets for the animal that
20 received a low dose $2e13$ GC/kg. And these were largely

1 unremarkable although at three weeks there was a slight
2 increase in transaminase.

3 When we went to a higher dose of AAV9, we
4 saw an increase in the transaminase but bilirubin was
5 fine, and platelets were not reduced. But this variant
6 factor of PHP.B at lose dose, again, showed a
7 reasonable toxicity profile. But at the higher dose,
8 7.5e13, this animal got severely ill like the other
9 case study. Large increase in transaminases, large
10 increase in bilirubin, reduction in platelets, and the
11 animal had to be euthanized.

12 Anticipating we may see toxicity like
13 this, we performed a more comprehensive review of the
14 coagulation parameters on the chance that this could
15 reflect a coagulopathy. And in the higher dose animal,
16 looking at baseline day 3 and day 5, we observed an
17 increase in PT and a slight increase in PTT, but a
18 reduction in fibrinogen and a transient increase in D
19 dimer suggesting that this may be more of a process
20 similar to DIC.

1 The pathology on the liver of the animal
2 that had to be euthanized is shown here. It was a
3 substantial grade 4 acute liver necrosis. And when
4 shown under higher magnification, there was evidence
5 for necrosis. Evidence for mitotic figures but
6 importantly the canalicular bile stasis as well. And
7 as we saw in the previous animal, marked intravascular
8 coagulation and deposition of fibrin.

9 So, with this as a potential model to
10 simulate high dose systemic toxicity of AAV, we asked a
11 couple of questions. And the first one, which is a
12 question that's been brought up by the committee on a
13 number of occasions, is to what extent is the way in
14 which the AAV is prepared could impact on performance
15 such transduction efficiency or safety? And so this is
16 an unpublished study that I'd like to share with you
17 today. We generated bulk materials of 89 using 293
18 cells using our GMP process. And then it was purified
19 using either a GMP-compatible column process or the
20 research approach of Iodixanol.

1 So, we had three animals that received the
2 Column Purified, three with Iodixanol. And these
3 animals were dosed at a high dose, 2×10^{14} that we thought
4 could lead to toxicity. And as Dr. Bern and others
5 have brought up, it's very important to the extent that
6 we can to try to get a handle on the purity, potency,
7 and residuals of our product. And this is some of the
8 key comparisons between these two downstream processes,
9 including the full to empty ratio, are pretty similar
10 and are toxin-free and potency was pretty similar. And
11 purity on a SDS Gel looked comparable.

12 So now the characterization of that
13 experiment. And what I have here is we've
14 characterized the transduction profile when these
15 animals were necropsied in heart, skeletal muscle,
16 liver, brain, spinal cord and the dorsal root ganglia
17 is something we'll talk about tomorrow. And which
18 we've quantified by morphometry the efficiency of
19 transduction.

20 The bottom line is that at this dose,

1 which is 2×10^{14} , which is something others have described
2 as well is the transduction efficiency of an AAV9
3 vector is very high. There may be some slight
4 differences, possibly the gradient was a little more
5 efficient, but the efficacy profiles would be the same.

6 But the question is what about the
7 toxicity? So, on this slide summarizes our evaluation
8 of toxicity in this experiment. First of all, all
9 animals survived, did not develop any clinical
10 sequelae. But what about clinical pathology? What I
11 have on the top here are the data from the Column
12 Purified experiment and the Gradient Purified
13 experiment, evaluating transaminase AST in which all
14 animals showed an increase. ALT, the gradient-purified
15 material may have a secondary spike. Bilirubin
16 elevated in the gradient-generated material. And
17 platelet reductions -- again, this is all within the
18 first week or so -- were demonstrated in bulk
19 processes.

20 During this time that we were conducting

1 the experiment, we learned of some of the data that
2 came out of the DMD studies. So, we asked the question
3 is there a correlation between these findings and the
4 activation of complement? Because there was a modest
5 increase in PTT.

6 So, in those same animals we evaluated the
7 samples for any evidence of activation of complement,
8 and there did seem to be, at day 3, an increase in MAC
9 SC5b. But when further analyzed for evidence of
10 classic pathway complement activation, there were no
11 increases. Although there was an increase in
12 Complement Bb in the Column Purified more than the
13 Gradient.

14 And as I mentioned, there was a
15 concomitant reduction in platelets. So we then asked
16 the question, is there a correlation between the
17 activation of Complement -- at least the alternative
18 pathway as demonstrated by Bb -- and the reduction and
19 platelets? And I'll show you that in the next slide.

20 Another question that has come up is, is it

1 possible that an activation of (audio skip) vector
2 could actually correspond to the activation of
3 Complement in the reduction in platelets and then the
4 hemolytic uremic syndrome? And when we look at our
5 antibody titer subsequent to vector, that there were
6 really no increases by day 3 where we saw the
7 activation of Complement. And, while it was
8 alternative Complement, this didn't seem to explain the
9 result.

10 If we summarize this experiment where we
11 compare the two groups Column versus Gradient with
12 respect to liver toxicity, what we go show is that the
13 Gradient Purified vector seemed to have a higher
14 increase in transaminases and bilirubin that were
15 statistically significant in these very small groups
16 with total bilirubin. But, then, when we evaluated the
17 same animals for reduction in platelets, what we saw
18 was that there was a greater reduction in platelets in
19 the Column, Purified animals, that did seem to
20 correspond with the activation of Complement. And when

1 we actually tried to correlate the increase in measures
2 of Complement Activation against the reduction in
3 platelets, it was not statistically significant but
4 there was a correlation.

5 So, what was interesting here is two different
6 methods of purification, controlled as best as we
7 could, did seem to impact on the performance of the
8 vector with respect to safety in very different ways.
9 Evidence of liver damage versus the thrombocytopenia
10 and the activation of Complement.

11 So, the last experiment, and I'll
12 summarize, was the notion of trying to abrogate both or
13 either of these toxicities with steroids, which is what
14 is generally used in the clinic. So, in this
15 experiment, we compared at a dose of $1e14$, an AV9
16 vector versus that PHP.B vector which at $7.5e13$ showed
17 toxicity.

18 And then we also dosed an animal that
19 received this more toxic vector with steroids, 1
20 mg/kg/day, to do basically the controlled experiment on

1 studying this steroid effect. These animals were
2 followed for two weeks.

3 So, what happened was the PHP.B animal
4 developed a severe clinical syndrome and had to be
5 euthanized, but the other two survived. And when you
6 compare PHP.B against the AAV9 vector in primates, very
7 similar transduction efficiency, although this animal
8 had to be euthanized.

9 And when you evaluate the liver of the
10 animal that received the AAV9 vector, there was
11 moderate to minimal degeneration infiltrates in bile.
12 But in the PHP.B animal, very severe hepatocellular
13 loss like we had seen in our initial study. But
14 steroid treatment with this vector pretty much
15 presented these severe findings, and we had similar
16 results to AAV9.

17 And then let's look at the clinical
18 pathology data in this experiment, because I think it
19 could be an interesting experimental tool. So we're
20 going to start with the impact of vector administration

1 at $1e14$ in terms of ALT, AST, bilirubin, and platelets.
2 And AAV9 relative to PHP.B capsid is really pretty
3 clean. And as we mention, the two animals that
4 received the PHP.B vector developed the syndrome that
5 required euthanasia at Day 3 or Day 6. Elevation of
6 bilirubin and substantial drop in platelets. But when
7 you treat that animal with steroids, you completely
8 abrogate the transaminase increase. But you have no
9 impact on the thrombocytopenia.

10 And the PHP.B animals that did develop
11 evidence for coagulopathy, as indicated by an increase
12 in PT, PTT, and a reduction in fibrinogen, and the
13 steroids had no effect at all on that.

14 So, one last data slide and then just the
15 summary and a model. It's something that's been very
16 striking to us which has been the impact of dose on
17 both gene delivery and transduction and toxicity.

18 I'm showing you here an experiment with AAV9
19 delivered at three doses: $1e13$, $3e13$, $1e14$ in a Rhesus
20 Macaque. And then evaluated in the heart for

1 transduction at these three doses. And what we've seen
2 in this model and many others, is there's a non-linear
3 response in terms of gene transfer and transduction.
4 Only a three-fold increase leads to a greater than
5 three-fold increase in transduction, and even more at
6 $1e14$.

7 And what we've done is we have generated data
8 here comparing the ratio -- the impact of the dose on
9 ALT, and this got somehow modified. But what it shows
10 is that there's a substantial increase in the
11 transaminase when you go from $1e14$ to $2e14$. In here
12 that got somehow messed up.

13 So, our model, and then just final
14 comments. How do we pull all of this together where we
15 start with vector? And in the clinic we observe a
16 spectrum of syndromes including hemolytic uremic
17 syndrome which we'll hear about, I think, tomorrow.
18 The cholestatic liver failure that we just heard about
19 from Dr. George in the Audentes study. And then
20 hepatocellular liver failure, for example, in some of

1 the SMA studies.

2 We think that there may be two inciting
3 events with a common pathway, but that's microvascular
4 thrombosis versus liver damage. And this could
5 definitely be influenced by a (audio skip). But then
6 the consequence of that, even in humans, varies pretty
7 significantly. And in animals it definitely can vary.
8 And this may be more influenced by the attributes of
9 the host.

10 So just to summarize about attempts to model
11 high-dose systemic toxicity in our experience with
12 Macaques is we've asked the question, where is there
13 agreement with at least some of the human studies? At
14 least for the acute host response of thrombocytopenia
15 and transaminase elevations, it seemed to simulate.
16 But that we believe there is evidence for microvascular
17 thrombosis in our non-human primate studies and likely
18 similar findings at least in some of the human studies.

19 But the development of hepatocyte or liver
20 failure we've demonstrated. And this non-linear sort

1 of dose-response of efficacy and toxicity, as I think
2 we are all we're also seeing in humans. But,
3 importantly, the threshold for severe toxicity
4 requiring at least euthanasia in primates, is almost
5 spot on with the threshold for human studies which is
6 2×10^{14} GC/kg.

7 But there are important differences to
8 point out in that the severe liver toxicity that are
9 observed in humans are often delayed and what we're
10 seeing in primates are a bit earlier. We've had no
11 evidence of hemolytic uremic syndrome in any of the
12 primates. We do have evidence of biostasis but not
13 severe cholestatic liver failure.

14 So just two limitations in the Macaques,
15 one of which is the most important. It's really hard
16 to evaluate the impact of disease factors in macaques
17 because they're generally animals that are without any
18 other co-morbid conditions. The other thing that I
19 thought about is that when an animal, a macaque, gets
20 sick early what we are required to do for animal

1 welfare considerations is to euthanize the animals.
2 And it would be interesting if the animals received the
3 same kind of supportive care -- pressors, assisted
4 ventilation, et cetera, to at least allow for the
5 evolution of these toxicity syndromes.

6 I think I made it through this with only a
7 minute for questions, and thanks for your help in
8 getting this thing rebooted.

9

10 **INVITED SPEAKER PRESENTATION Q&A**

11

12 DR. LISA BUTTERFIELD: Thank you very
13 much, Dr. Wilson. Thanks for persevering. So what
14 we're going to do is shorten the break to five minutes
15 to allow set-up for the OPH period. So, we do five
16 minutes for questions. And so, let's go to our first
17 committee member who's got their hand up, and that's
18 Dr. Zeiss, please.

19 DR. CAROLINE ZEISS: Thank you very much.
20 Dr. Wilson, I wonder if you could speculate on the

1 species differences and the tendency to get this acute
2 (inaudible) hepatic necrosis across dogs, pigs,
3 macaques, and humans?,

4 DR. JAMES WILSON: I think -- so our
5 experience in dogs -- we've worked with Charles Veet
6 (phonetic) on this -- as opposed to virtually any other
7 species is the tropism for AAV vectors across all
8 multiple serotypes is far less. For the
9 biodistribution in dogs our experience is less. We've
10 only done limited experiments with respect to high dose
11 and these are young (inaudible) models. And those
12 animals got very sick because of severe DRG toxicity
13 before we could actually evaluate other parameters.
14 So, each one sort of has its own story. And the other
15 model that you described was what? That you asked me
16 about?

17 DR. CAROLINE ZEISS: Well, on the
18 surface, it would appear that there are greater
19 similarities between macaque and humans. And I wonder
20 if some of that relates to interferon responses which

1 we know are evolutionarily diverse across species?

2 DR. JAMES WILSON: It's a really good
3 question because many experts, with whom I've discussed
4 this, believe that acute inflammation is an important
5 early factor in what subsequently happens. So you may
6 be right there.

7 DR. CAROLINE ZEISS: Thank you.

8 DR. LISA BUTTERFIELD: Thank you very
9 much. And we have also time for a question from Dr.
10 Roos, please.

11 DR. RAYMOND ROOS: I wondered whether
12 these macaques had co-morbidities with respect to the
13 liver, such as endogenous viruses or some injury in the
14 past. And whether that contributes to the phenotype
15 that you've seen once they get AAV?

16 DR. JAMES WILSON: They are captive bred,
17 but they're out-bred and they come to us with a history
18 for sure. I'm not aware of any co-morbidities that
19 we've identified that would impact on this effect.
20 Although what also is seen in macaques, as humans, is

1 there is substantial individual variability. During
2 that first study, one animal had to be euthanized in an
3 effort to (audio skip) resolve the syndrome. But I
4 don't know what I don't know, there. And everyone has
5 endogenous viruses for sure.

6 DR. RAYMOND ROOS: Well some of the non-
7 human primates have, perhaps, many more easily isolated
8 viruses than humans.

9 DR. JAMES WILSON: Mm-hmm. I mean,
10 they're screened for the classic ones that are
11 dangerous, but that doesn't mean there aren't others
12 that would be present that could confound this.

13 DR. RAYMOND ROOS: Thanks.

14 DR. LISA BUTTERFIELD: All right. Thank
15 you very much. I think we have addressed at least the
16 burning questions right after the presentation. So,
17 we're going to take about a six-minute break until five
18 after the hour. At that point, we'll begin the Open
19 Public Hearing and then we'll go on to the panel
20 discussion questions. Thanks again.

1 [BREAK]

2

3

OPEN PUBLIC HEARING

4

5 MR. MICHAEL KAWCZYNSKI: All right, thank you
6 and welcome back from that short little break. Again,
7 thank you for understanding when we had those little
8 technical difficulties with the presentation on Dr.
9 Wilson. Dr. Wilson did a great job adlibbing, and
10 great job to our board members as well. Now we're
11 going to hand it back to Dr. Butterfield as we start
12 with our OPH session. Dr. Butterfield, take it away.

13 DR. LISA BUTTERFIELD: Thank you. This is now
14 the Open Public Hearing for the second session, so this
15 is the same statement that I read before. Just to
16 reiterate that for the public speakers this time.
17 Welcome to the Open Public Hearing session. Please
18 note that both the Food and Drug Administration, FDA,
19 and the public believe in a transparent process for
20 information gathering and decision making. To ensure

1 such transparency at the Open Public Hearing Session of
2 the Advisory Committee Meeting, FDA believes that it's
3 important to understand the context of an individual's
4 presentation.

5 For this reason, FDA encourages you, the Open
6 Public Hearing speaker, at the beginning of your
7 written or oral statement to advise the committee of
8 any financial relationship you may have with the
9 sponsor of the product and, if known, it's direct
10 competitors. For example, this financial information
11 may include the sponsors payment of expenses in
12 connection with your participation at the meeting.
13 Likewise, the FDA encourages you at the beginning of
14 your statement to advise the committee if you do not
15 have any such financial relationships. If you choose
16 not to address this issue of financial relationships at
17 the beginning of your statement, it will not preclude
18 you from speaking. Thank you. Let's begin. Jarrod.

19 MR. JARROD COLLIER: Thank you very much, Dr.
20 Butterfield. For session two we have three OPH

1 speakers. We will start with the first, which is Dr.
2 Kaczmarek from Indiana University School of Medicine.
3 Dr. Kaczmarek?

4 DR. RADEK KACZMAREK: Thank you. Can you
5 please move to my second slide? Thank you.

6 MR. JARROD COLLIER: Your second slide is
7 there.

8 DR. RADEK KACZMAREK: Thank you. Good
9 afternoon again. My name's Radek Kaczmarek, I am
10 honored to present again today on behalf of the
11 National Hemophilia Foundation, Hemophilia Federation
12 of America, World Federation of Hemophilia and the
13 European Hemophilia Consortium. Thank you for this
14 opportunity. Next slide please.

15 As I mentioned earlier today, hemophilia is a
16 deficiency of coagulation factor 8 or factor 9, which
17 results in prolonged bleeding. AAV vectors use for
18 gene therapy of hemophilia failing factor 8 or factor 9
19 transgene's to hepatocytes. Notably, factor 8 is
20 naturally expressed from liver sinusoidal endothelial

1 cells as Dr. George previously mentioned, not from
2 hepatocytes. Next slide please.

3 Several ongoing Phase III programs have shown
4 great potential and generated a lot of interest in the
5 hemophilia community and continue to do so. This
6 community has long-awaited gene therapy as potentially
7 the ultimate cure. However, there are several
8 questions that remain unanswered. One of the issues is
9 the apparent liver toxicity observed in all hemophilia
10 gene therapy trials manifesting with elevated liver
11 transaminases.

12 These elevations are generally considered mild
13 and transient, one to three-fold above the upper limit
14 of normal. In some participants, they went up as high
15 as 10 to 20 times over the upper limit of normal.
16 Then, in some cases, these elevations may persist for
17 months and as long as over a year. Our understanding
18 of the mechanism of these elevations is limited and
19 compounded by inconsistent observations between factor
20 9 and factor 8 gene therapy trials. Next slide please.

1 One common observation is that these
2 elevations occur in both factor 8 and factor 9 trials.
3 They correlate with transgene expression loss and
4 adaptive cellular response, but this correlation is
5 weak in factor 8 trials. These liver enzyme elevations
6 don't always respond well to immuno-suppression. We
7 don't understand that variability and our lack of
8 understanding is somewhat symptomatic of how little, in
9 general, we know about what happens between the moment
10 the vector enters its target cell and the moment that
11 the transgenic protein is released from the cell. It's
12 largely a black box. Next slide please.

13 Between factor 8 and factor 9 gene therapies,
14 these elevations in the underlying liver toxicity may
15 result from one or any number of the many more or less
16 hepatotoxicity issues, including immune response,
17 transgene expression site, which is ectopic in the case
18 of factor 8, overexpression of the transgene leading to
19 the ER stress response, and perhaps vector overloads
20 plays a role too.

1 Understanding these events will likely be
2 essential for finding mitigation strategies. On that
3 point, some of these issues might have been a little
4 better understood already with more data transparency.
5 There's a clear need for timely reporting of animal
6 data, vector quality aspects, such as the proportion
7 empty capsids to full capsids and the presence of
8 absence of adventitious agents, and what has been done
9 to figure out and minimize their potential impact. Two
10 of the three vector manufacturing platforms, currently
11 in use today, may generate adventitious viruses,
12 potentially contaminating the final formulation. We
13 received (inaudible) reported about it. Better
14 transparency will be key as the field moves forward and
15 pursues those outstanding questions. Thank you.

16 MR. JARROD COLLIER: Thank you very much, Dr.
17 Kaczmarek. Now we'll move on to the next OPH speaker
18 who is Dr. Wolfgang Miesbach from University Hospital
19 Frankfurt, Germany. Dr. Miesbach?

20 MR. MICHAEL KAWCZYNSKI: Yes, we want to make

1 sure we have him connected properly, hold on a minute.

2 DR. WOLFGANG MIESBACH: Can you hear me?

3 MR. MICHAEL KAWCZYNSKI: Dr. Miesbach, you
4 there?

5 DR. WOLFGANG MIESBACH: Yes, I can hear you.
6 Now it's okay, can you hear me?

7 MR. MICHAEL KAWCZYNSKI: Yes, we can.

8 DR. WOLFGANG MIESBACH: Okay. Great. I would
9 like to start my presentation about hepatotoxicity in
10 AAV gene therapy trials of hemophilia. With the next
11 slide you can see my disclosures. The next slide
12 please.

13 Now, there is a great variability of liver
14 function of normal ALTs, what they have in common is
15 they present with ALT elevation, mostly mild or
16 moderate. In all cases this is asymptomatic and
17 transient and detected by routine follow-up
18 investigations. It's mostly said to occur the first
19 year of the treatment, mainly the first weeks.
20 Unfortunately, it's not predictable and as a problem

1 this is asymptomatic. ALT elevation is, let's say, it
2 may lead to reduced factor expression.

3 What we know from the Phase I to the Phase III
4 studies so far, it seems to be dose dependent and it
5 may occur more often after gene therapy in patients
6 with hemophilia A than hemophilia B, but it's treatable
7 and manageable with immunosuppressive agents.

8 There is a bit of variability. In the next
9 slide you can see there's a bit of variability of the
10 different treatment options throughout the trials.
11 Most of immunosuppressants alone is used either on
12 demand or prophylactically, but also other
13 immunosuppressive agents may play a role. Next slide
14 please.

15 The pathogenesis of this ALT elevation is
16 still unclear, and it may differ from patient-to-
17 patient. Of course, there are some mechanisms, at
18 least found from (audio skip) clinical investigations.
19 It may depend on an innate or adaptive immune response,
20 including cytotoxic T-cell responses. Remarkably in

1 several studies, liver function abnormalities occurred
2 also without (inaudible) T-cell response. But the
3 problem is the (inaudible) assay is not very well
4 standardized and has several limitations. Finally, the
5 translation of the transgene delivered via AAV gene
6 therapy can induce stress of the endoplasmic reticulum.

7 Now, with the next slide, you can see that
8 more insight is suspected from liver biopsies.

9 Recently, liver biopsy material from humans, treated
10 with AAV vectors, has been obtained and evaluated for
11 the present and (inaudible) form of AAV persistence.
12 But it also could be shown that in at least two
13 patients had biopsies between 201 and 140 after
14 administration of gene therapy. And even after this
15 effect, that one participant has been treated with
16 Prednisolone because of ALT elevation, the patient only
17 showed mild signs of steatosis and no persistent liver
18 inflammation or liver damage.

19 With the next slide we can see the (inaudible)
20 and histopathological findings of hemophilic dogs,

1 which has been published recently. They lived for over
2 10 years and mainly this was focused on integration
3 analysis. But again, it was shown that in these dogs
4 there was no altered liver function, no liver toxicity
5 and also, of course, no clonal expansion.

6 The next slide shows the longest follow-up
7 trial after intravenous gene therapy. Even after one
8 decade or even after 10, 12 to 15 years of follow-up,
9 there was no major safety outcome or no liver problems.
10 This has led me to summarize that liver-directed AAV
11 gene therapy can lead to trans-inhibitor toxicity,
12 which is indicated by ALT elevation. And this occurs
13 throughout all trials in hemophilia A and hemophilia B.
14 So far, all cases are manageable and immunosuppressants
15 are effective in limiting hepatocellular toxicity and
16 preserving transgene factor expression.

17 It is very much important to note that sets
18 the study criteria and very close follow-up are
19 effective in detecting ALT elevation as early as
20 possible to start immunosuppression as early as

1 possible. It is also very important that the strict
2 inclusion/exclusion criteria are followed. Especially
3 it would be important that no patient with uncontrolled
4 liver disease should be included into these trials.
5 These patients are clearly excluded. With this, I
6 would like to thank you for your attention.

7 MR. JARROD COLLIER: Thank you very much, Dr.
8 Miesbach. Our last OPH speaker for session two will be
9 Dr. Whiteley, who represents Pfizer. Dr. Whiteley?

10 DR. LARRY WHITELEY: Can you hear me?

11 MR. JARROD COLLIER: Yes, we can hear you.

12 DR. LARRY WHITELEY: This is Larry Whiteley,
13 I'm with the Pfizer Company, and let's go to the first,
14 next slide. Next slide. All right, so I want to make
15 the point since we're talking about liver toxicity that
16 we distinguish between the acute and severe liver
17 toxicity as discussed by Dr. Wilson, and the more
18 delayed toxicities that we're observing in people. I
19 think that's -- in our view they're two different
20 mechanisms. And I'll present some data here on the

1 acute liver toxicity in monkeys. We initiated a study
2 using an analogous vector to the ones used by Dr.
3 Wilson to assess whether we could see the same thing in
4 a cynomolgus monkey, since his work was done in rhesus
5 and cynomolgus is the standard non-human primate
6 species that we typically use for non-clinical safety
7 assessment.

8 The bottom line conclusion was that we were
9 able to reproduce the acute liver toxicity in
10 cynomolgus monkey, and in our experience with this, it
11 only takes a few animals to identify this potential
12 risk for a given gene therapy. Then, to further
13 emphasize the distinction between liver toxicity, or
14 elevations to the liver enzymes that we're seeing in
15 our hemophilia trials that are thought to be cell
16 mediated they typically occur several weeks to months
17 after dosing. They're not predicted by the non-
18 clinical studies that have supported the development of
19 these. In most cases, particularly for hemophilia B,
20 they are managed well by steroid intervention.

1 There is some indication that factor 7 gene
2 therapies are different from hemophilia B. With factor
3 7 you're seeing these low-grade, prolonged elevations
4 in liver enzymes that maybe is not necessarily
5 associated with a cell-mediated response but might be
6 related to an ER stress response related to the factor
7 7 production. Next slide.

8 The next slide is data from the cynomolgus
9 monkey study that we conducted to assess comparability
10 with the syndrome described by Dr. Wilson. In this
11 study, we dosed animals at 2 or 5e to 13th or 1e to the
12 14th. We did see the fulminant hepatotoxicity and we
13 saw one animal that was found dead at day three in the
14 mid-dose group. I think one of the key differences
15 that we're seeing than what Dr. Wilson discussed, was
16 we're not seeing any evidence of coagulation fibrin
17 deposition within the liver. We do have decrease in
18 platelets, but everything appears to be happening at
19 the same time that we're seeing severe liver toxicity,
20 as evidenced by the increase in liver enzymes.

1 We're also seeing complement activation, but
2 that also occurs concurrently with the elevations in
3 liver enzymes. And the lack of evidence of
4 intervascular coagulation on histology, and the lack of
5 cellular infiltrate, really points to a direct
6 mechanism of hepatocellular injury when we see this.
7 In our experience, we can see various degrees of this
8 early increase in liver enzymes that are asymptomatic.
9 Sometimes they will be symptomatic and animals have to
10 be euthanatized. Also, in our experience, steroids
11 have not been effective at mitigating this acute liver
12 enzyme increase that we see within the first few days
13 post-dose. That's it.

14 MR. JARROD COLLIER: Okay, thank you very
15 much, Dr. Whiteley. Thank you all OPH speakers for
16 this session. This concludes the OPH speakers for
17 session two. At this point I will now turn it over to
18 Dr. Butterfield.

19

1 **COMMITTEE DISCUSSION OF QUESTIONS**

2

3 DR. LISA BUTTERFIELD: All right, thank you
4 very much, Jarrod, and for all of the speakers in the
5 Open Public Hearing section. What we have now is the
6 opportunity for the committee to have a discussion on
7 the five key questions for this section of our two-day
8 meeting. This is session two, hepatotoxicity, and so
9 we'll follow the same format. This time we have not
10 four, but five questions. We also have about an hour
11 and a half to discuss them. So, we'll go in turn.
12 I'll read the questions, I'll look forward to the
13 committee and our guests discussion of these, and then
14 I'll do a quick sum up and then we'll move to the next
15 one.

16 With that, our first question is, please
17 discuss the merits and limitations of animal studies to
18 characterize the risk of hepatotoxicity and provide
19 recommendations on preclinical study design elements
20 such as animal species, disease models and in-life and

1 post-mortem assessments. A lot to address there. Our
2 discussant for this section is Dr. Theo Heller. Let me
3 start with Dr. Heller and then we will go to the
4 committee members.

5 DR. THEO HELLER: Perhaps I can say one or two
6 things in general first. I think that a lot of the
7 studies have missed opportunities to involve
8 hepatologists early on, particularly for
9 hepatotoxicity, such a common side effect of this
10 therapy. I would say it's a missed opportunity to look
11 at mechanism in humans, maybe the most authentic
12 mechanism, not a model. I would encourage early
13 engagement of hepatologists in the design of these
14 studies.

15 The second thing I would say is that there are
16 two approaches, I think, to toxicity. The first is the
17 acute severe toxicity that we've been talking about.
18 Then the second is the longer-term. What's interesting
19 for me is that both the SMA and the hemophilic biopsies
20 showed steatosis, the depletion of glutathione, the ER

1 stress, that all goes together. The question was
2 raised earlier, would that lead to longer-term toxicity
3 in and of itself? In other words, could patients
4 develop fibrosis, could we see long-term more
5 significant damage over -- remember, people take 20-30
6 years to get cirrhosis due to prior hepatitis or
7 alcohol or things like that. But could we see very
8 delayed side effects like that?

9 I would also put in a word of caution about
10 immunosuppression. There is decreased liver enzymes
11 even in hepatitis B, but the hepatitis B doesn't get
12 better, the fibrosis actually accelerates. We have to
13 understand whether we're really treating the underlying
14 disease by giving immunosuppression, or whether we're
15 doing something cosmetic and we're going to be
16 surprised later. I think that also goes to more
17 careful studies. A lot of this could be looked at in
18 animals.

19 The first point of merits and limitations of
20 animal studies is that we need to understand both. I

1 think we've heard very good data about the more acute,
2 severe hepatitis. But the longer, later toxicities, I
3 think, will become important. There are elements of
4 the immune system, previous endogenous exposure to
5 adenoviruses, preexisting liver disease, are they
6 intrinsic to the disease itself, like in the myopathy
7 that we heard of? Or unrelated to the disease but say
8 for example we heard about NASH in mice, that's very
9 relevant to NASH in humans. That's increasingly common
10 across many populations.

11 The real Holy Grail is animal models that will
12 mirror human toxicity in that sense. Also, interesting
13 that the immune response doesn't -- the adaptative
14 immune response doesn't always have a tight correlation
15 with the ALT. I would recommend that we think
16 carefully about preclinical models and separate out the
17 problems related to vector, and then problems related
18 to disease and bear a lot of these in mind. I would
19 strongly recommend liver biopsy and maybe bringing in
20 things like transcriptomics and to look at more

1 sophisticated methods of understanding what's happening
2 in the liver in these patients -- in the animal models
3 as we move forward. Thank you.

4 DR. LISA BUTTERFIELD: Thank you so much, Dr.
5 Heller, for initiating the conversation. Let's move to
6 some conversational points and discussion from the
7 committee members. Dr. Berns, Dr. Ken Berns. We have
8 two Berns and a Byrne, so trying to differentiate.
9 There you are.

10 DR. KENNETH BERNS: Okay. I really worry
11 about the animal model studies as really good models
12 for what to expect when we start treating patients. I
13 think that's really been brought out in terms of the
14 various therapies for hemophilia. There are obviously
15 toxicities, but they weren't apparent, interestingly
16 enough, originally from the animal models. It wasn't
17 until the patients were actually put into clinical
18 trials that some of the real problems became evident.

19 The concept of the animal model is extremely
20 appealing. The problem is I find it very difficult to

1 know when an animal model is really a model for human
2 therapy. I'm kind of limited on this. We have
3 hepatotoxicity here, we're going to talk about the
4 oncogenicity. I think that the toxicity, we're
5 probably getting some better idea on that finally.
6 Maybe it's more informed by the kinds of experiments
7 that Jim talked about with the non-human primates.

8 I really reserve (audio skip) for going all
9 out on the animal models because I don't know quite
10 what it tells us. I mean, anytime we start treating a
11 patient, I would hope that we are monitoring that
12 patient long-term. Just in terms of following the
13 patient in the clinical sense. That could easily
14 include a lot of things we're talking about in terms of
15 looking for these toxicities. So, I'm up in the air at
16 the moment in terms of this question. Not that we're
17 not going to use animal models, I don't have any
18 question about that.

19 DR. LISA BUTTERFIELD: Thank you very much,
20 Dr. Bern. Dr. Zeiss.

1 DR. CAROLINE ZEISS: So, concerning the acute
2 toxicity in primates, we don't see quite the same thing
3 in people. However, we don't know what the mechanism
4 is in primates. We don't know if it's an immunity,
5 complement activation, whether it's direct toxicity.
6 And because we haven't seen that yet in people, it
7 doesn't mean we won't see that. And we need just one
8 death of that kind of nature to derail the whole field
9 for several years.

10 So, I think they are worth studying and I
11 think that they are worth keeping alive, potentially
12 through long-term ICU-type treatment. There certainly
13 are examples of animals being sedated and intubated for
14 a week, that would allow you to follow some of this
15 pathology in a clinical course and do field biopsies
16 before euthanizing the animals. Or even seeing if they
17 recover.

18 I think that you could learn a lot about the
19 innate immune response or complement activation. It
20 may be worth treating some of these animals with

1 complement inhibitors and seeing if that either
2 prevents disease or helps. I think the animals could
3 be well-used to look at DRG toxicity at the same time,
4 because both are issues. That may combine the use of
5 one animal to look at a number of things.

6 I think this would be a long, uncomfortable
7 discussion with the IACUC, but I think that it
8 potentially is justified to try and get to the root of
9 the mechanism of the acute toxicity. I think ignoring
10 it could be problematic down the line.

11 DR. LISA BUTTERFIELD: Thank you very much.
12 Dr. Vite.

13 DR. CHARLES VITE: I would tend to agree very
14 much with what Dr. Zeiss and Dr. Berns said. One of
15 the questions -- or two of the questions -- that I have
16 is, what do you do if there's no decrease in the ALT in
17 some of these prednisone-treated animals or treated
18 people? How do you respond to that class of patient
19 who is having continued elevations in transaminase
20 level, and has no response to the prednisone? That was

1 brought up in a number of cases that it wasn't always
2 effective.

3 The idea of switching to something like
4 tacrolimus or switching to (inaudible) has its own
5 level of basic medical care without a reasoning of the
6 mechanism behind what's going on. I think there is
7 still very useful work to be done here, and as Dr.
8 Zeiss said, all you need is a small group of patients
9 that you don't understand who are then not responding
10 to therapy and end up in a spiral, in this chronic
11 instances, that that will be a problem.

12 I think it also brings up, responding to what
13 Dr. Bern said, in the few cases where there are
14 naturally occurring animal models, I think the idea of
15 using those models to understand why cholestasis might
16 develop in a chronic case, why transaminase levels and
17 hepatotoxicity in terms of cell death might occur. I
18 think there is a great use for the naturally occurring
19 models. Again, it requires, it sounds like from the
20 human studies, long-term therapies and long-term

1 studies to try to evaluate them.

2 I believe there is still some merit for long-
3 term studies in these animals. One of the things to
4 throw out there is potentially long-term studies of
5 animals that have potentially no immunosuppressants put
6 on them to see what develops. And to try to understand
7 more of the mechanism in the chronic cases, as well as
8 trying to understand whether there's a potentially
9 better therapy than throwing prednisone at them. I
10 think those are the main topics I just wanted to bring
11 up.

12 DR. LISA BUTTERFIELD: Thank you. Dr. Barry
13 Byrne please.

14 DR. BARRY BYRNE: Hi there. Thanks for going
15 through this extensively, Jim, in your presentation.
16 It raises several important points. And they're
17 relevant to this decision about what animal models and
18 what to measure. It should be emphasized it's really -
19 - actually was excellent to do the early time points in
20 this study. It's difficult to do in primates. It's

1 much easier, ironically, to do those early studies with
2 indwelling catheters in humans than it is in primates.
3 So many clinical studies miss those early time points,
4 acute time points, after dosing for these critical
5 parameters.

6 One of my questions is, was the cross-
7 switching studied in the primates to confirm these were
8 in fact, you know, probably selected for their
9 seronegative status. But until one measures the IGM to
10 IGG change you can't confirm that they were truly
11 naïve. The IGM component seems to be one of the key
12 parts towards activating a complement on day three or
13 day four. I don't know if you can comment or if anyone
14 else in the committee is aware?

15 DR. LISA BUTTERFIELD: Do we still have Dr.
16 Wilson? Do you want to address that?

17 DR. JAMES WILSON: Yeah, I'm here. Can you
18 hear me?

19 DR. LISA BUTTERFIELD: Yes. Yeah.

20 DR. BARRY BYRNE: Yeah. Yeah, we can hear.

1 Yeah.

2 DR. JAMES WILSON: Yeah, Barry, what was the
3 question again? Whether they were --

4 DR. BARRY BYRNE: The question is whether
5 there was evidence for greater IGM response early on in
6 that early time point that may have contributed to
7 complement fixation in the animals, in both the early
8 studies and the ones that were done with the different
9 quality characteristics of the vector product?

10 DR. JAMES WILSON: Yeah. Yeah, we screen them
11 all for neutralizing antibodies to be negative. But
12 they weren't stratified according to binding antibodies
13 because almost all (audio skip) have some level of
14 binding antibodies. So another variable could be the
15 level of binding. I know that's something that the
16 field is considering. And that would be another way to
17 kind of take that experiment to the next level.
18 Because it may be binding that could activate
19 complement, not necessarily neutralization.

20 DR. BARRY BYRNE: Yeah, correct. Because the

1 neutralizing assay doesn't differentiate IGM from IGG,
2 you know, so that probably is a critical point. Really
3 important to look at those early time points, so I do
4 appreciate that. That's an important aspect to
5 (inaudible) these primate studies. It adds to the
6 difficulty of doing them carefully.

7 DR. JAMES WILSON: Yep. Good suggestion,
8 okay.

9 DR. BARRY BYRNE: I would say too, I have
10 another point about what should be measured, and it's
11 really critical to know what to measure at what time.
12 But we focused a lot on transaminases and sometimes
13 there aren't concurrent GGT measurements, or GLEH
14 (phonetic) that help confirm that those evidence of
15 cellular injury are specifically from hepatocytes. So
16 it's probably going to be important also to measure CK
17 and troponin at these times points because the membrane
18 attack complex can damage muscle in the same way it
19 could damage liver.

20 I think it was correctly stated you really

1 have to think if these are the acute toxicities that
2 occur within the first five to seven days, and those
3 chronic toxicities, they are more related to cell-
4 mediated immunity. The early time points are clearly
5 all innate responses that actually amplify the adaptive
6 response that occurs later.

7 DR. LISA BUTTERFIELD: Great, thank you very
8 much. Then we have Dr. Venditti next.

9 DR. CHARLES VENDITTI: Yeah. Wonderful
10 presentations. I was wondering if the experiment of
11 giving a huge capsid load of either AAV 8 to PHP.B, at
12 least, something that someone did maybe worth
13 considering, in the primate studies, which are fairly
14 extensive, to see how much of this is a capsid-mediated
15 phenomenon?

16 Perhaps that was done, I'm not an expert on
17 the primate studies. I just wondered if somebody could
18 comment on that? Maybe Dr. Wilson, if you guys did
19 that study. What was seen? Did those animals all get
20 very, very sick or did they just have an immune

1 response to that?

2 DR. JAMES WILSON: Yeah. Yeah, this is Jim,
3 can you hear me?

4 DR. CHARLES VENDITTI: Yeah. Yeah. Yeah.
5 Yeah.

6 DR. LISA BUTTERFIELD: If you could turn your
7 camera on as well, Dr. Wilson, that would be great.

8 DR. JAMES WILSON: Oh, sorry. Okay.

9 MR. MICHAEL KAWCZYNSKI: You can talk while it
10 comes up, there it comes.

11 DR. JAMES WILSON: Okay. Got it. All right.
12 We have not isolated just capsid and done the same
13 experiment. That has not been done. It's a little
14 hard to quantify and to compare apples to apples since
15 we quantify the capsid by genomes. But another way to
16 look at that could be we don't enrich for fulls and you
17 evaluate a dose that has a lot of empties versus one
18 that doesn't. Because that's driving a lot of the
19 product (inaudible) and that would be a good
20 experiment, it wouldn't be that hard to do.

1 DR. CHARLES VENDITTI: Thank you.

2 DR. LISA BUTTERFIELD: Great. Thanks very
3 much for that discussion. If there are no other new
4 points, let me try to summarize this for question one.
5 In terms of merits and limitations of animal studies
6 for the risk of hepatotoxicity, the reviews, as always
7 with animal models, was mixed. But there were several
8 on the committee that felt that animal models were
9 better already for acute rather than long-term toxicity
10 analyses. And that animals models could be better
11 utilized for those long-term studies. Following them
12 longer, later in the disease or toxicity state, longer
13 into their treatment, and that that could be an
14 opportunity to learn more about mechanism for both
15 acute and the more long-term effects.

16 There was a call for earlier engagement of
17 hepatologists to look more in-depth in the human
18 patients, to really learn as much as possible from
19 their perspective in the human patients. Then, in
20 terms of additional assessments, the immune studies

1 that have been done to date could be built upon, given
2 the complexity of the immune response and the number of
3 things that there could be an immune response to. So,
4 class switching, for example, could also be examined.
5 Then additional assessments could include some muscle
6 toxicity instead of focusing more exclusively on liver
7 toxicity measures. Is there anything else to add to
8 that? I see hands up from Dr. Venditti. No, okay, Dr.
9 Kenneth Berns, did you want to add to that?

10 DR. KENNETH BERNS: Yeah, I wanted to raise a
11 question about what I've heard. Most of the
12 conversation we've had right now has had to do with
13 non-human primates and the kinds of studies that Dr.
14 Wilson was talking about. So the question is much
15 vaguer than that. And so, I think one of the issues is
16 to what extent. Are we talking about all kinds of
17 animal models, or are there better animal models? I
18 think that it might be really useful if we were to
19 define somewhat more, or suggest somewhat better, which
20 kinds of animal models we think would be more useful.

1 DR. LISA BUTTERFIELD: Great, thank you. I'm
2 not sure if someone has a quick response to that or
3 perhaps that can be also further developed in the other
4 sections. Dr. Vite?

5 DR. CHARLES VITE: In response to Dr. Berns'
6 comment, I think that it's a wonderful opportunity with
7 the hemophilia dogs. That's been a wonderful
8 opportunity to study and to compare to humans. I think
9 in many of the liposomal storage diseases there's also
10 a large cadre of affected dogs that can be used and
11 studied and compared chronically to long-term cases. I
12 think those are particularly excellent because they
13 tend to be phenotypically so similar to the human
14 diseases because they have the same -- we discovered
15 them by having the same phenotype.

16 I think Dr. Wilson's comments on differences
17 between dogs and between people is very relevant. But I
18 think that in the few instances where we're lucky
19 enough and fortunate enough to have natural occurring
20 models in large animals, such as in the hemophilic

1 dogs, such as the muscular dystrophy dogs, such as in
2 many of the lysosomal storage diseases. I think it's
3 possible to get at these questions because the science
4 is so much more advanced in understanding physiology in
5 dogs, for example, than it is in pigs.

6 I think those would be the ones that would be
7 predominantly the most useful for comparison. And I
8 think letting people know that those are available, and
9 studies can be completed in a lifelong history of a dog
10 in 10 years, I think have their own merit in this field
11 of models that could be used.

12 DR. LISA BUTTERFIELD: Thanks so much. All
13 right. Let's move to our second of five questions for
14 this session two on hepatotoxicity. That is, how
15 should patients be screened and categorized based on
16 their risk for developing liver injury before AAV
17 vector administration? Please discuss whether pre-
18 existing hepatic conditions may predict for the risk of
19 serious liver injury. Let's begin with Dr. Heller
20 please as discussant.

1 DR. THEO HELLER: I think this is a very
2 important question. I think this is where careful
3 evaluation is essential, and not to just rely on liver
4 enzymes, but on a more formal assessment. Fibrin scan
5 was mentioned earlier, non-typical liver enzymes were
6 mentioned. I think that the differences in toxicity
7 between the different diseases that we heard about
8 earlier most likely relate to underlying liver disease.
9 I think patients should be very carefully screened and
10 categorized. The concepts in hemophilia of not
11 enrolling people with fibrosis -- with bridging
12 fibrosis or greater -- is something that could be
13 emulated in other diseases.

14 I think that we have strong suggestions that
15 pre-existing hepatic conditions may predict the risk of
16 serious liver injury in multiple human models, which
17 would make this even more important to do carefully and
18 not to just rely on fluctuating enzymes or bilirubin,
19 and then enroll patients when they're normal. I'll
20 stop there.

1 Oh, I wanted to add one other thing.
2 Previously, the question of prednisone was raised and
3 then patients who don't respond to prednisone. I think
4 this is an important category, and thinking about it,
5 because those are the patients who have non-immune-
6 mediated injury and that's an opportunity to tease
7 apart different mechanisms.

8 DR. LISA BUTTERFIELD: Great, thank you. All
9 right, that starts us off. Dr. Roos.

10 DR. RAYMOND ROOS: I think the idea that pre-
11 existing liver problems set the stage, perhaps, for
12 contributing to AAV hepatotoxicity is important. When
13 it comes to investigations of animals, it might be
14 valuable to not only use the AAV on the animal, but
15 also perhaps in addition, stress the animal a little
16 bit. For that reason it might be valuable to look at
17 more than one animal species. It'd be nice to have
18 non-human primates, although they may have some
19 toxicities different from mice that are bred and kept
20 in very standard conditions.

1 On the other hand, working with non-human
2 primates is somewhat limited, whereas mice one could
3 look more broadly, different dosages, different liver
4 injuries. So, having more than one species is
5 valuable. There's always problems and limitations in
6 animal studies. For example, integration in the mouse,
7 in this particular locus, may be an issue because that
8 locus doesn't exist in humans. So, having more than
9 one species is good, and having species in which one
10 could do multiple experiments that aren't too
11 expensive, is valuable.

12 DR. LISA BUTTERFIELD: Thank you. Dr. Barry
13 Byrne please, you're next. We can't hear you yet.

14 MR. MICHAEL KAWCZYNSKI: Do you have your
15 phone muted, sir?

16 DR. BARRY BYRNE: Sorry about that. Sorry
17 about that.

18 MR. MICHAEL KAWCZYNSKI: There you go.

19 DR. BARRY BYRNE: I'll just comment from
20 questions that were raised during Lindsey George's

1 presentation regarding the MTM study that's pertinent
2 to this question. In that program, patients were
3 excluded for AST/ALT greater than five times the upper
4 limit of normal, and they did have liver ultrasounds to
5 exclude hepatic (inaudible). Recently the exclusion
6 criteria have been changed to exclude those with any
7 clinically significant underlying liver disease.
8 Ofcourse that's partly in the opinion of the
9 investigator and their consultants, if they involve
10 them at screening. I think many studies do take this
11 into account now because of the label for monitoring
12 and evaluation of liver toxicities in (inaudible) use.

13 My comments are just around -- you know, the
14 typical laboratories with the expanded panel using GLDH
15 and GGT and going with them as part of screening
16 measures. It's hard to do liver ultrasounds in those
17 conditions where there's no prior history of liver
18 disease, or where there's an underlying abnormality,
19 like in other muscular dystrophies where the
20 transaminases come from muscle so the baseline is high

1 for treatment. That's why that becomes a bit more
2 challenging to (inaudible) exclusion criteria. I think
3 most sponsors are considering the appropriate
4 laboratory assessments prior to inclusion in the study.

5 DR. LISA BUTTERFIELD: Thank you. Dr. Fox.

6 DR. BERNARD FOX: Thank you, Dr. Butterfield.
7 I was kind of struck by what Dr. Zeiss had said and Dr.
8 Wilson's response about the antibody tests and the fact
9 that it was a viral neutralization test. I'm a little
10 bit naïve in terms of what screening is done for
11 patients that are enrolled in these trials. But
12 thinking about what titers or what types of antibodies
13 might be there that might be a complement, are these
14 all viral neutralization tasks? I wonder if somebody
15 can respond to that. And whether or not there are some
16 classes of immunoglobulins that are characterized in
17 patients that enroll in these trials for potential
18 complement fixation?

19 DR. LISA BUTTERFIELD: Thank you. Does
20 someone want to jump in to specifically address that

1 before we move on?

2 DR. BARRY BYRNE: Sure. I can comment. This
3 also pertains to the question that came up during the
4 first invited speaker. The inclusion criteria for
5 commercial Zolgensma, for example, requires a total
6 binding antibody to the left and -- can you hear me
7 now?

8 DR. LISA BUTTERFIELD: Yes.

9 DR. LINDSEY GEORGE: I can jump in if Dr.
10 Byrnes is having connection issues.

11 DR. BARRY BYRNE: Yeah, I don't know if you
12 can hear me, but the webcam won't come on. Can you
13 hear me all right?

14 DR. LISA BUTTERFIELD: Actually, we're having
15 Dr. George jump in to specifically address Dr. Fox's
16 question.

17 DR. BARRY BYRNE: Okay.

18 DR. LISA BUTTERFIELD: Then we'll carry on.

19 DR. BARRY BYRNE: Okay.

20 DR. LISA BUTTERFIELD: Thank you, Dr. George.

1 DR. LINDSEY GEORGE: Okay. I think what Dr.
2 Byrne was going to say is that Zolgen- -- neutralizing
3 antibody threshold, I believe it's 1 to 50, is the
4 cutoff. I think that not completely universally, but
5 nearly universally, people are using AAV neutralizing
6 antibodies as the threshold. These are typically
7 transduction-based assays or, in some cases, ELISA-
8 based, but it's typically transduction-based assays.
9 There's variable thresholds people are using.

10 Many are using no detectible neutralizing
11 antibodies, some are using less than 1 to 5, some are
12 using less than 1 to 50, et cetera. Then, of course,
13 there's no uniformity to how these tests are done, so
14 they're not comparable across cohorts. To my
15 knowledge, people aren't using anything beyond that as
16 any additional layers to look at pre-existing AAV
17 immunity for enrollment criteria.

18 DR. LISA BUTTERFIELD: Thank you.

19 DR. BARRY BYRNE: Yeah, I can answer that.
20 It's actually -- the screening test for Zolgensma is

1 actually a total binding antibody assay. Just to put
2 it in context, that 1 to 50 level is about the lower
3 limit of detection and, in fact, post-exposure, the
4 antibody levels go to the millions. Just to keep that
5 in mind.

6 DR. LISA BUTTERFIELD: Great, thank you. All
7 right, let's go back to our list. Dr. Crombez please.

8 DR. ERIC CROMBEZ: Great, thank you. I agree
9 with the opening comments on ALT and AFT and their
10 limitations. I think while it may be good to monitor
11 for patients post-dose, I think with inclusion and
12 exclusion criteria you do need to go far beyond that.
13 I can't claim to have seen all inclusion and exclusion
14 criteria for active studies or studies that have been
15 completed, but I do think most of them do exclude any
16 patient with active liver disease or long-standing
17 hepatic disorders. I think it is a fairly aligned view
18 that the underlying liver disease associated with their
19 primary disease in those MTM patients did contribute to
20 the outcome there.

1 DR. LISA BUTTERFIELD: Thank you. All right,
2 don't forget to put down your hand after you've spoken.
3 Next, we have Dr. George and then Dr. Venditti and then
4 I'll sum up.

5 DR. LINDSEY GEORGE: Great, thanks. I want to
6 make two comments of the same vein. The first is with
7 respect to ascribing the observations in the XLMTM
8 (inaudible) myopathy due to some kind of pre-existing
9 liver disease, presumably that could be a contributor,
10 but I think it's important to acknowledge that -- you
11 know, maybe there are some studies that have documented
12 this as part of natural history, but in several of the
13 pre-existing, reasonably small cohort natural history
14 data, that hasn't been an overwhelming feature of the
15 disease, although it certainly could be part of it.

16 I think there should be a little bit of
17 caution in saying that this was observed because these
18 patients had pre-existing liver disease. Certainly,
19 that could be the case, but it clearly wasn't
20 overwhelming liver disease because they were able to be

1 enrolled in the trial and many of them had normal
2 values at enrollment. I think it's a little tough to
3 say, oh, they had pre-existing liver disease and that's
4 what the problem -- to completely ascribe it to that.

5 I just want to state the obvious that might be
6 worthy of consideration, which is that significant
7 hepatotoxicity hasn't been observed at doses less than
8 $1e14$ vector genomes per kilogram. And so maybe one of
9 the important points of this is to continually strive
10 for using lower vector doses as part of the clinical
11 development of these programs.

12 DR. LISA BUTTERFIELD: Thank you. Then,
13 finally, Dr. Venditti.

14 DR. CHARLES VENDITTI: Yeah, I was just going
15 to offer comment related to the pre-existing hepatic
16 conditions. Again, from the perspective of inborn
17 errors metabolism, I think there a number of (audio
18 skip) inborn errors metabolism where patients (audio
19 skip) their underlying condition with acute liver
20 failure. And the ones that come to my mind, of course,

1 are the urea cycle disorders at the top of the list.
2 With OTC, having that as part of the presentation in
3 some patients. Then, also, as far as the urea cycle,
4 almost all of them have had some form of chronic liver
5 disease are getting succinic acid urea. Of course, in
6 those patients, they can almost have a primary
7 cirrhotic-like syndrome.

8 One considered capsid choice and the
9 underlying target, and we didn't talk a lot about that
10 yet, whether or not there's certain capsids that are
11 more prone to induce (audio skip) . I don't know if
12 it's a capsid-dependent process, but whether those
13 patient populations need special consideration up
14 front. For example, I think the suggestion of
15 involving hepatologists in these patients is really
16 important. Whether those patients need more beyond
17 just (audio skip) borderline (audio skip).

18 DR. LISA BUTTERFIELD: I'm sorry, you're
19 cutting out.

20 DR. CHARLES VENDITTI: Many patients can have

1 at baseline, abnormal AST/ALT, and that's where they
2 live. That urea cycle disorder and the other ones I
3 think of are glycogen storage is used. I think there
4 has to be special consideration about these patient
5 populations. And whether there's a capsid metabolic
6 disorder interaction that could precipitate an acute
7 liver failure is something that we should probably look
8 out for in the trials. Again, whether or not just,
9 quote/unquote, doing a liver screening in some of these
10 patient populations is enough, or we have to go beyond
11 that to mitigate risk.

12 It didn't come up, but certainly for some
13 groups of patients this could be a really important
14 consideration as we move to the trials, different
15 serotypes and different products. We don't want people
16 to have acute liver failure if there's some, let's just
17 say related to metabolic control, or their oxidative
18 stress balance, which is sort of things that in
19 patients who have nutritional problems can be prone to.

20 I was just going to offer that comment for the

1 minutes because I do think that on the subject of pre-
2 existing hepatic condition, even though it's not
3 fomented in the patient, is a very important
4 consideration when we think about hepatic toxicity in
5 some of the patient populations.

6 DR. LISA BUTTERFIELD: Thank you. I'm going
7 to try to summarize question two, and there's more
8 opportunity to flesh this out in question three. Keep
9 your hands up about other strategies, but in terms of
10 screening and categorizing patients for their risk of
11 (audio dropped).

12 MR. MICHAEL KAWCZYNSKI: We lost your phone.
13 Dr. Butterfield, we lost your connection. Hold on.
14 Dr. Butterfield. Jarrod, you want to jump in here?
15 Hold on. I'm trying to get her attention. There we
16 go, she got it, all right. She's coming back in.

17 MR. JARROD COLLIER: Okay.

18 MR. MICHAEL KAWCZYNSKI: Be right in just a
19 moment. There you go. Okay.

20 DR. LISA BUTTERFIELD: Okay.

1 MR. MICHAEL KAWCZYNSKI: That was good. We
2 didn't hear what you said, but it was good.

3 DR. LISA BUTTERFIELD: That's super. Did you
4 miss the whole sum up?

5 MR. MICHAEL KAWCZYNSKI: A little bit, yes,
6 ma'am. Yes, Dr. Butterfield, we apologize. We were
7 trying to wave at you. All right.

8 DR. LISA BUTTERFIELD: Yeah. My apologies.
9 All right. I'll be brief. Yes, pre-existing hepatic
10 conditions are important and can predict serious liver
11 injury. One such criterion that was mentioned was
12 bridging fibrosis as something that might preclude
13 treatment, but really there are a lot of opinions about
14 what does and doesn't constitute a condition, given the
15 diverse clinical settings in the different disease
16 states that could all lead to different baseline
17 characteristics. There is human data here to learn
18 from. We do need careful screening and we need to
19 focus on more than just fluctuations in bloodwork.

20 Of note, both total antibody and neutralizing

1 antibody measures are performed, but how they're
2 performed, and their cutoffs and acceptance criteria
3 are all variable. So,, there might be some
4 standardization opportunity there.

5 All right. That's the short version. Our
6 next question gives us more time to dig into this. Are
7 there any quick comments here or should we move to the
8 third question? Okay.

9 All right, then the third question is really
10 also thinking about what we should we do before or
11 after. What additional strategies could be implemented
12 before or after AAV vector administration to prevent or
13 mitigate the risk of liver injury? Let's go to Dr.
14 Heller to start us off.

15 DR. THEO HELLER: I think one of the most
16 important things we can do is have careful exclusion
17 criteria until we understand exactly what's going on.
18 I'm not suggesting that the reason for cholestatic
19 injury was the underlying liver disease in the previous
20 discussion, I'm saying that it's a possibility, but it

1 can't be excluded, or the patients weren't adequately
2 evaluated. Now that there are publications saying that
3 cholestatic liver disease is part of the natural
4 history it raises the question.

5 This sort of thing can be answered if careful
6 exclusion criteria are considered. Ultrasound isn't
7 adequate. Liver enzymes aren't adequate. It requires
8 a little bit more than that, in some patients, but not
9 in all patients. I don't want to make it so complex
10 that we can be nihilistic about it and say there's no
11 point in anything. I do think that careful set of
12 exclusion criteria, a careful pre-evaluation, will
13 exclude most of those patents with pre-existing liver
14 disease or at least classify it so that they can be
15 followed carefully.

16 The second thing is that we could standardize
17 post-treatment evaluation. There are simple things
18 that can be done non-invasively with threshold
19 criteria, switching to a liver biopsy or to something
20 more invasive or more frequent monitoring if patients

1 start exhibiting danger signals. But that's not
2 something we would have to establish for every patient,
3 only if patients across the threshold, we could be
4 practical about it.

5 I think that the single most important thing
6 is meticulous delineation of these sort of procedures,
7 and then follow-up. That way we could anticipate most
8 of the injury -- or not just anticipate but find it
9 early before it's advanced. I don't think the things
10 we saw in the animals with the acute and severe failure
11 can be predefined, and that would be a completely
12 separate category.

13 DR. LISA BUTTERFIELD: Great, thank you. All
14 right. Let's go to the committee discussion. Dr.
15 Crombez please.

16 DR. ERIC CROMBEZ: Thank you. I think
17 applicable here, but I think harking back a little bit
18 to what Dr. Venditti was talking about earlier, it's
19 raised a question in my mind. If we need to think
20 about patients with pre-existing liver conditions, at

1 least two different -- maybe more than that -- but at
2 least two different buckets. And using his reference
3 to patients with urea cycle defects, and particularly
4 OTC, where those patients really have elevation in
5 liver function tests only when in metabolic crisis, but
6 when well-managed not really showing those signs and
7 symptoms. So, really almost a secondary part of that
8 disease, and more related to the metabolic
9 decompensation.

10 And really trying to dose those patients when
11 they are clinically stable. And I think that probably
12 applies to a handful of inborn errors of metabolism,
13 versus those diseases who have, I guess, what I'll call
14 primary liver disease, liver signs and symptoms that
15 are really core to their disease. And even if they're
16 not showing elevated biomarkers or other lab tests,
17 they maybe are still predisposed or more at risk with
18 dosing of gene therapy. In my mind, looking at those
19 patient populations a little bit differently.

20 DR. LISA BUTTERFIELD: Thank you. Dr. Heller.

1 DR. THEO HELLER: There was one other thing I
2 neglected to say. I've noticed that many patients get
3 given steroids. I'd like to see the evidence of that.
4 I tried to look through the literature, I didn't see
5 any real evidence-based approach. The assumption then
6 is that everything is immune mediated and adaptive
7 immune mediated.

8 It would be nice to use animal models,
9 perhaps, to tease some of that apart, because I'm not
10 sure that giving steroids to everyone prophylactically
11 really makes a difference. We've seen that despite
12 steroids, some patients have persistent elevation in
13 their enzymes. I want to be careful not to make
14 steroids mandatory or mycophenolate or mTOR inhibitors
15 a standard approach to these patients without
16 understanding more. Thank you.

17 DR. LISA BUTTERFIELD: Does anyone want to
18 comment on the point about steroids?

19 DR. LINDSEY GEORGE: Hi there, I can comment.
20 I wholeheartedly agree. I think that -- I can't speak

1 for all the scientific rationale that's gone into all
2 the clinical protocols, but I think overwhelmingly
3 there's been a kneejerk response that when you see LFT
4 elevations it's a capsid knee response. But clearly --
5 I mean, there's some evidence of that in some of these
6 trials. But then there's clearly diversion from that
7 in other work. So really understanding the etiology of
8 what's going on is probably quite important.

9 Lastly, in some cases if you see they're not
10 working or not making a difference, then also it's not
11 insignificant to have patients on steroids for several
12 months. In some cases, over a year, particularly in
13 the SMA maybe. So no, I think that's a great point.

14 DR. LISA BUTTERFIELD: Thank you. Mr.
15 DeFilippi please. We can't hear you yet.

16 MR. MICHAEL KAWCZYNSKI: You have to unmute
17 your phone, sir. You're unmuted in Adobe, so it's just
18 you have to make sure your phone's unmuted.

19 MR. JAMES DEFILIPPI: (Audio distorted).

20 MR. MICHAEL KAWCZYNSKI: Let's go to the next

1 one, Lisa, it looks like he dropped off.

2 DR. LISA BUTTERFIELD: Okay. We don't have
3 any other comments right now. Any other comments from
4 the committee members? Okay, I'm not seeing any other
5 hands up, so I can try to summarize this question and
6 its discussion while looking up periodically to make
7 sure I don't go quiet. Okay. One more comment from,
8 okay, here we are. First Dr. George and then we'll
9 finish with Mr. DeFilippi, who has called in again.
10 Dr. George.

11 DR. LINDSEY GEORGE: (Audio skip). The
12 toxicity that's been observed has been observed in the
13 higher dose of AAV vector used. It seems like a
14 potential mitigating strategy certainly could be using
15 lower vector doses. I just wanted to state that for
16 the group again.

17 DR. LISA BUTTERFIELD: Great, thanks for
18 reiterating that point. All right. Let's try again,
19 Mr. DeFilippi.

20 MR. JAMES DEFILIPPI: Can you hear me now? My

1 apologies. I wanted to talk quickly, Dr. Heller had
2 brought up a point when he was opening the questions
3 about use of prednisone as kind of like a cosmetic
4 treatment, not necessarily addressing an underlying
5 issue of hepatic toxicity. I think when asked about
6 elevated toxicity levels and then using prednisone for
7 bringing them down, it makes me wonder if we're making
8 a value tradeoff where we're taking away, at least in
9 hemophilia patients, the use of one type of treatment,
10 the clotting factor, and then trading it for another
11 type of treatment. I would say, if we're implementing
12 gene therapy, then maybe we should be also tracking,
13 and really including any secondary treatments that have
14 to be used to manage the implementation of the gene
15 therapy as a consideration and the value of the gene
16 therapy overall.

17 DR. LISA BUTTERFIELD: Thank you. All right.
18 In terms of question three, in terms of additional
19 strategies that could be implemented before or after
20 vector administration to mitigate the risk, there was a

1 further reiteration of the disease specific liver state
2 that this is all -- sometimes it's tied to a genetic
3 disease, sometimes it's tied to other things and needs
4 to be treated in different ways. The workup can be
5 complex, that just ultrasound or just bloodwork may not
6 be enough. And there's a certain amount of
7 individuality about these measures that need to be
8 considered, including biopsy, but again, in patients
9 who are beginning to show signs of toxicity.

10 In terms of strategies, we need to consider
11 the role of vector dose. And that lowering the dose
12 may help mitigate the toxicity. And the immune-
13 mediated toxicity, like the immune system, is also
14 complex and needs additional investigation.

15 Any quick comments about that before we move
16 to question four? Dr. George, your hand is up. Is
17 that a new comment or just the hand still being up?

18 DR. LINDSEY GEORGE: Oh, sorry, I'll take my
19 hand down. That wasn't a new comment. Thank you.

20 DR. LISA BUTTERFIELD: Okay. All right. Then

1 let's move to question four of our five questions for
2 session two. This is, what factors (e.g. level of
3 disease severity) other than weight should be
4 considered to determine the vector dose for systemic
5 administration? We'll start our discussion with Dr.
6 Heller.

7 DR. THEO HELLER: I think this is beyond my
8 expertise. It's not really liver-related, so I'm going
9 to keep my comments short. The only thing I will say
10 is that the site is obviously important. If you're
11 injecting into the eye it's very different than giving
12 it intravenously.

13 DR. LISA BUTTERFIELD: Thank you. Let's hear
14 thoughts from the committee about what other factors,
15 other than weight, should be used to determine dose.
16 Dr. Barry Byrne please.

17 DR. BARRY BYRNE: Hi, Lisa, can you hear me?

18 DR. LISA BUTTERFIELD: I can hear you, I can't
19 see you, but I can hear you.

20 DR. BARRY BYRNE: The camera never went back

1 on, but that's okay. Just to comment about weight-
2 based dosing. Obviously, even in the context of
3 enrichment for full capsids, one can't then go -- as
4 Dr. Wilson showed, there really is a threshold for
5 transduction outside of the liver, which are many of
6 the targets that are being considered. Certainly, in
7 inherited muscle diseases and then some neurological
8 diseases where the dose is given systematically.

9 Just to put it in context, the amount of
10 protein associated with those full vector genomes is
11 between 2 and 200 milligrams in a typical dose from an
12 infant to a school age child. And could be even more
13 in adult patients unless, as was stated by someone
14 earlier, that you consider the agile body weight or the
15 lean body weight and lean muscle mass.

16 So, it's hard to go below those levels when
17 considering what the antigenic burden is of the capsid,
18 and still have a therapeutic benefit. You can't delink
19 the two because you obviously have to deliver the
20 transgene that generates the active product, which is

1 the therapeutic protein. That's just something to
2 consider. That is between 10 and 100 times more than a
3 typical vaccine. So, there are always going to be
4 immune responses associated with this type of therapy.

5 DR. LISA BUTTERFIELD: Thank you. Dr.
6 Venditti.

7 DR. CHARLES VENDITTI: Yeah, I just had to
8 comment that, again, in the pediatric population,
9 particularly in those patients, many that have
10 neurologic conditions or metabolic disorders, have
11 grossly abnormal BMIs. It's something that goes beyond
12 just measuring height and weight. Again, in the NIH,
13 in the intermural program, we're privileged to be able
14 to (audio skip) patients. Whether or not, in some
15 populations, if there is an aberrant BMI, it would be a
16 consideration to at least perform a DEXA scan to get an
17 idea about how much fat somebody has, what's their lean
18 muscle mass, how dense their bones are. These are
19 things you can get from a DEXA scan. There's even more
20 sophisticated measures you could get depending on the

1 nature of the scan and how you do the analysis.

2 Then, another question -- again, for high dose
3 applications where we're concerned about toxicity,
4 whether or not you can employ volumetrics in the
5 analysis of the patient, something like a whole-body
6 MRI scan. Again, this is not the sort of thing you
7 would just order on somebody, it'd be part of a
8 research protocol. But then you could more precisely
9 determine, for any given patient, (audio skip)
10 composition. But sort of, how big is their liver,
11 what's the volume of it, how about their heart, how
12 much muscle mass do they have?

13 (Audio skip). before we -- as part of the
14 clinical characterization of the patients, it might
15 help just to get information for the field as we move
16 forward for the trials. I'm not making any firm
17 recommendation about dosing someone based on their DEXA
18 scan, but it's something that has come up in
19 discussions. And as we think about, at least (audio
20 skip) in my section about patients with abnormal body

1 habits. And there's quite a number of them, as I
2 mentioned, that are excellent patient populations for
3 AAV gene therapy because they don't have any other
4 treatment options. It is something we should think
5 about and try to keep track of, but I wanted to offer
6 that comment to the group.

7 DR. LISA BUTTERFIELD: All right, thank you.
8 Dr. Roos please.

9 DR. RAYMOND ROOS: There's different serotypes
10 of the AAV vectors and different transgenes, so it's
11 hard to generalize about a particular vector dose. I
12 think it's important to do animal work to get some idea
13 about this, but then, ultimately, I think in the
14 clinical trial, looking at dose response would be
15 important. Starting at a low dose and challenging that
16 patient population with increased amounts of AAV
17 vector. Thanks.

18 DR. LISA BUTTERFIELD: Thank you. All right,
19 so I don't see any other raised hands at this time.
20 Let me summarize the discussion about question four,

1 factors other than weight to be considered to determine
2 the vector dose for systemic administration. For
3 administration that is not systemic, obviously the dose
4 would change for intraocular or other locations. There
5 was a recommendation to perhaps factor in the capsid
6 antigen burden relative to the transgene expression as
7 something that factors into dose.

8 Then, one can start with animals, but very
9 carefully follow for dose response in patients during
10 the early toxicity dose escalation studies. Then,
11 noting that BMI abnormalities can exist in a number of
12 the disease states, and so more detailed information
13 gathering about bone, muscle, fat, organ composition
14 and volumetrics could help define doses that could lead
15 to toxicity versus efficacy. Any comments about
16 anything I left out? All right, seeing none, let's
17 move to our fifth and final question for session two on
18 hepatotoxicity.

19 This is a two-part question. Considering the
20 risk of hepatotoxicity observed in clinical trials with

1 high doses of AAV vectors. A) please discuss whether
2 an upper limit should be set for the total vector
3 genome dose per subject, and, given that many AAV
4 products contain significant amounts of empty capsids,
5 please discuss whether an upper limit should be set on
6 the total capsid dose. For this let's again start with
7 Dr. Heller to set the stage.

8 DR. THEO HELLER: Again, this is a little bit
9 outside of my expertise. It's not quite what I do. It
10 makes sense from the discussion today that an upper
11 limit might be reasonable. I would just caution that
12 the upper limits in the one study with the three deaths
13 is maybe affected by underlying liver disease. We
14 don't know the answer to that, but going to humans, it
15 would be prudent to be cautious.

16 DR. LISA BUTTERFIELD: Thank you. All right.
17 Dr. George.

18 DR. LINDSEY GEORGE: The only caveat I would
19 have in this is it might be, if there is a decision or
20 a consensus about an upper limit, it might be more

1 relevant to have it per kilogram or for BMI or whatever
2 body assessment we want to use. Mainly just because
3 the doses that -- and presumably, like Dr. Byrne was
4 saying, there's presumably modifying factors involved
5 in various patient populations. It's important to note
6 that the pediatric subject, there are adult subjects
7 that have gotten -- the dosing is much lower on a --
8 you know log folds lower on a vector genome per
9 kilogram dosing. But if the patient themselves is a
10 log fold higher weight, then the same amount of vector
11 is being infused. You know, that had vector-related
12 toxicities in a pediatric patient but didn't in an
13 adult patient. I think it might be -- limiting to a
14 total overall vector dose might be a little bit
15 difficult, and the ranges of sizes, so to speak, of
16 people receiving AAV vectors.

17 Then, the other point that might be worthy of
18 consideration -- and I don't know if other members have
19 input on this. But it is interesting to me that these
20 toxicities have only been observed in pediatric

1 patients. If there's anything relevant to pediatric
2 hepatic physiology or something of that nature that's
3 relevant here, or perhaps it's the modifier of the dose
4 in the underlying patient population disease. But just
5 to reemphasize the point that marked hepatotoxicity has
6 only been observed in pediatric patients, which of
7 course are in the trials with the highest AAV vector
8 doses. But I am curious if the group has input on the
9 potential role of liver development or the pediatric
10 liver hepatic physiology, and if that somehow
11 contributes to the observed toxicities.

12 DR. LISA BUTTERFIELD: Thank you. Let's move
13 to Dr. Venditti and then, the committee, think about
14 please the last question about pediatric -- anything
15 specific to pediatric versus adult patients.

16 DR. CHARLES VENDITTI: Yeah. As we think
17 about offering comments for this specific question, I
18 do think we have to harken back to the discussion we
19 had a bit earlier today about vector QC and about
20 standards. And about comparing different AAV products

1 for different indications, at different doses, of
2 different serotypes. As we think about tox- (audio
3 skip). For example -- and again, I would defer to my
4 colleagues that are in the manufacturing space. I
5 don't know that it's widely known -- any AAV products
6 that are early phase trials -- the QC sheets, the specs
7 (audio skip) for each thing. In other words, how many
8 empties or full are in that AAV they're giving the
9 patients?

10 If a patient gets a certain dose, and we go
11 back to our example of abnormal body habitus in
12 pediatric patients, maybe they're getting a lot more of
13 an empty product, which there is some suggestion, based
14 on preliminary data that's been presented at least at
15 ASUCT this past year, that those empty capsids,
16 quote/unquote, empty capsids, they may have something
17 in them. They may have contaminants, they may have
18 DNA, they may have plasma remnants. They may have
19 cellular debris. That could be a contaminate.

20 If a patient gets dosed based on the vector

1 genome copy number of the titer -- which is typically
2 how you would titer an AAV, by genome copy -- but that
3 one AAV has a lot of empty capsids. Again, I would be
4 interested to hear from others on the line. What do
5 people think could be in an empty capsid? I don't know
6 that the capsids are zero on the inside. I think
7 there's something inside them, and whether or not they
8 are toxic is a question that I think is not fully
9 resolved.

10 So, it confounds this question of
11 hepatotoxicity and toxic side effects of AAV preps.
12 Because, again, going backwards, we don't have
13 referenced standards from the fields, if we're not
14 doing next-gen sequencing to see what the preps are, if
15 people are using different production methods, if
16 there's differing amounts of empty capsids that are in
17 the preps, and those empty capsids -- for example, as I
18 mentioned, have something in there that could stimulate
19 the innate immune response or that in themselves are
20 hepatotoxic -- how can we make a prediction about a

1 dose? We need to have that real basic information,
2 which again, I look to my colleagues in manufacturing
3 and industry, I don't know that people widely share
4 that information before they have their marketing
5 authorization.

6 I think this is a very hard one. It's also
7 going to be influenced, as we talked about extensively
8 earlier today in this discussion, by the underlying
9 disease state. Whether or not a patient -- whether
10 they are an OGCB (phonetic) patient who's well-
11 controlled but then all of a sudden gets (audio skip)
12 capsid load, is that enough to precipitate an acute
13 liver failure (audio skip) this well-controlled
14 patient?

15 I don't know the answer to that, I'm just
16 throwing it out as a hypothetical. If we change (audio
17 skip) over the different dosing regimen. I think this
18 is a really tough one to provide real clarity and
19 guidance on because of this confounding factor of the
20 biologic complexity problem. I would welcome (audio

1 skip) opinion in the trenches on this, thank you.

2 DR. LISA BUTTERFIELD: Thank you. Dr.

3 Crombez.

4 DR. ERIC CROMBEZ: Yeah, thank you. I agree.

5 I also find the idea of a blanket upper limit on dose
6 very challenging. I mean, I think dose finding is very
7 important and a key part of drug development. While,
8 in some instances it may be reasonable to start at
9 doses below the e14 range, but some targets, such as
10 muscle, may require relatively higher doses. I think
11 this is where benefit/risk, informed consent, specific
12 indications, specific vector becomes very important,
13 and that really, I think, it's best to keep these on a
14 case-by-case and program-by-program basis.

15 DR. LISA BUTTERFIELD: Thank you. Dr. Heller.

16 DR. THEO HELLER: One of the difficult things
17 for me in looking at this question before today was
18 that it was hard to find standardization, and it was
19 hard to find clear evidence that everything had been
20 done in the same way in every study, that when we talk

1 about the upper limit, we're comparing apples to
2 oranges in most places. And then, I agree completely
3 with Dr. Venditti, viruses don't like to assemble
4 around empty spaces, they usually assemble around
5 something. The empty capsids, it varies so much. Then
6 when we talk about injecting in different sites and
7 different underlying diseases, I honestly felt it might
8 be a little bit premature to try and predefine this
9 sort of answer.

10 DR. LISA BUTTERFIELD: Thank you. Dr.
11 Venditti? Okay, Dr. Barry Byrne please. Can't hear
12 you yet.

13 DR. BARRY BYRNE: Just want to reiterate what
14 was just said, that when there's dose finding that
15 establishes a certain level of efficacy, that's
16 anticipated for a clinical study, that it would be
17 unwise to have exposed patients to subtherapeutic doses
18 of vector based on an arbitrary limit set for total
19 capsids. Of course, it's very important to fully
20 characterize the product. I think we have to be

1 careful not to characterize any in-process impurities
2 as contaminants. They're really very, highly
3 characterize products to the best of any sponsors
4 ability to understand both the purity and the potency
5 of the material.

6 But that is going to mean that enrichment for
7 the biologically active particles, and not the empty
8 capsids is going to be important to ongoing
9 development. Right now physical methods are used to do
10 that, and some ion exchange chromatography methods have
11 been established and they'll continue to evolve. But
12 that should not meant that we can pick an arbitrary
13 limit which might impact effectiveness.

14 In one of the areas we've been investigating
15 is the notion of incremental dosing. Where under the
16 cover of a brief period of immune protection, you don't
17 have to expose the patient to what appears to be the
18 upper -- really the MTD has been reached in some of
19 these studies, especially if there are empty capsids in
20 the preparation. So, that's a consideration for some

1 studies. Or where the dose can be split between the
2 compartments where it's needed because that would avoid
3 overexposure in one area or another. That's a
4 consideration, I think, as a way to manage this
5 problem.

6 DR. LISA BUTTERFIELD: Thank you. Dr. Herzog
7 please. Can't hear you yet.

8 MR. MICHAEL KAWCZYNSKI: Make sure it's your
9 own phone, Dr. Herzog. There you go.

10 DR. ROLAND W. HERZOG: Yeah, so one point just
11 to reiterate what was already said, is that we don't
12 have standards for vector titers right now. Ten to the
13 14th of one product made by one company may not be what
14 another in their product calls 10 to the 14th. (Audio
15 skip). Of course, a company (audio skip). But on the
16 other hand, we talked about steroids versus other kind
17 of immune modulation (audio skip) adjunct therapy to
18 reduce toxicities and immunogenicity.

19 I think as the field is learning more about
20 the underlying mechanisms that are causing these

1 toxicities, and finding ways to get around them, a dose
2 of 10 to the 14th or 10 to the 15th per kilogram may
3 not be as problematic anymore. Hopefully, some of the
4 knowledge gained, that will help develop these kind of
5 adjunct therapies that work together with gene therapy
6 to achieve efficacy and reduce toxicity.

7 Of course, it'll be much further into the
8 future, well, hopefully, it'll improve our vector
9 technologies (audio skip) down to (audio skip). But I
10 think a specific number across the board would be very
11 (audio skip).

12 DR. LISA BUTTERFIELD: Great, thank you. All
13 right. Let's finish up with Dr. Ken Berns please.

14 DR. KENNETH BERNS: Yes. Can you hear me?

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

17 DR. KENNETH BERNS: I think this is a very
18 interesting basic problem, because I like to think that
19 the good part of the vector is probably the DNA. Now
20 you're talking about genomes. But we don't really know

1 what the toxic component of the vector is, and to the
2 extent that it may be the capsid, it may be that the
3 overall number one wants to consider is the total
4 capsid load being delivered. Now I think it's an open
5 question as to whether empty capsids have the same
6 conformation and detail that full capsids do, and where
7 it rings with partial genomes or with other kinds of
8 DNA in capsids these are, we don't know.

9 But I really think that regardless of how
10 purified we think a preparation is in terms of having
11 full capsids, that probably it behooves us to really
12 consider the total number of capsids that we're putting
13 into the patient. Although I agree completely with the
14 comments that having a standard number is probably
15 inadvisable, because you don't know if different kinds
16 of vectors and different preparations, a priori what's
17 too much or too little? I really do think that we do
18 have to consider the total capsid quantity that's
19 present in what you're giving the patient.

20 DR. LISA BUTTERFIELD: Great. Thanks very

1 much. All right, let me sum up the discussion of key
2 points for this question, our last question for the
3 day. Then that will leave a couple of minutes at the
4 end for our FDA colleagues to see if they would like to
5 get greater clarity from the group before we adjourn.

6 Considering the risk of hepatotoxicities, in
7 terms of should an upper limit be set for total vector
8 genome dose per subject, that was not suggested to be
9 the best approach. Total vector genome dose per
10 kilogram or factoring in some measure of BMI for the
11 patient, was better received. We also need to know
12 whether the deaths that have been seen, the extent to
13 which vector dose and capsids played a role there or
14 what role was exacerbated by underlying liver disease.

15 As to the empty capsids, or total capsid dose,
16 again the committee did not discuss support for
17 arbitrary capsid limits, but instead some
18 standardization and measures of vector QC, including
19 vector titer, the number of empty capsids, should they
20 be there (audio skip) or capsids around other

1 contaminants in the preparation. So, measures of those
2 are evolving, but need more standardization and
3 discussion in order to really know what's being
4 measured and then to better consider limits, if any.
5 But carefully following patients with a dose
6 escalation, and also there may be differences in that
7 for the pediatric setting versus the adult setting.

8 Are there any new points to be made on that
9 sum up before I turn it over to additional questions?

10 Dr. Kenneth Berns, your hand is still up, but you were
11 the last speaking, so is that a new hand or is that the
12 previous hand up? There, that must have been the
13 previous one. All right. Then with that, I'll ask if
14 our FDA colleagues need greater clarity or expansion on
15 any of these questions? There is, Dr. Wilson Bryan.

16 DR. WILSON BRYAN: Yes. Let me check with my
17 folks. First the Pharm/Tox team, Dr. Urban, any
18 further questions? Dan, we didn't hear that, but you
19 went so quick I'm guessing that all's good with your
20 team, is that right?

1 DR. DAN URBAN: I forgot to unmute my phone.
2 Sorry, yes, we are good. We do not have any more
3 questions regarding the design of preclinical programs.
4 Thank you.

5 DR. WILSON BRYAN: Thank you. Let me check,
6 there's been a lot of chatter among the clinical group
7 in the background. Let me see, Dr. Sherafat, anything
8 more from the clinical group?

9 DR. ROSA SHERAFAT-KAZEMZADEH: No, I think we
10 are very pleased with all the answers. Thank you for
11 the discussion.

12 DR. WILSON BRYAN: All right. Thank you.
13 Then CNC team, Andrew Byrnes, Dr. Byrnes?

14 DR. ANDREW BYRNES: Thanks for all the great
15 discussions. We've been talking a little bit behind
16 the scenes here about question 5 b. on empty capsids.
17 We've been faced with the question pretty frequently,
18 recently, as we see toxicity with high dose AAV
19 vectors, that some of them are very pure and have very
20 few empty capsids and some of them have much higher

1 amounts of empty capsids. It's difficult for
2 manufacturers to turn on a dime and make a new lot that
3 has much greater purity, so we often meet resistance
4 when we suggest reducing the amount of empty capsids.

5 Can the committee comment on the importance of
6 empty capsids? We have uncertainty about their
7 toxicity, but at the same time we're faced with these
8 situations where we seem to be bumping up against the
9 maximum tolerated doses and we're looking for
10 opportunities to improve the safety of these vectors,
11 thank you.

12 DR. LISA BUTTERFIELD: All right, thank you,
13 Dr. Byrnes, for that additional question. About
14 capsids toxicity, are there members of the committee
15 that would like to specifically go into that in greater
16 detail? Perhaps members who have some experience with
17 manufacture and doing those measures? Dr. Barry Byrnes
18 please.

19 DR. BARRY BYRNE: Yeah, I can comment to
20 amplify on what I said before. The stoichiometry

1 between the vector genome and the three principle
2 capsid proteins is known, so that, in fact, on an ideal
3 basis you can actually measure in a preparation that
4 is, let's say, greater than 95 percent capsid proteins
5 assessed by either (inaudible). You can determine what
6 the total protein concentration is.

7 That's one way to level set across these
8 various trials where there may be genome-specific
9 titers -- or the product-specific titers determined by
10 genome content may not be representative of the total
11 protein content across various products. That would be
12 an important release specification to consider.
13 That'll really detect what the total capsid burden is
14 in the dose.

15 DR. LISA BUTTERFIELD: Dr. Andrew Byrne, did
16 that help?

17 DR. ANDREW BYRNE: Was there any more
18 discussions because we have assays, sponsors of assays
19 to measure empty capsids, but the uncertain thing is
20 how toxic they are and how hard to push when it looks

1 like there's toxicity.

2 DR. LISA BUTTERFIELD: Please.

3 DR. BARRY BYRNE: Sorry, can you hear me now?

4 Yeah, some of these points will come up tomorrow when
5 we discuss thrombotic microangiopathy because the same
6 factors apply. That the capsid burden will control the
7 total protein delivered with a dose, but the antibodies
8 made in response to that antigenic exposure can be
9 infinite as long as there are cells making antibodies
10 that acutely bind to the capsids. So, those antibody
11 complexes are what influenced the activation of the
12 classical pathway.

13 It may be that some of these same mechanisms
14 that influence TMA, as Dr. Wilson alluded to, they can
15 be linked to the acute toxicity that effect the liver
16 as well. These may be mitigated by strategies to
17 lessen or remove the antibodies from the equation
18 altogether. I think that'll be discussed further
19 tomorrow.

20 DR. LISA BUTTERFIELD: Thank you. We have a

1 hand up from Dr. Venditti as well.

2 DR. CHARLES VENDITTI: Yeah, I was just going
3 to add the comment, again, this is more of a chicken
4 and an egg question because I think we don't really
5 know what is in an empty capsid. If one manufacturer
6 has a certain process and they have empties that are
7 full of some sort of unwanted cellular product, you can
8 use your imagination of different classes of compounds
9 that maybe can get inside an AAV particle. That might
10 be very different than someone else that has possibly
11 the same amount of empties, but they might not, you
12 know, --who knows what, if there would be a toxicity.

13 The other comment that I was just going offer,
14 again, is that -- maybe it'll come up tomorrow -- about
15 the reverse packaging of plasmid and other types of
16 vectors that get into the full particles. Whether
17 that's the balance that we have to also look at very,
18 very carefully, which is the manufacturing process, the
19 things that Dr. Byrne just mentioned. But then also
20 how much backbone plasmid was reversed packaged? We

1 know that those things are very inflammatory for the
2 innate immune response and they're sort of toxic -- it
3 could be toxic contaminants of a prep.

4 I think this goes back full circle to the
5 question that we had on the QC of AAVs and what should
6 we be doing as a field? Implementing a wide range of
7 nucleic-acid, maybe proteomic technologies, to QC all
8 the different AAVs. These different products (audio
9 skip). The genome (audio skip) capsid. But it could be
10 that these byproducts of manufacturing that are present
11 at a much lower amount, that's what's driving the
12 toxicity.

13 I'm saying, possibly, that's a theory, I don't
14 know that. I'm throwing it out there as a point of
15 discussion when someone -- a comment about trying to
16 reduce the amount of the empties and does it matter in
17 their models. I just was going to offer that comment
18 at the end. Could just be that it's not really (audio
19 skip). And again, (audio skip) that it's not really
20 the empties (audio skip) it could be fortuitously

1 packaged contaminants in the full capsid vector (audio
2 skip) that could be equally toxic. That's it. I was
3 just going to offer that comment, thank you.

4 DR. LISA BUTTERFIELD: Thank you. Dr. Andrew
5 Byrnes, anything else?

6 DR. ANDREW BYRNES: Great points. Thank so
7 much and we'll have a very similar question at TMA
8 session tomorrow again. Thank you.

9 DR. LISA BUTTERFIELD: All right, then with
10 that, if there are no other questions from our FDA
11 colleagues?

12 DR. WILSON BRYAN: No, that's all from us.
13 Again, thank you very much for the discussion today.
14 It's been very helpful.

15 DR. LISA BUTTERFIELD: Terrific. Then with
16 that, I turn the meeting over to Jarrod.

17 MR. JARROD COLLIER: Okay. Thank you very
18 much, Dr. Butterfield. Can you all hear me?

19 DR. LISA BUTTERFIELD: Yes.

20

1

ADJOURN

2

3 MR. JARROD COLLIER: Okay, great. Okay, so at
4 this time this concludes day one of the two-day virtual
5 meeting. I would like to thank all the participants
6 for their time and effort to conduct today's meeting.
7 I would also like to give special thanks to Michael
8 Kawczynski for managing the Adobe Connect Platform for
9 today, and to our esteemed chair, Dr. Lisa Butterfield,
10 for facilitating today's meeting.

11 Before we adjourn, I would like to ask that
12 all advisory committee members and temporary voting
13 members stay online after adjournment to discuss the
14 updates for tomorrow. With that said, the meeting is
15 now adjourned. I want to thank you all and we will see
16 you tomorrow for day two. Have a great evening.

17

18

[MEETING ADJOURNED FOR THE DAY]