Food and Drug Administration
Center for Biologics Evaluation and Research
Summary Minutes
70th Cellular, Tissue and Gene Therapies Advisory Committee Meeting
September 2-3, 2021

Committee Members
Lisa Butterfield, Ph.D. (Chair)
Tabassum Ahsan Ph.D.
Kenneth Berns, M.D., Ph.D.
Christopher Breuer, M.D.
Bernard Fox, Ph.D.
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Roland Herzog, Ph.D.
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Carlos Sanchez, M.D.
Charles Venditti, M.D., Ph.D.
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Industry Representative
Eric Crombez, M.D.**

Consumer Representative
Randy Hawkins, M.D.*

*Consumer Representative
** Industry Representative
***Patient Representative

Speakers and Guest Speakers
Deepa Chand, M.D. (Guest Speaker)
Ronald Crystal, M.D. (Guest Speaker)
Lindsey George, M.D. (Guest Speaker)
Denise Sabatino, Ph.D. (Guest Speaker)
Mark Sands, Ph.D. (Guest Speaker)
Rosa Sherafat-Kazemzadeh, M.D. (FDA)
James Wilson, M.D., Ph.D. (Guest Speaker)

FDA Participants
Rachael Anatol, Ph.D.
Wilson Bryan, M.D.
Andrew Byrnes, Ph.D.
Denise Gavin, Ph.D.
Leila Hann
Gaya Hettiarachchi, Ph.D.
Vijay Kumar, M.D.
Peter Marks, M.D., Ph.D.
Steven Oh, Ph.D.
Raj Puri, M.D., Ph.D.
Tejashri Purohit-Sheth, M.D.
Mercedes Serbian, M.S.
Zenobia Taraporewala, Ph.D.
Daniel Urban, Ph.D.
Celia Witten, Ph.D., M.D.
Lei Xu, M.D., Ph.D.

Designated Federal Officers (DFO)
Jarrod Collier, M.S.
Christina Vert, M.S.

Committee Management Specialist(s)
Joanne Lipkind, M.S.

Director
Prabhakara Atreya, Ph.D.

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These summary minutes for the September 2-3, 2021 meeting of the Cellular, Tissue and Gene Therapies Advisory Committee were approved on November 17, 2021.

I certify that I participated in the September 2-3, 2021 meeting of the Cellular, Tissue and Gene Therapies Advisory Committee (CTGTAC) meeting and that these minutes accurately reflect what transpired.

/S/
Christina Vert, M.S.
Jarrod Collier, M.S.
Designated Federal Officer

/S/
Lisa H. Butterfield, Ph.D.
Chair

On September 2-3, 2021 at 10:00 a.m. Eastern Standard Time (EST), the 70th meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) took place in open session to discuss the toxicity risks of AAV vector-based gene therapy products. The discussion topics include oncogenicity risks due to vector genome integration and safety issues identified during preclinical and/or clinical evaluation. Given the topic of this meeting, it was determined to be a Particular Matter of General Applicability (PMGA).

On Day 1, September 2, Dr. Lisa Butterfield, the Chair, called the meeting to order. The DFO, Mr. Jarrod Collier, made administrative remarks, conducted roll call and invited the committee members to introduce themselves, and read the Conflict of Interest (COI) statement into the public record. There were four conflict of interest waivers issued under 18 U.S. Code Section 208 in connection with this meeting: Dr. Kenneth Berns (Committee Member), Dr. Barry Byrne (TVM), Dr. Roland Herzog (TVM), and Dr. Charles Vite (TVM). During the open session, CTGTAC members, consultants, FDA speakers, Guest Speakers, staff, and the public speakers all participated via the Adobe Connect web conference.

Dr. Wilson Bryan, Director of the Office of Tissues and Advanced Therapies, provided FDA Opening Remarks. This was followed by a presentation from the FDA Speaker, Dr. Rosa Sherafat-Kazemzadeh, on "Toxicity Risks of Adeno-associated Virus (AAV) Vectors for Gene Therapy (GT)." Immediately following this presentation, there was a Q & A session for the FDA Speaker.

Following the FDA Q&A, Session 1 on Vector Integration and Oncogenicity Risks commenced. For the start of Session 1, the Guest Speaker, Dr. Mark Sands, gave a presentation on "rAAV Integration: *In Vitro* & Mice." Immediately following the presentation, there was a 15-minute Q & A session for Dr. Sands. Next, the Guest Speaker, Dr. Denise Sabatino, gave a presentation on "AAV Integration Studies in Large Animal Models: Non-Human Primates and Dogs". Immediately following the presentation, there was a 15-minute Q & A session for Dr. Sabatino.

Once the Committee returned from lunch, a 30-minute Open Public Hearing (OPH) session was held from 12:45 p.m. to 1:15 p.m. in which 4 pre-registered public speakers provided presentations. The names of OPH speakers and their remarks may be obtained from the transcript posted on the website. Following the OPH session there was a Committee Discussion of Questions session with Committee Members and Temporary Voting Members.

During the Committee Discussion of Questions for Session 1, the following discussion questions were presented to the Committee:

Discussion Question #1:

1. Please discuss the merits and limitations of animal studies to characterize the risk of AAV vector-mediated oncogenicity, and provide recommendations on specific preclinical study design elements, to include:
    a. Animal species, healthy vs. disease models, and animal age
    b. In-life and post-mortem assessments, including methods for integration analysis
    c. Duration of follow-up, post-dose

Summary of Discussion: *The extent to which animal studies can translate oncogenic risk associated with a specific AAV vector-based product to a particular patient population is unknown; more clinical long-term follow-up data are needed to better assess this issue. However, animal studies may be helpful to better understand the mechanism by which AAV vector-mediated integration may lead to oncogenicity and how factors such as vector construct, dose levels, route of administration and animal age when dosed may influence study outcome. It is important that animal study designs recapitulate these aspects of the intended clinical use, be of adequate duration to detect potential tumor formation, and use appropriate methods for analysis of integration and clonal expansion. Current scientific gaps/limitations, emerging technologies for integration analysis, and the value of developing and standardizing methods were discussed.*

Discussion Question #2:

2. Current literature suggests that various factors may affect AAV-mediated vector genome persistence, vector integration, and the risk of oncogenesis. Please discuss benefit-risk considerations for AAV vector-mediated oncogenesis, such as patient age at the time of treatment, pre-existing liver conditions (e.g., infection with hepatitis B or C virus), and high vector dose.

Summary of Discussion: *Benefit-risk evaluations of AAV gene therapy (GT) products should be made on a case-by-case basis, and take into consideration the risks related to:*

- *Available alternative treatments such as liver transplant for inborn errors of metabolism*
- *Patient's age at the time of gene therapy*
- *Pre-existing conditions such as chronic viral hepatitis prior to systemic administration of gene therapy*
- *Route of administration, e.g., considering the risk of liver toxicities with intravenously administered AAV vectors, patients with pre-existing conditions such as viral hepatitis may wait until their liver shows improvement before receiving the AAV GT*

Discussion Question #3:

3. Considering the risk of oncogenesis,
    a. Please provide recommendations on safety monitoring measures that should be included in clinical trials.
    b. Please provide recommendations on duration, frequency, and method of long-term follow-up (LTFU) for recipients of AAV vectors.

Summary of Discussion: *Monitoring for signs of hepatocellular carcinogenicity could be done by adopting standard approaches, but computerized tomography (CT) scans are not recommended due to the radiation risk, and MRI is not cost effective. Ultrasound every 6 months with lifelong follow-up may be an option but could be challenging in some cases due to patient's age, or the cost. Newer approaches such as analysis of cell-free DNA for vector integration screening may be a promising monitoring tool.*

Data sharing across registries and electronic health records (EHRs) for long-term (lifelong) follow-up and data catalogues or repositories generated across AAV vector serotypes could be considered to assist in identifying signals. There are challenges related to such approaches/surveillance (complicated and labor-intensive) which should be considered in the context of the potential risk of oncogenicity due to vector integration.

Discussion Question #4:

4. Please discuss whether some vector designs may enhance the frequency of vector-mediated integration and the risk of oncogenesis. For example, how is the risk affected by promoter-enhancer elements, genome-targeted nucleases, or novel AAV vector designs for which there is limited clinical experience. Because AAV vectors can carry significant levels of co-packaged DNA impurities from the manufacturing process, is the risk of oncogenesis increased due to potential integration of non-vector DNA, and what types of studies should be performed to assess this risk?

Summary of Discussion: *Some animal studies have shown that certain promotor-enhancer combinations lead to higher risks of oncogenicity. Studies are needed to analyze and eliminate sequences in vectors that increase the risk of oncogenicity. Better analytical approaches are also needed to look at vector sequences, characterize contaminants in the vector preparation, and identify any contaminating sequences.*

After the Committee Discussion of Questions for Session 1 concluded, the Committee moved immediately to Session #2: Hepatotoxicity.

For the start of Session 2, the Guest Speaker, Dr. Lindsey George, gave a presentation on "Systemic AAV: Clinical Findings of Hepatotoxicity". Immediately following the presentation, there was a 15-minute Q & A session for Dr. George. Next, the Guest Speaker, Dr. James Wilson, gave a presentation on "Adeno-Associated Virus-Related Toxicities in Nonhuman Primates". Immediately following the presentation, there was a 15-minute Q & A session for Dr. Wilson.

Once the Committee returned from a 10-minute break, a 30-minute Open Public Hearing (OPH) session was held from 4:05 p.m. to 4:35 p.m. in which 3 pre-registered public speakers made presentations. The names of OPH speakers and their remarks may be obtained from the transcript posted on the website. Following the OPH session there was a Committee Discussion of Questions session with Committee Members and Temporary Voting Members.

During the Committee Discussion of Questions for Session 2, the following discussion questions were presented to the Committee:

Discussion Question #1:

1. Please discuss the merits and limitations of animal studies to characterize the risk of hepatotoxicity and provide recommendations on preclinical study design elements, such as animal species / disease models and in-life and post-mortem assessments.

Summary of Discussion: *Concern was expressed over whether available animal species/models are good models of the AAV-mediated hepatotoxicity observed in humans. However, animal studies could potentially be used to understand the mechanisms of acute and long-term hepatotoxicity, the impact of underlying disease pathophysiology, the role of the immune response, impact of complement activation, effects of immune suppression, and alternatives to corticosteroids. Recommendations included incorporation of early assessment timepoints, liver biopsies, transcriptomics, and evaluation of biomarkers for muscle or liver injury toxicity.*

Discussion Question #2:

2. How should patients be screened and categorized based on their risk for developing liver injury before AAV vector administration? Please discuss whether pre-existing hepatic conditions may predict the risk of serious liver injury.

Summary of Discussion: *Hepatologists should be engaged early in clinical development to ensure careful screening and identification of patients with pre-existing liver conditions, which could predict serious liver injury. Screening for bridging fibrosis might be informative but would depend on different disease states that affect baseline characteristics. Total and/or neutralizing antibody titers are screened in many clinical studies, but how such testing is performed, the cut-offs, and the acceptance criteria are all variables that may need standardization.*

Discussion Question #3:

3. What additional strategies could be implemented before and/or after AAV vector administration to prevent or mitigate the risk of liver injury?

Summary of Discussion: *Disease-specific liver state should be considered to enable screening and monitoring to be individualized, if necessary. Bloodwork may be sufficient for some study subjects, while other study subjects may need ultrasounds or a biopsy, e.g., in cases where subjects are showing signs of hepatic toxicity. Strategies to mitigate risk of liver injury should include lowering the vector dose and investigating the immune responses.*

Discussion Question #4:

4. What factors (e.g., level of disease severity) other than weight should be considered to determine the vector dose for systemic administration?

Summary of Discussion: *Dose escalation studies should be carefully performed to monitor for toxicities.*

The capsid antigen burden should be considered, but it may not be possible to delink the antigenic burden from the levels of transgene (in the therapeutic dose). Body mass index (BMI) abnormalities can exist in many disease states, in which case bone, fat, muscle and organ composition and volumetrics might help define doses that lead to toxicities versus efficacy.

Discussion Question #5:

5. Considering the risk of hepatotoxicity observed in clinical trials with high doses of AAV vectors,
    a. Please discuss whether an upper limit should be set for the total vector genome dose per subject.
    b. Given that many AAV products contain significant amounts of empty capsids, please discuss whether an upper limit should be set on the total capsid dose.

Summary of Discussion: *An arbitrary upper limit of the total vector genome dose or total capsid dose is not recommended, as it is hard to standardize vector measurements across studies or to determine if there is an appropriate upper limit. The dose should be based on a measurement of total vector genome dose (vg/kg) or by factoring in BMI, while also considering that risks may be exacerbated by underlying liver disease.*

Assays for empty capsids need better standardization. More effort is needed to comprehensively characterize empty capsids and other byproducts of AAV manufacturing; data from such studies are needed before discussing limits on the total vector genome dose.

Carefully following subjects may facilitate understanding the differences between pediatric and adult subjects.

The meeting was then adjourned on September 2, 2021 at 6:00 PM EST.

On Day 2, September 3, Dr. Lisa Butterfield, the Chair, called the meeting to order. The DFO, Mr. Jarrod Collier, made administrative remarks, conducted roll call and invited the committee members to introduce themselves, and read the Conflict of Interest (COI) statement into the public record. There were four conflict of interest waivers issued under 18 U.S. Code Section 208 in connection with this meeting: Dr. Kenneth Berns (Committee Member), Dr. Barry Byrne (TVM), Dr. Roland Herzog (TVM), and Dr. Charles Vite (TVM). During the open session, CTGTAC members, consultants, FDA speakers, Guest Speakers, staff, and the public speakers all participated via the Adobe Connect web conference.

Dr. Wilson Bryan, Director of the Office of Tissues and Advanced Therapies, provided FDA Opening Remarks.

For the start of Session 3: Thrombotic Microangiopathy (TMA), the Guest Speaker, Dr. Deepa Chand, gave a presentation on "Clinical Findings of Thrombotic Microangiopathy (TMA)". Immediately following the presentation, there was a 15-minute Q & A session for Dr. Chand. Once completed, a 30-minute Open Public Hearing (OPH) session was held from 11:00 a.m. to 11:30 a.m. in which 1 pre-registered public speaker made a presentation. The name of OPH speaker and their remark may be obtained from the transcript posted on the website. Following the OPH session there was a Committee Discussion of Questions session with Committee Members and Temporary Voting Members.

During the Committee Discussion of Questions for Session 3, the following discussion questions were presented to the Committee:

Discussion Question #1:

1. Please discuss factors that may increase the risk of TMA following AAV vector administration.

Summary of Discussion: *There seems to be an association between the frequency of TMA events and total viral capsid exposure. Immune activation is likely a common event and antigen-antibody complex triggering is critical. While patients are tested for pre-existing neutralizing antibodies for AAV, some assays cannot differentiate immunoglobulin class to determine whether a sero-negative result means that the patient is truly naïve. Measurement of immunoglobulin class at early timepoints after AAV GT administration is helpful because IgM plays an important role in mediating complement activity. Early laboratory testing for D-dimer will also help monitor for liver injury.*

Discussion Question #2:

2. Please provide recommendations on strategies that could be implemented before and/or after AAV vector administration to prevent or mitigate the risk of AAV vector-mediated TMA.

Summary of Discussion: *In addition to monitoring the timeline of vector copy number decline, strategies focusing on targeting IgM-mediated complement activation and implementation of B-cell-targeted therapies should be considered. Additional risk mitigation strategies to be considered include decreasing the amount of CpG levels in the vector sequence to potentially decrease stimulation of the innate immune system. The underlying disease could also impact the likelihood of developing TMA and possible*

need for prophylaxis. Prophylactic strategies should be carefully considered in the context of associated risk.

Discussion Question #3:

3. Considering the risk of TMA observed in clinical trials with high doses of AAV vectors,
   a. Please discuss whether an upper limit should be set for the total vector dose.
   b. Given that many AAV products contain significant amounts of empty capsids, please discuss whether an upper limit should be set on the total capsid dose.

Summary of Discussion: *An upper limit of vector dose per subject is not advised; however, an upper limit of vector per kilogram of body weight, or per kilogram of ideal body weight based on BMI, could be recommended. One challenge for recommendation of an upper limit on vector dose per subject is the lack of reference standards, limiting the comparison of critical quality attributes across sponsors and/or products. There is a need for development of better techniques, such as next generation sequencing and proteomics, to better characterize if the capsids are full, partially full, or truly empty capsids.*

Following the Committee Discussion of Questions, the committee had a 35-minute lunch break.

For the start of Session 4: Neurotoxicity: Dorsal Root Ganglion (DRG) Toxicities, the Guest Speaker, Dr. James Wilson, gave a presentation on "Nonclinical Findings of Dorsal Root Ganglion, Spinal Cord and Peripheral Nerve Toxicities". Immediately following the presentation, there was a 15-minute Q & A session for Dr. Wilson.

Once the Committee returned from a 10-minute break, a 30-minute Open Public Hearing (OPH) session was held from 1:50 p.m. to 2:20 p.m. in which 2 pre-registered public speakers made presentations. The names of OPH speakers and their remarks may be obtained from the transcript posted on the website. Following the OPH session there was a Committee Discussion of Questions session with Committee Members and Temporary Voting Members.

During the Committee Discussion of Questions for Session 4, the following discussion questions were presented to the Committee:

Discussion Question #1:

1. Based on the published data, please discuss the relevance of the non-human

primate cases of DRG toxicity to human subjects.

Summary of Discussion: *Questions remain regarding the mechanism of DRG toxicity and the relevance to humans. Further investigation in animal studies may be useful to better understand the mechanisms underlying the DRG toxicity, including the role of endoplasmic reticulum stress, transgene-mediated effects, and the threshold at which histopathological changes may lead to clinical manifestations.*

Discussion Question #2:

2. Please provide recommendations on preclinical study design elements, such as animal species/disease models, age, in-life and post-mortem assessments, and duration of follow-up, post-dose, that may contribute to further characterization of DRG toxicity.

Summary of Discussion: *Healthy animals or disease models in an animal species sensitive to the DRG changes, such as non-human primates, dogs, cats, and pigs, could be informative, and ideally would reflect the age of study subjects. Longer study durations may be appropriate given the chronic nature of the findings, particularly for novel vectors and subtypes. Comprehensive histopathological assessments by a neuropathologist, nerve conduction studies (including functional assessments, such as H-reflex testing), responses to von Frey filaments, dynamic-brush assays, and urinary reflex assessments could be important for more comprehensive assessment of nerve function and pain. Given the unknown translational relevance and challenges with detecting sensory neuron toxicity, it is important to evaluate this risk in the context of the overall benefit-risk profile for an AAV vector product.*

Discussion Question #3:

3. In addition to periodic neurological examinations, please provide recommendations on other methods to mitigate the risk of DRG toxicity in clinical trials.

Summary of Discussion: *Assessments can be complicated by limitations due to age or cognitive and/or communication skills. Identification of potential biomarkers in peripheral blood or in cerebrospinal fluid (CSF) might be helpful in assessing risk of DRG toxicity. Evaluation of potential DRG toxicity should be performed at baseline and at regular intervals, particularly in children.*

For the start of Session 5: Neurotoxicity: Brain MRI Findings, the Guest Speaker, Dr. Ronald Crystal, gave a presentation on "Clinical and Nonclinical Consequences of Direct CNS Parenchymal Administration of AAV Vectors". Immediately following the presentation, there was a 15-minute Q & A session for Dr. Crystal.

Once the Committee returned from a 10-minute break, there was a Committee Discussion of Questions session with Committee Members and Temporary Voting Members.

During the Committee Discussion of Questions for Session 5, the following discussion questions were presented to the Committee:

Discussion Question #1:

1. Please provide recommendations for any preclinical in-life and post-mortem assessments (e.g., behavioral and neuropathological assessments) and duration of follow-up, post-dose, to identify and further characterize the risk of neurotoxicity following intraparenchymal administration of AAV vectors.

Summary of Discussion: *Preclinical studies in small and large animals, including models of disease, can provide neuropathological and behavioral information for safety evaluation prior to a clinical trial. Large animal species may facilitate the use of specialized delivery techniques that can be translated clinically. Further investigation into contributing effects of the vector and transgene, immune response, and the impact of immunosuppression could be valuable in providing a better understanding of the mechanism of toxicity, while inclusion of endpoints such as serial imaging to assess recovery from any lesions, electroencephalograms (EEGs), and behavioral assessments may be useful to better characterize the toxicity.*

Discussion Question #2:

2. Please discuss the clinical significance, if any, of brain MRI abnormalities observed in clinical trials of AAV vector gene therapies. Please discuss whether the delivery procedure vs. AAV vector may have contributed to the abnormal brain MRI findings.

Summary of Discussion: *Local delivery may be effective to deliver the GT vector for the treatment of diffuse CNS disease. Combined delivery methods may be necessary to more effectively treat diffuse CNS disease.* The *abnormal MRI findings may be due to the AAV vector and/or the delivery device/ procedure.*

Discussion Question #3:

3. Please provide recommendations on strategies that could be implemented before and after vector administration to prevent or mitigate the risk of central nervous system injury.

Summary of Discussion*: Intra-operative MRI monitoring with gadolinium contrast infusion may decrease procedure-related risks by allowing for more accurate cannula placement, tracking of infusion, and early identification of infusion leaks. Follow-up neurological examinations could be supplemented by serial electroencephalograms (EEGs) to detect and manage subclinical seizures.*

Discussion Question #4:

4. Please recommend a duration of monitoring for subjects who have abnormal brain MRI findings, or factors to consider for the determination of an appropriate duration of monitoring.

Summary of Discussion: *Serial MRIs at 1, 3, and 6 months after product administration, and then annually, would be appropriate for consideration. MRI follow-up can be challenging in many pediatric settings and can possibly require general anesthesia. If immunogenicity to the vector is being considered as a potential cause of the abnormal MRI findings, identification and development of biomarkers of immune response, in the CSF or blood, may allow for monitoring of the immune response.*

Discussion of Follow-up Question:

Dr. Wilson Bryan asked the committee for any additional recommendations if clinical manifestations associated with the abnormal MRI findings are reported in the future.

Summary of Discussion: The committee noted that if clinical manifestations are associated with MRI findings, this would be concerning, and recommended evaluation of the particular clinical case(s) to determine whether the clinical findings were due to the intraparenchymal route of administration and thus warrant modification of the route of administration or modification of the catheter design, or whether the clinical findings were due to an immunogenic reaction to the vector or transgene.

After the Committee Discussion of Questions, Dr. Peter Marks, Director of the Center for Biologics Evaluation and Research, provided closing remarks.

The meeting was then adjourned on September 3, 2021 at 5:58 PM EST.

Additional information and details may be obtained from the transcript and the

recording of the webcast of the meeting may be viewed at:

- **https://youtu.be/58KjL9_p9Tw** (Day 1)
- **https://youtu.be/yLggQF0XUUY** (Day 2)