
RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 9, 2015

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health**

Contents

I.	Call to Order and Opening Remarks	2
II.	Minutes of RAC Meeting, March 10, 2015	2
	A. Committee Motion 1	2
III.	Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products.....	2
	A. Presentation by Dr. Taraporewala	2
	B. RAC Discussion	5
	C. Public Comment.....	6
IV.	Gene Transfer Safety Assessment Board Report	6
	A. GTSAB Report	6
	B. RAC Discussion	6
	C. Public Comment.....	6
V.	Outgoing Members Award Ceremony	6
	A. Presentation of Certificates of Appreciation to Retiring RAC Members	7
VI.	Review and Discussion of Human Gene Transfer Protocol #1504-1415: HD-CELL: MSC Engineered to Produce BDNF for the Treatment of Huntington’s Disease	7
	A. Protocol Summary	7
	B. Written Reviews by RAC Members	8
	C. RAC Discussion	12
	D. Investigator Response	14
	1. Written Responses to RAC Reviews	14
	2. Responses to RAC Discussion Questions	20
	E. Public Comment.....	22
	F. Synopsis of RAC Discussion and RAC Observations and Recommendations	22
	G. Committee Motion 2.....	23
VII.	Review and Discussion of Human Gene Transfer Protocol #1504-1396: A Phase III, Open-Label Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with β -Thalassemia Major by Transplantation of Autologous CD34+Stem Cells Transduced Ex Vivo with a Lentiviral β A-T87Q-Globin Vector (LentiGlobin BB305 Drug Product) in Subjects \geq 5 and <12 Years of Age	23
	A. Protocol Summary	23
	B. Written Reviews by RAC Members	24
	C. RAC Discussion	28
	D. Investigator Response	29
	1. Written Responses to RAC Reviews	29
	2. Responses to RAC Discussion Questions	32
	E. Public Comment.....	34
	F. Synopsis of RAC Discussion and RAC Observations and Recommendations	34
	G. Committee Motion 3.....	35
VIII.	Update on Protocol #0810-946: Phase I Trial of Intratumoral Injection of Vesicular Stomatitis Virus Expressing Human Interferon Beta in Patients with Sorafenib Refractory/Intolerant Hepatocellular Carcinoma and Advanced Solid Tumors with Liver Predominant Locally Advanced/Metastatic Disease.....	35
	A. Presentation by Dr. Borad	35
	B. RAC Discussion	37
	C. Public Comment.....	38

IX. Vector Design Influences Hepatic Genotoxicity after Adeno-Associated Virus Gene Therapy 38
D. Presentation by Dr. Venditti 39
E. RAC Discussion 41
F. Public Comment..... 42

X. Closing Remarks and Adjournment..... 43

Attachment I: Recombinant DNA Advisory Committee Roster Att-I-1
Ad Hoc Reviewers and Participants Att-I-3
Ad Hoc Presenters and Speakers Att-I-4
Nonvoting Agency and Liaison Representatives Att-I-5

Attachment II: Public Attendees Att-II-1
Attachment III: Abbreviations and Acronyms Att-III-1

Appendix A: Verbatim Public Comments App-A-1

[Note: The latest Human Gene Transfer Protocol List can be found on the Office of Biotechnology Activities website at <http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment-recombinant-dna-advisory-committee/human-gene-transfer-protocols-registered-oba>.]

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting¹**

June 9, 2015

The Recombinant DNA Advisory Committee (RAC) convened for its 142nd meeting at noon on June 9, 2015, at the National Institutes of Health (NIH), Building 45, Conference Room E1/E2, Bethesda, Maryland. Dr. Donald B. Kohn, RAC Chair, presided. In accordance with Public Law 92-463, the meeting was open to the public from noon until 5:47 p.m. on June 9, 2015. The following individuals were present, either in person or by teleconference, for all or part of the June 2015 RAC meeting.

Committee Members

Michael Atkins, Georgetown University School of Medicine
Saswati Chatterjee, City of Hope National Medical Center
William Curry, Harvard Medical School
Kevin Donahue, University of Massachusetts Medical School
Rebecca Dresser, Washington University School of Law
Marie-Louise Hammarskjöld, University of Virginia School of Medicine
Angelica Hardison, Georgia Regents University (*via teleconference*)
Patrick Hearing, Stony Brook University
Howard Kaufman, Robert Wood Johnson Medical School/Rutgers, The State University of New Jersey
Hans-Peter Kiem, University of Washington School of Medicine/Fred Hutchinson Cancer Research Center
Donald Kohn (RAC Chair), University of California, Los Angeles
Joseph Pilewski, University of Pittsburgh
Lainie Ross, University of Chicago (*via teleconference*)
Michel Sadelain, Memorial Sloan-Kettering Cancer Center
Richard Whitley, University of Alabama at Birmingham
Dawn Wooley, Wright State University
Laurie Zoloth, Northwestern University

NIH Office of Biotechnology Activities (OBA)

Lyric Jorgenson, Office of the Director (OD), National Institutes of Health (NIH)

Nonvoting Agency Representatives

Kristina Borrer, Office for Human Research Protection, NIH
Denise Gavin, U.S. Food and Drug Administration (FDA)

NIH/OD/OBA Staff Members

Linda Gargiulo
Morad Hassani
Robert Jambou
Cheng Luan Li
Maureen Montgomery
Chris Nice
Marina O'Reilly

¹ The Recombinant DNA Advisory Committee is advisory to the NIH, and its recommendations should not be considered as final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

Gene Rosenthal
Kate Saylor
Aparna Singh

Attendees

There were 62 attendees at this 1-day RAC meeting.

Attachments

Attachment I contains a list of RAC members, nonvoting agency and liaison representatives, and ad hoc participants, reviewers, presenters, and speakers. Attachment II contains a list of public attendees. Attachment III contains a list of abbreviations and acronyms used in this document.

I. Call to Order and Opening Remarks

Dr. Kohn, the RAC Chair, called the meeting to order at noon on June 9, 2015. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* was published in the *Federal Register* on May 13, 2015 (80 FR 27330). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), recognition of outgoing RAC members, public review and discussion of two gene transfer protocols, an update on one gene transfer protocol previously reviewed by RAC, and presentations and public discussions on design and analysis of shedding studies and on the influence of vector design on hepatic genotoxicity after adeno-associated virus (AAV) gene therapy.

RAC members introduced themselves by name, affiliation, and research interests.

Dr. Jorgenson reminded RAC members of the rules of conduct that apply to them as Special Government Employees, read into the record the conflict of interest statement, and suggested that related questions be addressed to the OBA committee management officer.

II. Minutes of RAC Meeting, March 10, 2015

RAC Reviewers: Dr. Chatterjee and Ms. Hardison

Dr. Chatterjee and Ms. Hardison found that the minutes accurately reflected the discussion that was conducted during the March 2015 meeting. No changes to the document were suggested by the two reviewers or other RAC members.

A. Committee Motion 1

Dr. Kohn asked the RAC to approve the minutes of the March 10, 2015, RAC meeting. The RAC voted unanimously by voice to do so.

III. Design and Analysis of Shedding Studies for Virus- or Bacteria-Based Gene Therapy (VBGT) and Oncolytic Products

Presenter: Zenobia Taraporewala, Ph.D., Center for Biologics Evaluation and Research (CBER), FDA

A. Presentation by Dr. Taraporewala

Dr. Zenobia Taraporewala presented an overview of the draft guidance titled “Design and Analysis of Shedding Studies for Virus- or Bacteria-Based Gene Therapy and Oncolytic Products,” which was published in June 2014. The guidance applies to oncolytic and gene therapy products that are regulated by FDA’s Office of Cellular Tissue and Gene Therapies (OCTGT) and derived from infectious viruses or bacteria. The shedding profile for these products is not always predictable from historical or preclinical data due to product-specific variables and patient-specific factors. Guidance on shedding is therefore needed to assess the potential for transmission of product-derived viruses and bacteria to untreated individuals and to provide a clear and comprehensive understanding of the shedding profile of each product in the target population(s). The objective of the guidance is to convey FDA’s current thinking on the conduct of shedding studies during preclinical and clinical development, specifically how and when (at what phase of product development) to collect shedding data, and the analysis of shedding data to assess the potential for transmission to untreated individuals.

Before drafting the current draft guidance, FDA staff participated in the development of a shedding consideration paper titled “General Principles to Address Virus and Vector Shedding,” which was published by the International Conference of Harmonization (ICH) in June 2009. The ICH report was intended to be a brief document to set out general principles in virus and vector shedding. The 2009 ICH paper was built on input from an ICH public workshop on shedding convened in October 2007 and provided the framework for the FDA guidance. The current FDA guidance uses a definition of gene therapy products as delineated in the long-term follow-up guidance published in November 2006 titled “Gene Therapy Clinical Trials—Observing Subjects for Delayed Adverse Events.”

Per the 2006 guidance, gene therapy products are defined as those products that mediate their effects by transcription and/or translation of the transfer genetic material and/or by integrating into the host genome. These products are administered as nucleic acids, viruses, or genetically engineered microorganisms. They may be used to modify cells in vivo or transfer to cells ex vivo before administration to the recipient. Gene therapy products can be replication competent, incompetent, or deficient. Oncolytic products refer to replication-competent viruses or dividing bacteria that are used as therapeutic agents to mediate the loss of tumor cells. Some oncolytic products carry foreign genes (e.g., immune-modifying genes, genes that enhance oncolysis) and mediate part of their anti-tumor effect by transcription and/or translation of these foreign genes and the host. Hence, oncolytic products that carry foreign genes can also be classified as gene therapy products.

There currently are 35 active investigational new drugs (INDs) for replication-competent products, which include adenovirus, herpes simplex virus type 1 (HSV1), measles virus, reovirus, Newcastle disease virus, vaccinia virus, *Listeria monocytogenes*, and others. There are an additional 450 INDs for replication-incompetent products, including adenovirus, AAV, HSV1, retrovirus, lentivirus, and others. Dr. Taraporewala noted that the majority of retrovirus and lentivirus vectors are used for ex vivo transduction of cells.

Dr. Taraporewala provided a brief summary of each section of the draft guidance. The introduction section of the guidance defines shedding as “the release of the product from the patient through either or all of the following: secretions (urine, saliva, nasopharyngeal fluids, etc.), excreta (feces), and skin (pustules, sores, wounds, etc.)” Shedding is distinct from biodistribution, because the latter describes the extent to which a product is distributed in the body from the site of administration but not how it is released from the body. The introduction also addresses oncolytic and gene therapy products derived from infectious viruses or bacteria. In the guidance, gene therapy products derived from infectious viruses or bacteria are referred to as VBGT products. Since infectious (product-based) viruses and bacteria may be shed by patients, product shedding raises safety concerns related to the risk of transmission of viruses and bacteria to untreated individuals

The guidance applies to VBGT products regulated by OCTGT. It does not apply to plasmids, peptides, and genetically modified mammalian cells regulated by OCTGT. The rationale for these exclusions is because unlike viral or bacterial products, plasmids, peptides, and genetically modified mammalian cells are neither infectious nor transmissible. The scope of this guidance also does not include shedding as it may relate to potential environmental concerns; this aspect is covered in another draft guidance released

in June 2014 titled “Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines, and Related Recombinant Viral or Microbial Products.”

Collection of shedding data is generally referred to as shedding studies. As such, clinical shedding studies are not standalone studies but are integrated into the design of a safety or efficacy trial(s). The background for the guidance on shedding studies for VBGT and oncolytic products clarifies these points and notes that product-specific and patient-specific factors that can influence the design of a shedding study should be taken into account when designing a study. The purpose of collecting shedding data during clinical development is to inform patients and physicians of whether shedding could occur with the use of an oncolytic or VBGT product, the potential for transmission, and the measures to prevent such transmission. Shedding data may also be collected in preclinical studies. Shedding data may be described in the Investigator Brochure and informed consent document (ICD) for IND studies and/or the package insert for an approved Biologics License Application (BLA).

The main considerations in the study design include the choice of clinical samples, periodicity of sample collection, duration of the monitoring period, and assay methodology. The key guiding principles for designing a study include the biological characteristics of the product (e.g., replication competence, immunogenicity, tropism) and the route of administration. In the preclinical setting, shedding data may be requested for an oncolytic or a replication-competent VBGT product if humans have not been previously exposed to the product (e.g., a nonhuman bacterial or viral strain), if the product was administered previously to humans but has been modified for a different *in vivo* tropism than the parent strain, if a change in the route of administration is proposed, or if the route of administration differs from the natural route of exposure or infection. In the clinical setting, collection of shedding data for replication-competent products should begin in Phase I studies and continue in later phases after a dose and regimen have been determined to better characterize the shedding profile. For replication-incompetent products, collection of shedding data should start after a dose and regimen have been determined (e.g., during Phase II). Additional data should be collected in subsequent clinical trials any time the dose, route, regimen, or indication is modified. The guidance includes specific recommendations for frequency and duration of sample collection, types of sample to collect, and provisions for sample storage.

The guidance also provides detailed examples of many of the factors that can influence the choices involved in design of a shedding study. With respect to frequency and duration of sample collection, the FDA recommends starting sample collection immediately after product administration and continuing sampling in the initial days and weeks after treatment (e.g., sampling on days 1, 3, 7, and 10 and then weekly). Sampling should continue until three consecutive data points are at or below the limit of detection. Regarding the type(s) of samples to collect, investigators should take into consideration factors that can affect shedding (e.g., route of administration, tropism [of the product-based virus or bacteria], natural route of transmission, shedding of the parent virus or bacterium) and biodistribution or shedding data from preclinical studies.

Regarding assays to measure shedding, the FDA recommends a stepwise approach based on the replication competence of the product. For replication-competent products, initial analysis may be done by using *in vivo* amplification. Clinical samples that are scored positive for product-specific nucleic acids may be analyzed further with infectivity or growth-based assays. Some products may be shed as infectious virus or bacteria, but detection of nucleic acids by quantitative polymerase chain reaction (PCR) may not indicate the presence of infectious virus. In contrast, replication-incompetent or -deficient products shed to a lower extent than replication-competent products and are not capable of establishing an infection and *in vivo* amplification. For these products, PCR is adequate as the primary assay. Regardless of the product being tested, the analytical assays used should be qualified to meet minimal performance capabilities (e.g., sensitive, robust, repeatable) and be suitable for the intended purpose. To assess the potential of transmission to untreated individuals due to shedding, the analysis of shedding data for oncolytic or VBGT products should address the nature of the shed product (i.e., as infectious or noninfectious) and the extent of shedding. For each sample type, the degree of shedding should be defined in terms of time, dose, and regimen.

A full shedding report should be provided in the BLA and include the following:

- A comprehensive shedding profile of the product in patient population that includes analysis of shedding data,
- Data and analysis of clinical monitoring for transmission (if conducted),
- The sponsor's assessment of the potential for transmission, and
- Other relevant information on the potential of the product and/or parent strain to infect humans and cause disease.

Several components of the data collected can be used to assess the potential for transmission to untreated individuals due to shedding. Such an assessment should consider whether the product was shed, whether the shed product was infectious, whether the amount of infectivity was comparable to that needed to initiate an infection, and whether the clinical sample containing the shed product represents the natural route of transmission. Monitoring of untreated individuals for transmission depends on the type of product. For example, the probability of transmission of VBGT and oncolytic products is low, and monitoring for transmission of these agents in clinical trials is usually not required. If more data are needed to assess the potential for transmission, investigators should consult with OCTGT in developing a monitoring plan.

The draft FDA guidance is available in the guidance section of the CBER website at <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm404050.htm>. The comment period for the draft guidance is closed. Staff is in the process of reviewing submitted comments.

Regulatory questions can be directed to the OCTGT regulatory management staff via email (cberoctgrms@fda.hhs.gov) or telephone (240-402-8361) or by contacting Dr. Lori Tull at lori.tull@fda.hhs.gov.

B. RAC Discussion

Dr. Wooley requested more detailed information on the nature of and recommendations for positive controls such as those used in the recommended PCR and other assays. She noted that samples used in the standardized assays are not always the same fluids collected in the actual shedding studies and analyses. Thus, the results from the test assays are not directly applicable or comparable to the samples being collected for analysis. This discrepancy underscores the importance of positive (spiked) controls in establishing a limit of detection by using the same type(s) of specimens being collected in the clinical study. Dr. Taraporewala explained that while the guidance asks sponsors to consider the sample matrix and other aspects that could affect that recovery, the guidance does not include specific details for these assays because of the broad range of products covered by the guidance. The type of samples used to determine detection limits will vary on a case-by-case basis and would be an issue for further discussion with the sponsors.

Per a question from Dr. Kohn, Dr. Taraporewala confirmed that the guidance applies to replication-incompetent products and AAV vectors administered in vivo. Dr. Kohn also asked whether shedding studies on these products should be started once the dose is determined and the product is expected to proceed to a Phase II trial. In addition, he asked whether these recommendations apply to each product, including those with similar vectors or if the same product is being administered by a different route. Dr. Taraporewala commented that for replication-incompetent products, the FDA generally recommends that the sponsor consider doing shedding studies once the dosing regimen has been established, especially if it is not a first-in-human experience. Shedding data should be collected to take into account patient-specific characteristics, potential differences based on route of administration, and modifications to the individual product. This approach allows for the best translation of findings from one clinical study to another.

Dr. Whitley noted the potential confounding of shedding resulting from inadvertent inoculation of virus into a space that is not considered therapeutic (e.g., into the ventricular space for treatment of glioblastoma multiforme events). In such cases, evidence of the agent may be detected, but it may not be replicating virus, and provisions for data collection and assaying would need to be negotiated between the sponsor

and FDA. In addition, Dr. Whitley inquired about monitoring for potential phenotypic and genotypic changes in the replicating agents. For example, because of selective pressure, herpes simplex virus could become a thymidine kinase–negative virus, but clinicians could not treat cases that involve the central nervous system (CNS) due to the lack of available therapeutics that can penetrate the blood-brain barrier. Dr. Taraporewala noted that the advisory committee that met November 2014 to discuss the draft guidance considered these points and made recommendations to address these issues.

Dr. Kohn supported the inclusion of the statements regarding the low risk of shedding from some agents, such as plasmids, and said that shedding studies are not needed for those products. Such statements will help inform concerns regarding risk of transmission in studies of gene-modified cell with a replication-incompetent vector or a plasmid.

C. Public Comment

No public comments were offered.

IV. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Atkins, Curry, Donahue, Kaufman, Kiem, Kohn, Pilewski, Sadelain, and Whitley

A. GTSAB Report

Dr. Kohn presented the GTSAB report for the first quarter of 2015. Within the past 3 months, the OBA received a total of 40 protocol submissions, 38 of which were not selected for public review at this RAC meeting. Of the 38 protocols not selected for public review, 26 were oncology protocols, six were monogenic disease protocols, two were infectious disease protocols, one was a heart failure protocol, one was a brain injury protocol, one was an arthritis protocol, and one was an amyotrophic lateral sclerosis (ALS) protocol. Among these 38 protocols, nine used plasmids, eight used retroviruses, seven used lentiviruses, five used AAVs, four used adenoviruses, two used RNA, two used attenuated *Listeria monocytogenes*, and one used HSV. For the first quarter of 2015, the GTSAB reviewed initial and follow-up reports on 21 serious adverse events (SAEs) from 14 protocols. (Information about these trials was made available on the OBA website after this RAC meeting and will be available in the NIH Genetic Modification Clinical Research Information System, also known as GeMCRIS.) During this quarter, OBA received notification that nine new protocols opened, two of which were publicly reviewed and one of which previously responded to the RAC's public review. An update on another publicly reviewed protocol, OBA protocol #0810-946, "*Phase I Trial of Intratumoral Injection of Vesicular Stomatitis Virus Expressing Human Interferon Beta in Patients with Sorafenib Refractory/Intolerant Hepatocellular Carcinoma and Advanced Solid Tumors with Liver Predominant Locally Advanced/Metastatic Disease,*" was reviewed and discussed later during the current meeting.

B. RAC Discussion

No comments were offered by RAC members.

C. Public Comment

No public comments were offered.

V. Outgoing Members Award Ceremony

Presenter: Lawrence Tabak, D.D.S., Ph.D., Principal Deputy Director, NIH

A. Presentation of Certificates of Appreciation to Retiring RAC Members

Dr. Tabak presented the following departing RAC members with certificates of appreciation for their service on the RAC: Dr. Chatterjee, Professor Dresser, Dr. Hammarskjöld, and outgoing RAC Chair Dr. Kohn. A fifth departing member, Dr. Sadelain, had not arrived by the time that the certificates were presented; and a sixth departing member, Dr. Tianxi Cai of Harvard University, could not attend the current RAC meeting. Dr. Jorgensen recognized Dr. Kohn's extended tenure on the committee, which began in 2010, and thanked him for his leadership as RAC Chair, the position that he has held since 2013. Dr. Kiem will be the new Chair beginning with the next RAC meeting.

VI. Review and Discussion of Human Gene Transfer Protocol #1504-1415: HD-CELL: MSC Engineered to Produce BDNF for the Treatment of Huntington's Disease

Principal Investigator (PI): Vicki Wheelock, M.D., UC Davis Medical Center

Co-investigator: Jan Nolte, Ph.D., UC Davis

RAC Reviewers: Drs. Curry, Kohn, and Zoloth

Ad Hoc Reviewer: Dr. Galpern, NINDS

A. Protocol Summary

Huntington's disease (HD) is an incurable, inherited genetic disorder causing progressive neurologic deterioration and death within 15 to 20 years of onset. It is an autosomal dominant disorder caused by a gene mutation in the huntingtin gene, leading to production of the mutant huntingtin protein (mHTT). Children that have one of an affected parent have a 50 percent chance of inheriting HD. The median age of onset is between 30 and 40 years, but symptoms of the disease may start as early as childhood or not until later in life. Approximately 30,000 (one in 10,000) people in the United States are known to have HD, and some 150,000 are at risk. About 2,000 new cases are diagnosed annually.

HD causes selective loss of medium spiny neurons, which are critical components in brain networks controlling movement, cognition, and emotion. As the disease progresses, uncontrolled involuntary body movements may ultimately be replaced by a rigid, immobile state. In the later stages of HD, patients lose the ability to swallow or speak. During the final phase of the illness, patients are entirely bedbound, unable to move their body, and completely dependent on others for care. While cognitive changes may lead to profound dementia, many HD patients retain awareness of self and of their plight. Palliative drugs can reduce the involuntary movements typical of HD and may reduce psychiatric symptoms, but there currently are no drugs or therapies that can delay the onset or slow the progression of the disease. Screening for the mutant HD gene is available, but at-risk children of an affected parent often do not wish to be tested, given the lack of any early prevention strategies and effective treatments.

Several areas of stem cell research offer promise for the development of possible treatments for HD. Research shows that brain-derived neurotrophic factor (BDNF), which is needed by neurons to remain alive and healthy, plummets to very low levels in HD patients due to interference by the mHTT protein. A major impediment for clinical use of neurotrophic factors is their inability to cross the blood-brain barrier. Implanted mesenchymal stem cells (MSCs) have been demonstrated to be very effective delivery vehicles, moving robustly through the tissue and infusing therapeutic molecules into each damaged cell that they contact. Another advantage of MSCs is that their administration does not have to be done with concomitant administration of an immunosuppressive agent. A large number of peer-reviewed proof-of-concept studies have demonstrated efficacy for MSCs, BDNF, or MSCs engineered to produce BDNF (MSC/BDNF) in HD mouse models. Several published articles have implicated improvement of either behavioral or neuropathological deficits in rodent models of HD following treatment with MSC, especially when modified to express BDNF. In randomized, controlled, blinded preclinical studies conducted by the UC Davis team, intrastriatal injection of human MSC and MSC/BDNF in HD mouse models reduced the

rate of striatal atrophy, induced neurogenesis, and enhanced behavioral performance. The team has evaluated the safety of MSC and MSC/BDNF implantation in the brain of mice and nonhuman primates. Furthermore, no stem cell-related SAEs have been reported in clinical studies of MSCs and MSC-like agents, including Phase I and II trials in which MSCs were infused into the intrathecal space of the spinal canal or introduced via intracranial implantation.

The candidate investigational product for the proposed research is allogeneic human MSCs engineered to secrete brain-derived neurotrophic factor (MSC/BDNF). The planned Phase I trial (“HD-CELL”) will be an open-label dose-escalation safety and tolerability study of MSC/BDNF in adult patients with early-stage Huntington’s disease. Delivery of the agent will be via neurosurgical implantation into the bilateral striatum. The target enrollment is 20 to 26 subjects, with five to seven subjects in each of four dose cohorts. Participants will be recruited from subjects who have completed at least 12 months of pre-operative assessment under a lead-in observational study, the “PRE-CELL” protocol, to establish disease evolution. Enrollment to each group will occur in a sequential manner. Subjects will be followed per protocol for 12 months after cellular therapy implantation. Participants must have a caregiver or other close individual willing to report observations about the patient on standardized forms. The design will allow for comparison of disease trajectory pre- and post-implantation. As an added safeguard, the MSC/BDNF product will have a suicide switch to eradicate the modified cells if any adverse events (AEs) occur.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of this protocol. The study was found to warrant public review because it involves the first known trial proposing to use a lentivirus to express BDNF and the first gene transfer trial for individuals with HD. Also warranting review is the plan for intrastriatal administration of mesenchymal stem cells transduced with this vector, which is proposed specifically for research purposes and would not otherwise be performed.

Three RAC members and one ad hoc reviewer provided written reviews of this proposed Phase I trial.

The reviewers found the study to be well designed and the proposed intervention to be appropriate for the target patient population and disease, for which there currently is no effective treatment. The ICD is clearly written and includes extensive detail on the study time points and what participation entails for the subjects.

Dr. Curry found the protocol to be very well written and the information provided in Appendix M to satisfactorily address the cellular engineering and gene transfer aspects of the research.

Dr. Galpern raised the following points and questions regarding points made in the synopsis and/or elsewhere in the protocol document:

- Supporting information should be provided for the statement, “MSC have innate neurorestorative properties when implanted into the nervous system.”
- It is stated that “human MSC and MSC/BDNF reduce the rate of striatal atrophy and enhance behavioral performance in the YAC128 mouse, and induce neurogenesis in the R6/2 mouse model.” The protocol does not specify whether all parameters were assessed in each model. If not, why not? If so, why is there a differential response between models?
- The rationale for the study is “based on the hypothesis that our investigational product will provide reduce striatal cell death and increase neurogenesis.” However, it does not seem that these parameters would be measurable. This point needs to be clarified.
- How “early-stage HD” will be defined needs to be delineated.
- It is stated, “The rate of change in clinical, structural magnetic resonance imaging (MRI) and biomarker measures has been analyzed and will be carried forward into HD-CELL.” It is not clear that changes in the parameters will be linear. As such, comparison of rates of change between periods may not be appropriate.
- The introduction states, “Although MSCs will not persist longer than several months, the duration of BDNF production should be adequate enough to produce a beneficial effect in the HD striata.

We hypothesize that the neurorestorative effects of BDNF will outlast the survival of MSCs.” Is it known how long the current cells would produce BDNF, and are there data to support this amount and duration as being sufficient to mediate an effect?

- Similarly, subjects will be followed for 12 months after implantation. However, the implanted cells are expected to be functional for a shorter period. Is there a need to inject additional cells to maintain a benefit?
- With respect to the study design and planned doses, what is the rationale for the number of subjects per cohort and for the dosages of cells selected?

Dr. Galpern requested clarification regarding the following eligibility criteria:

- Since this is an early-phase study with the primary aim of assessing safety and tolerability, it would be helpful to further justify why early rather than late-stage subjects should be included in this initial study.
- How will “minimal cognitive impairment” and “stable psychiatric status” be defined, including whether participants need to be stable on medications for a specific period?
- Subjects must be able to give informed consent, but individuals with a Montreal Cognitive Assessment (MoCA) score greater than 13, which suggests significant cognitive impairment, are eligible to participate. Since a score below 26 suggests impaired cognition, assessment of the subject’s capacity per the proposed MoCA cutoff to give informed consent seems warranted.

Further information was requested with respect to the introduction section of the protocol:

- Provide supporting references where appropriate (e.g., “BDNF levels are very low in HD transgenic mice and humans with HD. In rodent models of HD, BDNF has been shown by many groups to ameliorate symptoms and to extend survival”).
- Are improvements in the behavioral phenotype and reduction in striatal degeneration after MSC/BDNF administration in HD mouse models also seen with nonengineered MSCs? If not, what are the supporting data for use of nonengineered MSCs as an active arm of the study (rather than as a placebo arm)?
- Reference is made to ongoing studies that are not yet published or are in the process of being completed. The protocol does not appear to specify data from the “in-press” article. Dr. Curry asked whether the investigators should have the results from these ongoing studies before proceeding with a human trial.
- Per the protocol, no infusion-related effects have been reported in clinical trials involving MSC infusion into the brain. How do these trials compare with the proposed study with respect to cell number and volume?
- The investigators should consider presenting the results of their double-blinded studies (protocol p. 21) in tabular form so that the data are easier to follow.

Dr. Galpern posed the following concerns about the study objectives, design, and procedures:

- The goal of this research is “to restore levels of BDNF in HD patients closer to the levels seen in unaffected controls.” Levels that are considered “normal” need to be defined, and data to suggest that a “normal” level of BDNF is achievable by using the suggested cell numbers should be provided.
- The rationale for not blinding the study is not clear. The investigators should consider blinding the rater and not informing subjects which dosing cohort they are assigned, in order to reduce bias and potential confounding of the study outcomes.
- It is not clear why the total number of subjects per cohort would range from five to seven rather than be a specific number.
- Precisely where the cells will be injected (e.g., caudate, putamen) and whether there will be more than one needle track should be specified.
- A delay between dosing cohorts is planned, but the rationale for a 2-week pause in enrollment between cohorts is not provided. The investigators should consider staggering enrollment between subjects within a dosing cohort for safety.
- The protocol states that one of two delivery systems will be used for cell implantation. To reduce technical variability, why has one system not been selected?

- Per the protocol, “the subject will be considered enrolled once all eligibility criteria are met and the cell product is administered.” However, baseline data are collected before a subject receives the cells. It is important that those who receive cells are not different than those who met eligibility criteria but did not receive cells; as such, all subjects should be considered enrolled if they sign the ICD.
- Post-surgery lumbar punctures are proposed, but it is not clear whether pre-surgery or baseline CSF samples are collected for comparison.
- The time window after screening that surgery would be done should be specified.
- The current list of expected AEs includes such things as fractures and motor vehicle accidents. It is not clear that such events would be expected to occur due to this intervention. The list should be updated to reflect anticipated research-related events. In addition, it would be helpful to provide further details regarding the types of behavioral and cognitive effects that might be expected in relation to BDNF.

Dr. Galpern noted the following issues relating to statistical methods and analyses:

- As currently proposed, the data and safety monitoring board (DSMB) will plan to meet 2 weeks after the last subject in each cohort has been treated. For a Phase I study, it would be more reasonable for the DSMB to review the AE data in real time.
- The proposed stopping rules seem rather liberal. For example, the protocol states that the study would stop if a CNS infection, a clinically significant hemorrhage, or an inflammation were noted in two or more subjects and if significant neurological, cognitive, or psychiatric deterioration occurred in three or more subjects. The investigators should consider stopping the study if clinically significant events are noted in a single subject.
- The planned sample size is not well justified. For a safety and tolerability study, the sample size could be based on a threshold of acceptable tolerability. There should be a statistical rationale for the number of subjects enrolled (e.g., “X subjects will be enrolled in order to ensure with at least 85 percent probability that the tolerability rate is at least 75 percent”).
- The protocol states, “No imputations will be performed for missing data.” Such an approach can result in biased conclusions, because there may be important reasons that the data are missing. This approach should therefore be reconsidered.
- It is not clear whether the analyses will involve within-subject or between-group comparisons. The presentation of the YAC128 mouse model data (protocol p. 21) is somewhat misleading. Upon close review, these data did not show overall significance in the cited studies. Thus, any post hoc analyses for between-group differences are not appropriate.
- How will efficacy data be analyzed if subjects begin medication for treatment of one or more of the outcomes being assessed (e.g., chorea)?

Some additional minor comments, clarifications, and questions were identified:

- The protocol section on the dosing administration plan and regimen should indicate that the activities described will take place after the subject has signed the ICD.
- The protocol needs to consistently specify whether it is the participant or the investigator(s) who make an assessment regarding the Clinical Global Impression (CGI).
- Initiation of new medications will be evaluated by the “clinical team,” but it is not clear who these individuals or clinicians are.
- The protocol needs to clarify that the MoCA will be obtained not only at visits 1 through 8 but also at screening, because the score on this assessment is an enrollment criterion.
- Assessments done at the enrollment visit and post-implantation assessments are included under the section titled “Screening Assessments.” Post-implantation assessments should be moved to the appropriate section of the protocol.
- Clarification is needed on whether a statement describing tests to enhance safety evaluations in the future planned Phase I cellular therapy trial refers to the proposed study or a different study.
- The number of planned lumbar punctures (LPs) and the visits at which LPs will be done need to be noted consistently throughout the protocol.
- Standardize terminology for AE documentation should be added to the protocol (e.g., in a Common Terminology Criteria for Adverse Events table).

- As currently proposed, unexpected SAEs that are related to the study drug and occur after the study will be reported as expedited reports but will not be included in the clinical study. The investigators should re-consider this plan and include all such events in the clinical study report.
- The items listed under the “Primary Endpoints” section of the protocol are written as aims rather than study endpoints and should be rewritten accordingly.
- The protocol states, “No data from the study will be published, presented, or communicated without the mutual agreement of the Sponsor-Investigator.” It would be preferable for the investigator to ensure that the data are published.

Dr. Kohn noted that the biosafety of the recombinant DNA component of the investigational product, ex vivo transduction of MSCs with a replication-defective lentiviral vector, does not raise significant concerns. The more relevant safety issues concern potential adverse neurological complications from implanting the BDNF-expressing cells into the CNS as well as potential immune or inflammatory responses to the allogeneic cells.

Dr. Kohn requested that the investigators describe and/or provide maps for the lentiviral packaging plasmids that will be used in the proposed research. He also asked whether they are second generation with *rev* (or *rev* and *tat*) expressed from the same plasmid as *gag/pol* or are more similar to the third-generation packaging system, with *rev* from a fourth plasmid.

The release test for replication-competent lentivirus (RCL) testing of the vector preparation and for testing the subjects is not clear. The bioassay for RCL (as described in Figure 23 of Appendix M) indicated absence of detectable HIV p24 *gag* by enzyme-linked immunosorbent assay. Additional details about the assay were requested, including how long the transduced MSCs were passaged before testing for RCL, what indicator cell line was used, and whether this assay has been validated for sensitivity.

Dr. Kohn asked the investigators to comment on the expected duration of persistence of the allogeneic MSCs in subjects who will be treated with immune suppressive medicine. The murine studies seem to be either matched murine cells in immune-intact disease models or xenogeneic murine cells in immune-suppressed or immune-deficient mice. Documentation should be provided for any studies done by using the equivalent of allogeneic MSCs in non-immune-suppressed mice (e.g., C57 into *balb/c*) to determine persistence duration in a setting resembling the clinical scenario. Dr. Kohn also asked whether the retention study presented to assess persistence of cells was the only such study to use a sensitive method, such as PCR of injection sites at necropsy, in addition to the less sensitive noninvasive bioluminescence to assess persistence of human MSCs in vivo. If so, what is the level of detection by the PCR method for human DNA sequences?

Is the recombinant human BDNF protein produced completely wild-type in amino acid sequence so that risks of immunological sensitization to it are minimal? Is there any way that participating in this study could preclude participation in future studies of a similar approach? For example, could a subject in a low-cell dose arm become unable to receive the high dose if there is evidence for efficacy at that level?

Statements in the ICD are inconsistent regarding the potential for direct benefit. Some statements imply prospect of benefit beyond that appropriate for a first-in-human study. Such statements may need to be consistent and should be softened in order not to overstate or overpromise the benefit of participating in the study. An alternative phrasing might be, “It is not known whether you will benefit from this research study.”

Use of the words “treatment” and “therapy” could be misleading to patients. The investigators should add a descriptor such as “experimental” or use other phrasing that alludes to the intervention’s investigational status.

Since the duration of persistence of the modified cells is unknown, statements concerning withdrawal from the study need to indicate that withdrawal from the study may not be equivalent to withdrawal from the “drug,” which could persist long term. Thus, “withdrawal” describes withdrawal from monitoring and evaluations that may be important for safety assessments.

Drs. Galpern and Zoloth commented that the number of evaluations and the length of some study visits (e.g., 7 to 9 hours for the screening visit) seem burdensome. The follow-up process is rigorous and will include five spinal taps and six MRIs in addition to other procedures and tests, on top of a minimum of a year in the PRE-CELL study. Subjects and caregivers must be clearly informed about how intrusive the study might be. Patients may have only a brief period of capacity, and this project will take much of their attention. The benefit of this altruism should be noted, given the sustained intensity of ongoing participation. In general, study participants will be fully competent individuals with informed consent and decision-making capacity. Having the care provider's informed consent is important, however, should the patient's condition decline over the course of the study.

Dr. Zoloth identified additional issues for further clarification, as follows:

- The animal murine model is male. Since HD may have different effects in men and women, why was the intervention not tested on females? Furthermore, the information about nonhuman primates seems to describe the use of humans. Were nonhuman primates used? In addition, many of the animals reportedly died prematurely, but the reasons for their early death are not specified.
- The Total Functional Capacity (TFC) Scale should be included with the protocol materials.
- Add the provided graphic of the implantation device into the ICD so that subjects will have a better idea of what will be done during surgery. The picture will make the phrase "A small hole will be drilled into your skin" more vivid.
- Exposure to BDNF seems a potentially significant risk to the fetus. Women who are pregnant and/or lactating will be excluded. Women of childbearing potential undergo routine pregnancy testing, but requirements and provisions for birth control need to be clearly delineated. In addition, the suggestion of another invasive procedure (i.e., an IUD) would be important to mention if it is included in the protocol.
- This project takes on several high-risk ideas that require complex, novel methods and theories to work. These ideas need to be clearly described to participants so that they understand the goals of the research and how those will be achieved.
- The ICD does not include a real discussion of what it means to "engineer" the MSCs to produce BDNF. A description of the vector and how a gene is changed needs to be added, using language that a layperson will understand.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers found the presentation to be clear and their concerns and questions to be well addressed.
- Clinical research experience with MSCs extends to patients with diseases other than HD, such as stroke. This experience is described in the response memo and the protocol.
- The primary outcome for this Phase I dose escalation study is safety. Dr. Curry noted that an underlying hypothesis or assumption is that as the dose increases, the investigators anticipate not only increased efficacy but potentially more toxicity. Thus, a goal is to determine a maximum tolerated dose (MTD) of cells injected bilaterally. As written, however, the protocol does not predefine "dose-limiting toxicity" (DLT) or how many of those events would be needed to pause or stop the study from proceeding to the next higher dose or enrolling additional subjects in a dose cohort. Such definition and criteria would help guide the DSMB and the study team to assess the safety and MTD of the experimental products. The NIH Common Toxicity Criteria could be used to specify the frequency and severity of events before patients enroll. These parameters, in turn, could be used to justify a statistically precise sample size. Then, if the investigators want to treat more patients, they can include an expansion cohort at any of the chosen dose levels.
- Dr. Galpern agreed with Dr. Curry's comments and suggestions and questioned the plan to have only five to seven subjects per dose cohort when the power calculation suggested that nine subjects are needed from a statistical standpoint. It might be helpful to revisit the design with the

statistician to ensure that the study is appropriately powered so that the opportunity to answer the research question is not lost.

- Dr. Galpern remained concerned about the MoCA cutoff at a score of 12, which, by definition on that scale, indicates substantial cognitive impairment. She questioned whether it would be reasonable to consider a higher cutoff. The proposed cutoff of 12 could be justified if there are data showing that those with a low MoCA score have the capacity to give informed consent and perform satisfactorily on other cognitive batteries. The investigators may want to consider using the McArthur capacity assessment tool, which has been used for persons with Alzheimer's disease and early-stage Parkinson's disease.
- Dr. Galpern also asked whether a patient without consent capacity at the time of enrollment could enroll with proxy consent from a family member.
- The protocol and consents discuss having an initial group that is given MSCs that are not modified to support BDNF. The rationale for including this group is not clear.
- Dr. Galpern expressed concern about the bias that will be introduced in the absence of blinding the subjects. Although the dose escalation will be sequential and subjects will likely have an idea of how many other patients have enrolled, it is not clear why subjects need to know which cohort they are in. It would be preferable to try to minimize access to this information to the extent possible. The investigators should consider not providing specific information about dose group while recognizing that subjects may find out anyway, particularly when interpreting efficacy outcomes.
- Dr. Galpern asked whether the criterion for depression for the proposed study is the same as that for the PRE-CELL trial and whether there is any flexibility for this criterion, given the possibility of progression of HD over the course of the study.
- Dr. Zoloth requested further information and clarification regarding whether only male mice or both male and female mice were studied by using the investigational product. Studying only one sex raises concerns regarding generalizability and potential problems or effects that could be missed in the other sex. For example, if one side effect of the intervention is weight gain, which is seen predominantly in female HD mice, and patients are being assessed for depression, how does the study control for potential confounding of this side effect in women? Dr. Zoloth also inquired about the reasons that some mice died prematurely.
- Dr. Zoloth commented that the phrase, "You may not receive benefit in the study," in the ICD sounds like a subject could benefit from participating in the protocol because it introduces the possibility of benefit. Subjects need to know from the outset that because this is a first-in-human trial, there is no potential for direct benefit. This needs to be stated as clearly as possible, particularly given concerns about the placebo effect in an unblinded trial. The ICD could include a statement about the participant's altruistic gesture to contribute to medicine.
- The investigators need to make sure that the ICD does not refer to the investigational product and intervention as "therapy". Use of the term "therapeutic trial group" is also not appropriate.
- Participants need to understand that once the modified cells are infused, withdrawal from the study. Rather, as noted in the written reviews, "withdrawal" refers to withdrawal from monitoring and evaluations that may be important for safety assessments. Even if the suicide gene is activated, and the cells commit suicide, they are still in the body and can't be extracted. A discussion of any options to "withdraw" from the suicide gene component of the cells should also be considered.
- Dr. Zoloth pointed out that the sham surgery presents considerable burden and inconvenience and increased risks to subjects. Under this protocol, the sham surgery is not simply one intervention. It involves six MRIs and multiple spinal taps in addition to the three LPs done in the PRE-CELL study. Thus, the follow-up is quite arduous, especially for this population, which is at a fragile point in their disease.
- Dr. Zoloth inquired about the rationale for allowing enrollment of individuals with a TFC Scale (TFCS) score between 9 and 13, which includes a very broad range of capacity. There also appears to be a discrepancy between this eligibility criterion and what is anticipated for patients enrolled in the study. Patients who cannot give consent will not be screened, but there is a caregiver consent and references to the subject's legally authorized representative. The chart of the TFC Scale should reference the stage of the study, not (only) the stage number.

- Participants should be provided a graphic of the apparatus that will be used for infusion of the modified cells into the brain so that they have a clear understanding of what the process and device entail. As written, it sounds like there will be a small injection into one small hole in the skull. However, a lot of apparatus is used for the procedure, and the full set-up is relatively large.
- Dr. Zoloth restated her concerns about risks if a subject or partner of a male participant becomes pregnant after infusion since the cells cannot be removed from the brain. She asked how this exposure risk and contraception will be addressed, including whether birth control is provided and/or paid for and how the investigators plan to assure that pregnancy prevention measures are used.
- One aspect of this research that Dr. Zoloth found particularly interesting is the use of several high-risk technologies at once. The project involves genetic engineering and injection of modified cells directly into the brain in as part of an effort to develop a therapy for a fatal neurologic disease for which there currently is no effective treatment. Dr. Zoloth felt, however, that the study complexity and technological aspects of the research need to be more vividly expressed in the ICD. Patients also need to know about the other options available to them, including other trials that are investigating aggressive approaches to symptom control.
- Dr. Kohn considered the trial to be very well designed and for a very important medical disease. The PRE-CELL lead-in trial to obtain a comprehensive baseline status on the patients and for the HD-CELL study appears to be a novel approach and is a strength of this research.
- Dr. Kohn supported the decision to drop the MSC-only arm of the trial. It isn't clear that this aspect of the study would add much to the scientific value of the project.
- Dr. Whitley challenged the proposal to stop the study if clinically significant events are noted in a single subject. Such a stopping rule could be restricting for the events as defined, in particular for the events that are related to the procedure vs. MSCs. Dr. Whitley noted that while the complication rate for stereotactic procedures is relatively low, it is not zero. For example, the hemorrhage rate, depending on the study, is between 3 and 10 percent. The investigators may want to retain the stopping rules at two or more patients, per the written protocol. Dr. Kohn added that the investigators also need to consider adding separate stopping rules to temporarily pause enrollment while data are being reviewed (e.g., for the interim analysis).
- Dr. Donahue noted the amount of information being collected about the potential patient population before they even are screened for and enrolled in the proposed study. He expressed concern about the possibility of inadvertently introducing bias into the upcoming trial (e.g., enrolling patients with the most rapidly progressing disease first), even with the most altruistic intent. He asked about the transition from PRE-CELL to HD-CELL, including how the investigators decide when someone makes that transition, how they will avoid bias, and how they might use the information from PRE-CELL to introduce some balance into the study groups.
- The investigators plan to use a multiplicity of infection (MOI) of 10 to 20 with a goal of getting two copies of the integrant per cell. Per this information, Dr. Hammarskjöld asked whether that is the average or a range, with some cells having zero copies. Further detail as to how this is judged and how many copies result was requested. Dr. Hammarskjöld also asked about the timeframe for the expansion of cells after transduction and before the cells are frozen. An additional question was whether the investigators have done or are planning to do any kind of integration site analysis on the lentivirus vector. This analysis would be important not only for this specific study but also for better understanding the lentivirus vector. Dr. Hammarskjöld asked whether there is a *Tk* gene in the vector.

D. Investigator Response

1. Written Responses to RAC Reviews

The protocol has been revised to include supporting references for the statement that MSCs have innate neurorestorative properties when implanted into the nervous system. The PI noted that the team published a review on this topic in 2012 and that an additional review to support this finding is in press. Supporting references have also been added for other research findings as noted by the reviewers.

The protocol now states that the rationale for the proposed study is “based on the hypothesis that our investigational product will reduce striatal cell death and increase neurogenesis.” The design of the trial has been revised; only MSCs that have been modified to express BDNF will be administered, the trial will no longer have an MSC-only arm.

The PI noted that BDNF levels in human and HD brains are limited to data collected in the antemortem brain. The relationship between CSF and brain parenchymal levels of BDNF is not known.

The PI clarified that the double-blinded proof-of-concept studies in mouse models have been completed. The results were reported in the pre-IND package submitted to the FDA in May 2015. The protocol has been updated accordingly. The team tested the potential development candidates in two mouse models of HD (YAC128 and R6/2 [CAG 120]). Behavioral and histological analyses were performed in both models. The investigators stated that results using the human development candidate MSC/BDNF support the translation of the extensive body of literature showing BDNF efficacy in transgenic mouse models into clinical trials.

The models for the primary efficacy studies were chosen due to their unique features and disease progression as it relates to HD. The PI acknowledged that while there is no perfect animal model for HD or any neurodegenerative disease, the YAC128 and R6/2 models capture key phenotypes of HD. The YAC128 mouse model contains the full human huntingtin gene and presents a subtle disease progression resulting in transient behavioral deficits and a slow, progressive cell loss in the striatum. The YAC128 mouse model of HD (aged for 7 months in the team’s studies) does not demonstrate significant motor deficits compared to wild-type but does display increased levels of anxiety. Transplantation of MSC/BDNF significantly reduced levels of anxiety and striatal atrophy, both of which are hallmarks of HD. In contrast to the Normosol-treated (control) YAC128 mice, the mice treated with MSCs and MSC/BDNF did not have significant levels of striatal atrophy. The R6/2 model contains only the toxic fragment of exon 1 and displays the early onset of a severe behavioral phenotype with shortened survival. Striatal implantation of MSC/BDNF increased the lifespan of R6/2 mice. There also was a significant increase in neurogenesis-like activity in the subventricular zone in R6/2 mice receiving transplantations of either MSCs or MSC/BDNF. The R6/2 model does not display robust neuronal loss, most likely because the lifespan of these mice is too short for the neuronal dysfunction to result in a quantifiable and significant loss of cells.

The team has designed the test parameters for the proposed research for both the strengths and limitations of both models. For example, it would not be experimentally sound to study survival in the YAC128, as they do not exhibit a shorter lifespan than their wild-type littermates; or to study the ability of the MSC to prevent neuronal loss in the R6/2, as there is no robust cell loss in the striatum. Therefore, the investigators tested neurogenesis and lifespan in the R6/2 model and behavior and striatal atrophy in the YAC128 model.

The proposed study is designed as a single-site, open-label dose-escalation Phase I trial. The PI and Co-PI believe that given the complexity of symptomatology in HD patients, care for subjects following cellular therapy implantation should be provided at a highly experienced clinical site. The UC Davis–based program is uniquely qualified and has been designated as a Huntington’s Disease Society of America Center of Excellence since 2001. The multidisciplinary care team has followed more than 350 patients, and the investigators have 17 years’ experience in HD clinical trials. It will be difficult to blind the subjects to their dosing cohort, because they will be aware of when the trial starts and because the study is designed to progress from low- to medium- to high-dose treatment arms in a sequential manner after study initiation. The raters will be blinded, however. Unified Huntington’s Disease Rating Scale (UHDRS) motor examinations are videorecorded in the PRE-CELL studies, and a blinded rater will review both pre- and post-implantation examinations. In addition to clinical assessments, structural MRI and biomarkers analysis will be performed in a blinded fashion, and statistical analysis will be blinded.

As part of the inclusion criteria, early-stage HD is defined as a UHDRS TFC score of 9 to 13. The recently completed Creatine Safety, Tolerability, and Efficacy in Huntington’s Disease study used the same definition for early HD, while the 2CARE study defined early-stage HD patients as those with a TFC score between 7 and 13. The TFC score that the investigators chose for the proposed protocol corresponds to

“HD1” (TFC 11 to 13) and “HD2” (TFC 7 to 10) in the TRACK-HD study, a prospective observational study in more than 300 subjects with pre-manifest or manifest HD. The supporting reference for this determination was provided.

In the TRACK-HD study and other longitudinal HD studies (per references cited in the response memo), rates of change in many clinical and structural MRI measures were found to be linear across three years in early-stage HD. For structural brain imaging, Ross (2014) reported, “Studies using raw volumes to calculate longitudinal change suggest that once atrophy begins, the rate remains fairly constant and is significantly faster in those with higher CAG repeat lengths.” However, rates of change may show considerable variation between measures and between patients. The investigators are analyzing the rates of change for all subjects and for each individual in multiple measures.

Under the proposed study, participants will receive a single dose of cells and then will be followed closely for one year to assess safety, tolerability, and any potential signs of efficacy. Based on preclinical studies, the investigators hypothesize that MSC/BDNF may slow disease progression via two mechanisms: MSC will regulate inflammation, promote neuronal growth, and reduce apoptosis within the striatum, while BDNF may enhance the survival of medium spiny neurons and induce neurogenesis. Clinical and/or neuroimaging effects from MSC/BDNF implantation may be noted early or after delay and may be transient or persistent. This proposed Phase I study will permit exploration of the time course (both onset and duration) of clinical and imaging changes as well as dosage effects, with findings used to design possible future trials in which MSC/BDNF would be re-implanted to extend clinical effects.

The rationale for enrolling patients with early-stage HD is based on the proposed mechanism of action of MSC and MSC/BDNF, which is to slow the rate of medium spiny neuron loss and enhance neurogenesis. At the time of HD diagnosis, previous structural MRI studies have demonstrated that the striatal volume has already decreased by 50 percent. Therefore, the window for a potential therapeutic effect will be markedly reduced in later-stage patients. Furthermore, a previous HD neurotransplantation study in subjects with a mean TFC score of 6.57 reported symptomatic brain hemorrhage in three of seven subjects, suggesting that neurotransplantation in more advanced HD patients would likely present greater safety risk.

The inclusion criterion for cognitive status and capacity has been changed to be “mild cognitive impairment but not dementia.” This criterion is defined by performance on neuropsychological testing in combination with measures of everyday function including the TFC score, UHDRS independence score and the Everyday Cognition (ECog) scales. To be eligible, subjects must function independently in their daily life. Those who are not still gainfully employed or may require some assistance in complex instrumental daily activities will be eligible if their TFC scores are 9 or above. In addition, subjects must be able to engage meaningfully in neuropsychological testing. Participants may demonstrate some difficulty on the neuropsychological test battery but must not be near floor performance because of the impact on the ability to longitudinally assess subjects over time and monitor for further deterioration. The investigators have confirmed that cognitive performance of PRE-CELL subjects is comparable to that of subjects in other HD studies (e.g., cognitive assessment battery for HD, TRACK-HD).

For PRE-CELL, stable psychiatric status is defined as no evidence of any of the following conditions for at least one year prior to enrollment: suicidal ideation with intent of injury, or suicide attempt, psychosis, severe irritability or aggression, and severe depression. All PRE-CELL subjects undergo a formal psychiatric interview at screening and at visits 1 through 4 by a psychiatrist with extensive experience with HD. The interview is conducted with both the patient and their care partner and then separately with the care partner. The psychiatrist also completes the Columbia Suicide Assessment Scale and the Problem Behaviors Assessment-short form. The psychiatrist reviews subjects’ status at each visit and determines if they are psychiatrically stable. Changes in psychiatric medications and referrals for therapy are permitted for worsening of psychiatric symptoms. Any subject who develops new onset suicidal ideation with intent of injury, suicide attempt, onset of psychosis, or severe behavioral disorder will be withdrawn from PRE-CELL and will not be eligible for enrollment in HD-CELL.

The MoCA cutoff of 13 was recommended by a neuropsychology consultant who is an expert in HD cognition and has served as a PI or consultant on several HD trials. While a MoCA score below 26 does suggest impaired cognition, the MoCA score is dependent on educational level and baseline intelligence. Due to the potential for cognitive decline as an AE of MSC/BDNF implantation, subjects enrolled in the HD-CELL trial will be asked to designate a research proxy. A capacity assessment at screening and each subsequent visit will be added to the study.

The choice of bilateral implantation is based on the pathophysiology of HD involving bilateral striatal degeneration and the risk-benefit ratio for each subject. Although the proposed protocol is a safety and tolerability trial, the team also hopes that the investigational product may offer some benefit to patients. The PI notes the precedent for this consideration: Previous Phase I and II studies of neural transplantation in HD, Parkinson's disease, and other neurological diseases have performed bilateral implantation.

The rationale for the number of subjects per dose cohort is based on the investigators' intention from the outset of this project to treat five patients per dosage group, with a target enrollment of 26 to 40 patients from the PRE-CELL trial. The accrual ceiling takes into account a probable attrition rate of 20 percent based on previous HD clinical trials. Enrollment in the Phase I HD-CELL trial will be offered to as many patients as possible who have completed a minimum of one year's participation in PRE-CELL and meet the inclusion criteria for HD-CELL. The upper range of seven subjects per group was set to accommodate a higher number of PRE-CELL enrollees.

The team consulted a biostatistician regarding statistical justification for the sample size for the proposed open-label pilot study. The biostatistician modeled several scenarios (as presented in the response memo), which suggested that a sample size of nine subjects per cohort would provide an acceptable tolerability rate if one or two SAEs are allowed. However, given that this is an uncontrolled, open-label pilot trial of a very novel treatment that is neurosurgically delivered, nine subjects per cohort seemed an excessively large number of subjects. The investigators therefore have proposed the smaller sample size of five subjects per group to assess safety and tolerability and to reduce risks.

The PI clarified that the primary analysis will compare the rate of change in clinical, imaging, and biomarker measures between dosing groups. Secondary analyses will assess rate of change in these measures within subjects. It was determined a priori that post hoc analyses would be performed for each independent measure (i.e., behavioral and histological measures) between the wild-type (negative control) and Normosol-transplanted (positive control) YAC128 or R6/2 to validate that the chosen measure displayed a phenotypic profile consistent with the disease progression.

The investigators recognize that analysis of efficacy data is problematic in HD studies if subjects begin medication for treatment of one or more of the symptoms of the disease, such as chorea. Correcting for this could be done with an approach used for medication effects in Parkinson's disease. For example, tetrabenazine has been reported to produce a five-point reduction in the UHDRS total chorea score. Data have also been reported for the effect of neuroleptic medications on chorea. Other features of HD, including cognitive and behavioral changes, are likely more disabling than motor performance. To control as much as possible for these factors, efforts will be made to maintain stable levels of psychiatric and cognitive medications during the one-year period following treatment, unless such changes are necessary for the treatment of AEs arising during the trial.

The planned doses of MSC/BDNF, number of cells, and volume of the cell suspension to be delivered were derived empirically, based on the team's proof-of-concept studies in HD transgenic mouse models and prior data from human cellular therapy trials. The target will be the striatum (caudate and putamen). Unlike fetal or stem cells intended to integrate into the striatum as a cellular replacement therapy, MSC/BDNF cells are not expected to permanently engraft. The proposed study will use 5×10^6 , 10×10^6 , and 20×10^6 cells per striatum, which is similar to the number of fetal cells transplanted into the unilateral caudate and putamen in HD patients in a trial aimed for cellular replacement and a SanBio study of engineered MSC in stroke. The number of cells is lower than that used in the open-label trial of MSC implanted into the subventricular zone in Parkinson's disease (1.2×10^8 cells). The planned volume of

cells for the proposed study is 200 μL per striatum, which is comparable to the volume of 240 μL per striatum of fetal cell suspension in the European HD network trial. Based on these considerations, the investigators believe that the selected dosages and volumes are conservative and consistent with a primary aim of subject safety.

The doses of BDNF delivered to the striatum will be maximal at implantation and then likely decrease over time. While studies in both HD transgenic mouse models and in human HD postmortem tissue reveal reduction in BDNF levels, the level of BDNF in antemortem striatum is unknown. The investigators believe that the differences in mouse brain size and homology with human brain preclude a simple linear scaling up from transgenic mouse to human. The initial dosage of BDNF to be delivered to the striatum will be 62.5 ng/24 hr for 5×10^6 cells, 1.25 $\mu\text{g}/24$ hr for 10×10^6 cells, and 2.5 $\mu\text{g}/24$ hr for 20×10^6 cells. These doses have not shown toxicity in xenotransplant studies.

Because medium spiny neuron cell and volumetric loss are present in both the caudate and the putamen, the investigators plan to target both structures. The target regions of the brain include the motor circuit primarily localized to the post-commissural putamen, the frontal-striatal circuits involved in cognition, and the limbic-striatal circuits involved in behavior and mood. These circuits are affected in HD and responsible for the most devastating aspects of the disease. The investigational products will be delivered by using either the radial branched deployment (RBD) system, which is currently undergoing FDA review, or an alternative cannula system, not both. The investigators consider the RBD system the ideal system for cellular therapy implantation and anticipate that it will be approved before the initiation of this study. As for other options, multiple neurotransplantation centers have used the Hamilton syringe, and other cannulas may be adapted for use for cellular transplantation. The SmartFlow® cannula system received FDA clearance in 2011 and has been recommended by the team's neurosurgical consultants as an alternative to the RBD system. The advantage of the SmartFlow system is its compatibility with the ClearPoint® injection system, which is used for interventional MRI (iMRI), which the investigators intend to employ to optimize targeting of the striatum and enhance safety of cellular delivery.

If the RBD system is approved by the FDA, then one needle track per side of the brain will be used. If an alternative injection system is needed, then two or more needle tracks per side of the brain will be used. The European HD Network has implanted multiple HD patients with human fetal whole ganglionic eminence tissue fragments by injecting a total of six tracks into each striatum (two in caudate, two in precommissural putamen, and two in post-commissural putamen). The researchers in that network use the same stereotactic technique for targeting and report no post-operative complications.

The review in *Regenerative Medicine* (in press) discusses the use of nonengineered MSCs as a therapeutic option for HD. Per this review, the hypothesized mechanism of action for the majority of the papers showing efficacy is through release and increased levels of BDNF. In the UC Davis team's lab, nonengineered MSC also showed a beneficial effect, specifically when quantifying neurogenesis. While both engineered and nonengineered MSC transplantation displayed increased levels of endogenous neurogenesis in the subventricular zone, only the animals that received cells engineered to overexpress BDNF saw a functional improvement, in measures of anxiety, lifespan, and striatal volume. These outcomes were likely due to the ability of BDNF to aid in the chemotaxis to the striatum and maturation of immature neurons from the subventricular zone.

The human BDNF sequence was PCR-amplified from normal human MSC and is completely wild type. The risk for sensitization should therefore be minimal.

The lentiviral packaging system that will be used in the proposed study is a three-plasmid system, consisting of a *gag/pol* plasmid, an envelope plasmid (VSV-G), and a transfer vector plasmid. The *gag/pol* plasmid contains *tat* and *rev* on one plasmid. Since the pCCLc vector has an enhanced CMV 5' promoter, it is *tat* independent. The 8.9 *gag/pol* plasmid has *tat*, which is necessary for second-generation lentiviral vectors (for use with wild-type 5' long terminal repeat [LTR]) but not for the current third-generation lentiviral system. The 8.9 *gag/pol* plasmid is used in the three-plasmid packaging system, even though *tat* is not needed. A four-plasmid system (RSV-REV, pMDL *gag/pol*, VSV-G, and the transfer plasmid [i.e., the vector containing the gene of interest]) could be used, but there have not been any studies to show

that this is safer than the three-plasmid system. Two maps for the plasmids were submitted with the response to the reviewers' comments.

Two types of RCL tests are performed. For in-house evaluations, a p24 assay is used to detect RCL after exposure of the vector supernatant to a susceptible cell line. This assay is used to test research grade vector stocks only for the absence of RCL. The clinical grade vector will be extensively characterized, including analysis using an RCL test in which the lentiviral vector will be amplified on C8166-45 cells for five passages, with HIV as a positive control, uninfected cells as a negative control, and HIV-spiked vector as an inhibition control. A standard operating procedure for the RCL assay that will be used in release testing was provided.

Regarding the durability of BDNF production and effect, results of the team's cell retention studies using bioluminescence detected about 50 percent of the implanted cells surviving at 28 days. Cell retention could not be assessed in the proof-of-concept studies, in which animals were sacrificed 6 weeks after xenotransplantation with human MSC and MSC/BDNF implantation. However, results of these studies demonstrated improvement in behavioral performance, reduction in striatal volume loss, and increased lifespan even over this relatively short period. Studies of YAC128 mice transplanted with mouse MSC/BDNF at 4 months and sacrificed at 13 months were found to have significant improvement in rotarod performance and reduced striatal atrophy through the duration of the experiment. Subsequent histological analysis did not reveal surviving MSC after study conclusion.

The limit of detection by quantitative PCR and bioluminescence was determined in a study where the mice were intrastrially transplanted with MSC containing the luciferase gene at different concentrations. The animals were imaged the same day, and their brains were harvested for quantitative PCR at the conclusion of imaging. The quantitative PCR was performed with primers to detect single human genes in the mouse brain. Results indicated that as few as 500 transplanted cells could be detected by this method in a 4-mm cube of mouse tissue and that as few as 16,250 cells could be quantified.

The investigators do not plan to use immune suppression in the proposed Phase I trial due to the innate immune modulatory effects of MSCs. Regarding the expected retention of the allogeneic MSC, while the retention of hMSCs in xenotransplant mouse studies has been reported, the investigators cannot accurately predict retention of the hMSCs in study subjects. Theoretically, the allogeneic hMSCs should persist longer than the xenotransplanted hMSCs in the immune-suppressed mouse models. Due to the karyotypic instability of mouse MSCs, matched or allogeneic transplants in the mice have not been studied.

Regarding the large number of assessments and long study visits, the PI explained that the schedule of activities for PRE-CELL was designed with the Phase I trial in mind. No patients have chosen to withdraw from PRE-CELL in the last 18 months, indicating that the schedule of activities and assessments has been tolerable. A consultant to the project advised the investigators to conduct frequent clinical evaluations in the first weeks and months following implantation based on her experience with fetal cell transplantation in HD. The investigators want to detect early and potentially transient changes in subjects, as well as those that may appear months after MSC administration. One important goal of this study is to identify the time course of clinical, imaging, and biomarker changes. The team will review the HD-CELL schedule to determine whether the number of LPs can be reduced. Baseline CSF samples have been collected in the lead-in observational study, PRE-CELL, at 6-month intervals at visits 1 through 4. All potential subjects in HD-CELL will have undergone CSF collection at least twice before enrollment in the proposed study.

The list of expected AEs was developed from AEs observed in PRE-CELL and reports from other HD observational studies and clinical trials. The list includes fractures and motor vehicle accidents, because both of these AEs have occurred in subjects in PRE-CELL. The section on possible behavioral and cognitive effects of BDNF will be expanded to include possible sensory symptoms, sleep disturbances, agitation, hypomania or other behavioral effects, dry mouth, gustatory changes, and increased sweating, as reported in a prior trial of intrathecal recombinant methionyl human BDNF infusion for ALS.

The two main sources of risk for this study are the risks associated with neurosurgical implantation and the risks related to the development candidate. The investigators propose amending the stopping rules to include the following criteria, as defined in the memo, in one patient:

- Clinically significant intracranial hemorrhage on post-operative MRI;
- Evidence of central nervous system infection;
- Evidence of clinically significant CNS inflammation;
- Significant neurological, cognitive, or psychiatric deterioration from pre-surgical baseline; or
- Clinically significant change in safety-monitoring parameters at visits 1 through 8.

Surgery will be done within 60 days after screening.

The team plans to use a multidimensional CGI developed for HD trials that includes a subject, caregiver, and investigator CGI.

The investigators agreed with the reviewers' other recommendations and have modified the protocol and the proposed ICD accordingly, as described in the response memo.

2. Responses to RAC Discussion Questions

Dr. Wheelock noted the suggestion to define DLTs in advance for safety assessments and for consideration in determining sample size.

Dr. Wheelock found the suggestion and rationale to increase the MoCA score to be eligible to enroll in the proposed study to be reasonable. She noted that a subject in the PRE-CELL cohort has a MoCA score of 16, is working full time, and lives independently but also has a lower education level. The investigators will also consider using the McArthur capacity assessment to screen for ability to give consent.

There are no plans to enroll subjects who cannot give informed consent at the time of study entry. Thus, patients requiring proxy consent will not be eligible to enroll. Study participants need to be able to complete cognitive assessments at the start and over the course of the study. In addition, lack of consent capacity would likely be considered an adverse event, raising the question of whether it would be safe to go forward in that cohort. Persons who are cognitively impaired could be at increased risk of target or off-target effects of BDNF.

The PI explained that the team originally planned to do the first arm using MSC alone but reconsidered this approach after reviewing the proof-of-concept data. The revised design is to test only MSC/BDNF.

Dr. Wheelock acknowledged that blinding subjects to their assignment would be the best approach, and she recognized the potential problems with not blinding subjects. It will be difficult to minimize participants' knowledge of which group they are in, since all subjects will be recruited from the same single-site lead-in study and will have a sense of when they are enrolled relative to other subjects. The team discussed including a sham surgery component in the HD-CELL trial but decided against that idea, because it was not incorporated into the PRE-CELL study. Full blinding or a sham surgery arm will be considered if the research proceeds to a Phase II study.

The PI clarified that mice of both sexes were included in all studies of the YAC mouse model. Physical and behavioral parameters are especially difficult to study in female mice, however, because they become fat and, as a result, cannot move well. No sex-related differences were seen in the differences in the R6/2 model. Another member of the UC Davis team, Dr. Bauer, noted that one of the reasons that multiple mouse models have been used is that no perfect model of HD exists. Some animals have gene metabolic deficits not seen in the human HD gene or population; these deficits affect female mice, who rapidly gain weight, more than male mice. To try to uncouple weight gain from behavioral deficits, the team studied male mice. Assessment of histology and outcomes other than behavior can be done without separating results by sex. Dr. Bauer explained that the weight gain in female mice is due not to the treatment but to the artificial insertion of the full-length huntingtin gene into the mouse genome. The huntingtin gene can cause deficits in metabolism, and these deficits appear to affect female mice to a

much greater degree than male mice. Thus, such problems are related to the shortcomings of the transgenic mouse models.

Dr. Wheelock explained that the premature deaths in the R6/2 (CAG 120) mice occurred in both the treated and untreated (sham) arms and were unrelated to the investigational product. A breeding colony died at the same rate, suggesting that this strain of mice is very fragile. The team continues to work on developing better strains for use in future investigations.

With respect to the TFC Scale, Dr. Wheelock pointed out that this tool measures functional ability. Therefore, part of how individuals are judged to be doing involves the environment in which they're working. She noted that many of the patients in this study are still working, including some who have reduced capacity and are in an enlightened workplace that provides the opportunity and flexibility for continued employment. Others are on disability because they do not have these options. Functionality also takes into account the ability to manage finances. The range of the TFC score for eligibility for the HD-CELL trial is based on criteria for recently completed studies of early-stage HD; one used a TFCS range of 7 to 13, and the other used a range of 9 to 13. The investigators will modify the TFCS chart to reference the stage of the study, as suggested.

Dr. Wheelock explained that the reason care partners have a consent form is because they are asked questions about the subjects' day-to-day lives. As such, care partners are part of the study and need to give informed consent to be able to complete those assessments, which will provide another perspective and additional information on how the subjects are doing. The investigators will make this aspect of the study and the role of the care partner clearer to the patient-participants.

Birth control is not provided or assured, but women of childbearing potential and men capable of fathering a child must agree to use an effective, reliable method of contraception for the duration of the study. Women of childbearing potential have a pregnancy test at each PRE-CELL visit and will continue to be tested at each HD-CELL visit. In addition, participants are asked at each visit if they are still using birth control. Dr. Wheelock explained that the IUD is mentioned only as an example of one form of birth control that could be used. The investigators do not recommend any specific method to participants and leave that decision to the subject.

The investigators will include a discussion of how MSCs are produced, the various technologies used in this research, and other clinical trial options to the ICD, as suggested.

Dr. Wheelock noted that the team is still considering the transition from PRE-CELL to HD-CELL very carefully because of the inherent and potential biases delineated by the reviewers. One of the strategies being discussed is to use a first in the PRE-CELL to first in the HD-CELL approach, which would take away the investigators' bias associated with how the subjects are doing in the study. A key issue that needs to be taken into consideration is that the first group in the PRE-CELL study is now 18 months out. If these subjects are asked to wait to be enrolled in a later group, that individual may lose the opportunity to participate in HD-CELL if their disease has progressed to the point that they no longer meet the eligibility criteria for the proposed trial.

Regarding the number of integrants per cell, Dr. Nolta noted that there is a range and that the investigators are looking at the average of the cell population. Cell expansion will go through six passages, which is expected to take 10 to 12 days.

The team conducts detailed study biosafety studies and will consider adding site integration analysis. Dr. Nolta noted that long-term follow-up of transplanted mice has not shown any transformations in the MSCs, but such results do not necessarily mean that the cells cannot become transformed.

Dr. Wheelock explained that the *tk* gene was in the original vector. However, because of some concern that it might be a target for immune reaction in the brain, it was taken out.

The PI clarified that UC Davis is a not-for-profit academic institution.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- Currently, the stopping rule is defined as a significant toxicity in at least two or more participants in any cohort. During the discussion, however, you proposed that the stopping rule will be defined as a significant toxicity in only one participant in any cohort. Given the nature of the disease and the population, consideration should be given as to whether clinically significant events that are observed in a single participant in any cohort should result in stopping the trial. In addition, consideration should be given to distinguish between conditions that would lead to trial cessation versus those that would lead to pausing enrollment.
- Regarding sample size and statistics:
 - Suggest that the statistical design is reviewed to ensure that the trial is sufficiently powered.
 - This is a Phase I trial, and safety is the primary outcome. The study should have a true definition of a dose limiting toxicity before study begins with the aim of identifying the maximum tolerated dose. Concerns were raised that an underpowered study may result in inconclusive data and inability to answer the study question. Consult with a statistician who is versed in Phase I trial design.
- Vector integration site analysis may provide useful information about the pattern of lentiviral vector integration in mesenchymal stem cells (MSCs). Consider performing this analysis on the expanded MSCs (i.e., on the cells that will be administered to the participants).
- Participants in this single site trial (HD-CELL) must have been enrolled in the observational study (PRE-CELL, also conducted at this single site) and therefore any participant in the gene transfer trial will be known to the investigators. Care should be taken to minimize enrollment bias based upon familiarity with participants from the preceding study.

Ethical, Legal, and Social Issues

The following recommendations refer to the consent document and the consent process:

- Concerns about an individual's ability to give consent given the broad range of Montreal Cognitive Assessment (MoCA) scores that a potential participant may have and be eligible to enroll. Consider consulting with experts conducting clinical trials with participants with potentially impaired cognitive abilities.
- Consider not explicitly informing participants to which dose arm they have been randomized, in order for the trial to be at least partially blinded.
- Complex issues related to vector design, genetic engineering, stem cells, etc., should be explained in simplified language in the consent.
- Language should be modified to inform participants of the potentially transient nature of the cells infused.

- Language about study withdrawal should be strengthened to clarify that participants can leave the study, but the administered modified cells cannot be removed once administered.

G. Committee Motion 2

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn requested a vote, and the RAC voted to endorse these recommendations with 14 in favor, 0 opposed, and 0 abstentions.

VII. Review and Discussion of Human Gene Transfer Protocol #1504-1396: A Phase III, Open-Label Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with β -Thalassemia Major by Transplantation of Autologous CD34+Stem Cells Transduced Ex Vivo with a Lentiviral β A-T87Q-Globin Vector (LentiGlobin® BB305 Drug Product) in Subjects \geq 5 and $<$ 12 Years of Age

PI: Alexis Thompson, M.D., M.P.H., Ann & Robert H. Lurie Children's Hospital of Chicago

Sponsor: bluebird bio, Inc.
Robert Ross, M.D.

Additional Presenters: Robert Ross, M.D., bluebird bio, Inc.
Mark Walters, M.D., Oakland Children's Hospital

RAC Reviewers: Professor Dresser, Dr. Hammarskjöld, and Dr. Kiem

Ad Hoc Reviewer: Dr. Harvey Luksenburg

Drs. Kohn, Sadelain, and Zoloth were recused from consideration of this protocol due to conflicts of interest. As a result of Dr. Kohn's recusal, Dr. Kiem chaired this section of the June 2015 RAC meeting.

A. Protocol Summary

β -thalassemia major is an inherited blood disease found most commonly in persons of Mediterranean, Middle Eastern, Indian, and South Asian descent. Thalassemia is a relatively rare disease in the United States, but it is one of the most common monogenic disorders on the planet, affecting about 300,000 individuals worldwide. Thalassemia is caused by the absence or reduced production of the β chains of hemoglobin A (HbA), a heterotetramer consisting of two β -globin and two α -globin chains ($\alpha_2\beta_2$) that accounts for more than 95 percent of the hemoglobin in the blood of adults. Hemoglobin, in turn, is the protein used by red blood cells (RBCs) to carry oxygen throughout the body. As a result of the defect in the β -globin part of hemoglobin, persons with β -thalassemia major usually require lifelong transfusions of donated blood, need iron chelation therapy for the potentially fatal iron overload that develops due to transfusion, and may experience symptoms associated with inadequate amounts of oxygen in their blood such as anemia, fatigue, and shortness of breath.

The only cure for β -thalassemia is an allogeneic hematopoietic stem cell transplant (allo-HSCT; i.e., a bone marrow transplant [BMT]). However, a BMT is an intensive medical procedure with serious short- and long-term risks, including transplant-related mortality, graft-versus-host disease (GVHD), and graft rejection. Because of these risks, transplants are offered primarily to patients with available human leukocyte antigen (HLA)-matched sibling donors (less than 25 percent of cases). One study of 179 patients reported 5-year overall and disease-free survival rates following transplant from a matched sibling to be 91 percent and 88 percent, respectively. While these rates are reasonable, patients still face the prospect of graft failure and acute and chronic GVHD, even with post-transplant immunosuppression therapy. In addition, many persons with thalassemia do not have a suitable donor for this procedure.

Given these factors and complications, new treatments that have the potential to cure thalassemia are being sought and tested. To address this unmet medical need, the study sponsor, bluebird bio, is investigating the use of LentiGlobin BB305 Drug Product in the treatment of subjects with transfusion-dependent β -thalassemia major. The LentiGlobin BB305 lentiviral vector encodes a single amino acid variant of β -globin, β A-T87Q-globin, which conserves the protein's function while allowing for correction of the β -globin/ α -globin imbalance in erythrocytes through expression of the β A-T87Q-globin gene. Treatment of the subject's own HSCs with the LentiGlobin BB305 lentiviral vector through transduction should eliminate the risk of GVHD and graft rejection and avoid the need for long-term immunosuppression. Preliminary clinical data from studies in subjects with β -thalassemia major treated with LentiGlobin BB305 Drug Product demonstrate rapid and significant production of the modified protein, HbAT87Q, resulting in minimal to no transfusion support for otherwise transfusion-dependent thalassemia. These early data showing near normal levels of hemoglobin in multiple subjects, coupled with consistent production of β A-T87Q-globin, indicate that the transfusion-independent status is due to treatment with LentiGlobin BB305 Drug Product. The investigators report that the safety profile of LentiGlobin BB305 Drug Product is consistent with fully myeloablative conditioning used for autologous transplantation. As of March 2015, no drug product-related AEs at or above Grade 3 have been observed, results of integration site analyses show highly polyclonal reconstitution, and no SAEs considered by the investigator to be related to LentiGlobin BB305 Drug Product (including no leukemia or lymphoma related to insertional mutagenesis) have been reported in two clinical trials (studies HGB-204 and HGB-205). Results from these Phase I and II studies are for seven subjects with β -thalassemia major of various genotypes (majority β 0/ β 0 and β 0/ β E), aged 16 to 26 years, who were treated with the LentiGlobin BB305 Drug Product. All seven subjects infused with the drug achieved successful neutrophil and platelet engraftment after myeloablation. Taken together, these data demonstrate the potential of treatment with LentiGlobin BB305 Drug Product to eliminate or significantly reduce transfusions for patients with β -thalassemia major.

The proposed Phase III trial (HGB-208) is an open-label, multi-site, single-dose study to evaluate the efficacy and safety of treatment with LentiGlobin BB305 Drug Product in 15 patients with β -thalassemia major who are at least 5 and less than 12 years of age at enrollment. The primary efficacy endpoint will be the proportion of subjects who are "transfusion independent" (as defined per the protocol). The study has four distinct stages. Stage 1 involves screening to determine eligibility for treatment, stage 2 involves autologous CD34+ cell collection and LentiGlobin BB305 Drug Product manufacture and disposition, stage 3 involves myeloablative conditioning (4 days of conditioning followed by at least 72 hours of washout) and infusion of LentiGlobin BB305 Drug Product (day 0), and stage 4 includes follow-up through engraftment and up to 24 months after drug product infusion. The goal during the follow-up period is to maintain a hemoglobin level of at least 9 g/dL and to avoid transfusions unless medically indicated. Subjects will be permitted to restart chelation post-transplant if needed (based on iron overload). Data and safety monitoring will be done by an independent Data Monitoring Committee (DMC), which will be able to recommend halting the study at any time due to concerns for the safety of the subjects. No untreated control group will be included in this study. Individuals with HLA-matched family donors will be excluded, because these patients generally have better outcomes in allo-HSCT than those without HLA-matched related HSC donors. A sister trial, HGB-207 (OBA #1504-1395), is identical in design to study HGB-208 but will enroll patients between ages 12 and 50.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because of safety considerations related to the enrollment of children 5 to 12 years of age as research participants, including appropriateness of exposing children to a vector with limited safety and follow-up data in adolescents and adults and the associated risks of myeloablative conditioning therapy.

Three RAC members provided written reviews of this proposed Phase III trial. The ad hoc reviewer did not submit a written review of this study.

Each of the reviewers raised concerns regarding the age of the planned patient cohort. They agreed that despite the initial promising results in adults, data on potential harms and benefits to subjects are limited.

Dr. Hammarskjöld noted special concerns associated with expansion of trials to include young children and preteens at this point in time. β -thalassemia has significant morbidity but is not a life-threatening disease at this age. Furthermore, many children with β -thalassemia will be expected to live a relatively long life with alternative treatments. In addition, the potential genotoxicity of LentiGlobin BB305 is not clear. Another serious concern is that busulfan conditioning (potentially also the gene transfer) carries a significant risk for reproductive problems later in life. In the case of adults, this can be mitigated by preservation of sperm or eggs. However, this will not be possible in the proposed study population (5 to 12 years old). It is quite possible that more efficient gene therapy protocols may be developed in the relatively near future. Participation in the proposed trial may prevent the subjects from reaping the benefits of these future developments. This is of concern, especially with a non-life-threatening disease for which conventional treatments can “buy” time. These concerns are compounded by the fact that children may not be able to give truly informed assent.

Dr. Hammarskjöld identified several other potentially serious risks associated with this gene transfer protocol. The primary risk of gene transfer is that of leukemia due to insertional mutagenesis (as acknowledged in the protocol). There has been considerable development in the retrovirus vector field, since the two severe combined immunodeficiency (SCID) trials that caused leukemia in several children, but integration into the human genome always carries a potential risk for insertional mutagenesis that can lead to cancer. The vector used in the proposed and ongoing trial (LentiGlobin BB305) is a self-inactivating (SIN) vector that removes the LTR promoters. The vector also contains a strong promoter that drives transgene expression and might activate neighboring genes. The investigators state that the risk of insertional mutagenesis is mitigated, because the β A-T87Q-globin gene promoter drives expression solely in the erythroid lineage. Dr. Hammarskjöld inquired whether this limited expression has been verified by analysis of patient cells.

The original vector used in a previous β -thalassemia trial (HPV-569) contained insulator sequences designed to minimize this risk. However, it turned out that the insulator sequences provided a cryptic splice site that activated the HMAG2 gene in one of the human subjects. This outcome showed that the vector could integrate into genes and activate them, even though it is a SIN lentivirus vector. In addition, the HPV-569 vector was shown to have a low titer and modest transduction efficiency. The current vector (LentiGlobin BB305) has the insulator sequences removed, which has resulted in better transduction and expression. As the investigators point out, only a relatively small number of human subjects (around 100) have been transplanted with HIV lentivirus vectors to date. Thus, the overall risk for insertional mutagenesis is still unclear and needs to be evaluated for the new vector.

Although there are no reports of genotoxicity with LentiGlobin BB305, the vector has been used in fewer than 10 subjects, and not enough time has passed to be able to fully evaluate it. In the original retrovirus SCID trials, for example, 5 of the 20 patients developed T-cell leukemia but not until 2 to 5.5 years after gene therapy.

Integration site analysis data are available for only a small number of subjects. This analysis did not show any dominant clones or evidence for clonal expansion, but caution is needed when basing potential risk on these preliminary findings. Dr. Hammarskjöld asked whether the investigators have any additional integration site analysis data or other data that they can make available at this point.

Dr. Hammarskjöld noted that several sections in Appendix M have been marked as confidential or completely blacked out, making it very difficult to adequately review the responses. For example, the section “Structure and Characteristics of the Biological System” has been marked as confidential in its entirety, and the structure and the composition and derivation of the pBB305 vector have been redacted (only shown as black boxes). A recent publication describes pBB305 in some detail (Negre et al., 2015). It is not clear whether the vector described in this publication is the same as the vector that will be used in the proposed trial. Sections and questions about the animal and cultured cell models used to assess the in vivo and in vitro efficacy of the gene transfer system, how the experimental treatment will be

administered in the proposed trial, and how risks of the gene will be assessed and minimized in the clinical setting are similarly marked confidential and shown as black boxes.

In other examples, Appendix M refers to Table 3 as providing a complete list of the nonclinical studies that have been performed to support the use of LentiGlobin BB305 lentiviral vector. Assessment of these studies was difficult, however, because the table was completely blacked out. Table 4, which is described as providing drug product release criteria, also was blacked out.

The missing information is important to understanding the proposed research and risks of the investigational product. Dr. Hammarskjöld asked whether the investigators were able to reveal any of the important information that should be described in these sections or clearly explain why they cannot reveal any further details.

Dr. Hammarskjöld found several parts of the informed consent document for the parents/guardians to be very well written and informative and presented at the appropriate level. She noted the following issues and suggested changes:

- The only potentially curative treatment currently available for β -thalassemia, hematopoietic stem cell transplant (HSCT), is clearly explained early in the consent form. The statements regarding the goal of the gene therapy (i.e., to cure the disease by transferring a functioning copy of the gene) and that there is no risk of rejection with this intervention could be misleading, however. There is nothing near the start of the document that distinguishes the curative HSCT (that many parents/guardians are likely to be familiar with) from the experimental gene therapy protocol. Rather, it may give the impression that the gene therapy is “better,” since, as stated, there is no risk of rejection. The experimental nature of this protocol is clarified later in the consent form, but it would be helpful to make a clear distinction between HSCT and this experimental protocol closer to the start of the document. The investigators should consider including information about the potential risks in the same section of the document, since potential risks of the gene transfer are not mentioned until about 10 pages later. One option is to state up front that the potential known and unknown risks with this gene transfer protocol are further discussed on pages “x and y.”
- A statement or brief discussion of the potential seriousness of the infections and AEs (including death) that could occur with busulfan should be added to the section on conditioning treatment with busulfan. These risks are not mentioned until much later in the document.
- The consent should clarify under “Long-Term Follow-Up” that the lentivirus vector sequences may remain permanently in the body after transplantation.
- The consent includes a very brief summary of the outcomes to date in the seven subjects treated with the modified β -globin gene and the LentiGlobin BB305 Drug Product in protocols HGB-204 and HGB-205. Additional information about when these subjects were transplanted would be helpful in making informed decisions about participation. Because this section includes a specific date as a reference (“as of January 2015”), the information will need to be updated as more data come in. The statement that the infused stem cells received during transplant made new blood cells could be misleading and should be clarified to reflect successful globin expression.
- Reproductive risks are clearly spelled out, but sperm and ova banking are unlikely, given the age of the cohort (5 to 12 years old), in contrast with adolescents and adults who undergo transplantation. The information about banking is therefore misleading and should be changed. The information about pregnancy, abstinence, contraception, and related issues appears to have been largely lifted from an adult protocol and should be rewritten to be more appropriate and relevant to the 5- to 12-year-old age group.
- The word “can” should be replaced with “will” in the statement, “When the vector enters your child’s stem cells, the DNA (genes) from the vector can insert into the DNA from that cell,” because integration always occurs with a lentivirus vector.
- The investigators should qualify the statement that no subject to date in a gene transfer trial using a lentiviral vector has developed a cancer related to the lentiviral vector, to indicate that the number of subjects transplanted with lentivirus vectors is limited (e.g., less than 100).
- The statement “This does not mean that your child has developed HIV” should be revised to say “has been infected with HIV” or something similar, because one cannot “develop” HIV. In addition,

the section on HIV testing should clarify any provisions for follow-up testing to make sure that a future positive HIV test is not a result of an infection resulting from risky behavior. While the subjects are children at this point, they may be at some risk for HIV infection as they grow older.

Dr. Kiem found the protocol and consent documents to be very well written. He identified no major issues with the proposed vector or the manufacturing of the CD34 modified cells and noted that the same vector has been used in a number of patients without any obvious side effects. In addition, the vector has been previously reviewed by the RAC and the FDA. The main question that warrants further discussion is the issue of benefits versus potential side effects in the planned patient population i.e. children between 5 and 12 years of age. While it can be argued that the earlier the intervention occurs the better the outcome, it will be helpful for the study team to discuss the pros and cons of this approach.

Dr. Kiem noted the following specific issues and questions for the investigators to address:

- The advantage of enrolling children would be less preexisting organ damage, particularly to the liver. In this country, however, most patients have good access to appropriate chelation therapy. What is the typical progression rate in this country for children on adequate chelation therapy to progression (e.g., from a Pesaro risk class 1 to a 3)? Will the investigators limit enrollment to particular Pesaro risk classes or enroll all?
- The vector has been safe so far, but follow-up is limited. It would be helpful to provide an update on the follow-up and safety data available to date, in particular, the number and age of patients and any integration site data available in these patients who have received the gene-modified cells.
- Myeloablation will be required to facilitate the engraftment of the gene-modified cell and will most likely result in the patients being sterile (no eggs or sperm can be banked, unlike what can be done for the adults and adolescents who undergo this procedure). This is acknowledged in the consent document – but again one will have to weigh the pros and cons for waiting till the patients would have a chance to do that and also provide more informed input to this decision.
- Will there be backup marrow or stem cells in case the gene-modified cells fail to engraft?

Professor Dresser pointed out that children aged 5 to 12 have limited ability to understand what the study will involve and limited ability to make decisions independent of their parents. These children are not facing near-term serious health threats, but the proposed study will expose them to serious risks as well as appreciable pain and discomfort. She suggested making the study of this young population contingent on more solid findings of potential benefit and reasonable risk in older subjects.

Some of the language in the consent form could lead parents to think that this relatively new investigational intervention has a high likelihood of success. Use of the terms “treatment” and “therapy” can promote therapeutic misconception, in which those making decisions regarding the research confuse investigational interventions with proven therapies. Professor Dresser provided several suggestions and comments to address this problem:

- The goal of gene therapy could be explained more clearly with a statement such as “Gene transfer attempts to use functioning genes to help people who have a gene that is not working properly.”
- The investigational nature of gene therapy should be clearly noted with a statement such as “Although some results have been promising, gene transfer remains experimental and has not been approved for general patient use.”
- Instead of saying that stem cells will be returned to the child’s body, where “they are expected to grow and produce new cells,” the document should say, “We hope that they will grow and produce new cells.”
- The document needs to include a statement that the genetically modified cells cannot be removed from the child’s body once they are infused.
- The ICD needs to state clearly whether travel costs will be covered.
- Where applicable, change “treated” to “infused.”
- Replace the term “treatment” with “study procedures” or other terms that link risks to research or study interventions, not medical treatment.

- In relation to the section on reproductive tissue banking, Professor Dresser suggested informing the parents that the study procedures are likely to cause the child to be infertile.
- Professor Dresser noted that the institutions' legal departments probably require material on pregnancy and contraception but agreed with Dr. Hammarskjöld's suggestion to abbreviate the section and tailor the information to the participants' age.

Professor Dresser commended the investigators on the assent forms. She suggested the following minor edits. In the detailed assent form, consider changing "treatments that you need" to "things that we have to do for the study." In the simplified assent form, consider changing "this study will be good for you" to "this study will not hurt you too much."

C. RAC Discussion

The ad hoc reviewer, Dr. Luksenburg, raised the following issues and concerns during the meeting:

- A critical question is whether there is sufficient urgency at this time to begin to use the LentiGlobin BB305 Drug Product in younger children before waiting for additional safety and efficacy data from the team's ongoing trials. As noted by the other reviewers, with use of busulfan, infertility is nearly certain. The long-term benefit of the investigational agent is not known, and the potential for benefit to the individual child could be transient. Thus, it is not clear that the risk of infertility and other known and unknown risks outweigh the potential benefit of the intervention.
- Further clarification was requested on whether the use of busulfan could in any way compromise eligibility for other treatment options in the future (e.g., undergoing transplantation if an unrelated donor is identified at a later time). Dr. Luksenburg also asked about other potential risks of busulfan, such as an effect on future bone marrow fibrosis in a patient who does not undergo a BMT.

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- Dr. Kiem inquired about enrollment of thalassemia patients with an HLA-identical sibling or a matched unrelated donor. He noted poor outcomes and complications in patients following unrelated donor transplants and asked how the investigators advise parents with affected children between ages 1 and 10 who do not have a matched sibling.
- Dr. Hammarskjöld noted the team's extensive preclinical work and product development and testing and relayed her assessment of the proposed study as interesting and promising. The original protocol and parental ICD have been revised to address the issue of potential sterility with busulfan treatment and to clarify that sperm and ova banking are not options for these participants because of their age. She remained concerned, however, that the risk/benefit ratio is not favorable for younger children and supported waiting until more safety and efficacy data are available to determine whether younger children should be enrolled in a LentiGlobin BB305 or similar trial. A more complete safety profile of the vector is needed, and the basis for potential long-term benefit is not clear. Although the vector for the LentiGlobin BB305 Drug Product has been modified to reduce the possibility of insertional mutagenesis, integration into the human genome always carries the risk of cancer, as has occurred with prior vectors. More efficient gene therapy protocols may be opened in the future, and participation in the proposed trial could prevent the subjects from realizing the benefits of these future developments. A further concern is that the decision to undergo an experimental treatment is being made prematurely for these patients, who typically are not facing a life-threatening disease at their young age. Given these factors, children might be unnecessarily exposed to risk if the trial proceeds as proposed at this time. Ongoing collection and analysis of data for this specific vector in adolescents and adults will better inform this decision.
- Professor Dresser continued to have some reservations regarding the prospect for direct benefit given the higher risk of infertility for this younger population and the burden of transplant. She agreed with the investigators, however, that a long-term delay also would be burdensome and wondered whether parents will be inclined to wait after learning about the risks of the proposed clinical trial. She suggested that the consent include language to explain that the investigators

have safety and efficacy data from older kids and adults with thalassemia given this product and that they expect to have additional information in the next couple of years that could change the current understanding of the risks and benefits of the intervention.

- Dr. Wooley inquired about review and analysis of aggregate data compiled on the lentivirus vector and how results from disparate studies were assessed.
- Dr. Donahue noted that, given the small number of subjects studied to date, the range of risks may not be realized and caution should be used in assessing risks and potential. He mentioned that short-term toxicities are not known so far but long-term toxicities need to be elucidated. In addition, he asked what criteria are used for discerning whether to consider enrollment in the proposed trial or advise patients and families to wait.
- Dr. Hammarskjöld also advised against lumping results and data for all lentivirus vectors together, because each vector is different.

D. Investigator Response

1. Written Responses to RAC Reviews

Since the proposed protocol was submitted to the RAC, clinical data from ongoing studies conducted with LentiGlobin BB305 Drug Product have been accumulating and additional data are expected before initiation of the study. To date, a total of 12 subjects with β -thalassemia major, ranging from ages 16 to 35, have been treated with LentiGlobin BB305 Drug Product in clinical studies HGB-204 (OBA protocol #1204-1164) and HGB-205. The clinical data available demonstrate a positive risk-benefit balance and support studying LentiGlobin BB305 Drug Product in children with β -thalassemia major who do not have an HLA-matched sibling donor. Safety data are available for up to 1.5 years post-treatment and show no LentiGlobin-related SAEs. Efficacy data continue to provide the prospect of direct benefit to individual subjects. (Confidential efficacy information was provided to the RAC.) The investigators expect that 20 subjects (16 adults and four adolescents) will be treated with LentiGlobin BB305 Drug Product by March 2016 (the anticipated date of first consent in study HGB-208), from which 11 subjects will have 12 months of data post-transplant, 5 subjects will have 18 months of data post-transplant, and 3 subjects will have 2 years of data post-transplant. The investigators acknowledge that long-term risks associated with gene therapy remain unknown but note that many lentiviral vectors used to genetically modify hematopoietic stem cells ex vivo have been studied to date with no reported cases of gene product-related malignancy.

The short-term risks of myeloablation are of a lesser magnitude in pediatric subjects compared to adults due to better organ function and lower iron overload in children. The risks of myeloablation are also expected to be smaller than the overall risks associated with allogeneic transplantation, which is regularly offered to children with β -thalassemia major in this age group (under 12 years of age) with an HLA-matched sibling donor. Allogeneic BMT is currently recommended in young children before the development of iron overload and iron-related tissue damage. Transplant-related mortality associated with both allogeneic and autologous BMT is lower in younger patients due to better tolerance of myeloablative treatments.

The investigators are concerned that deferring participation in this study with an investigational agent that has the potential to reduce or eliminate transfusion dependence could come with a cost to pediatric patient-participants of accumulating clinically meaningful disease burden. Younger children without well-matched donors will continue to be exposed to the risks of chronic transfusions (e.g., progressive and irreversible organ damage from iron overload, risks of viral exposure and allo-immunization) and side effects of chelation. Other risks to this population include ongoing psychosocial burden, delayed growth and puberty, and continued tissue damage due to iron accumulation that may, in turn, affect overall prognosis and tolerance of myeloablation in future.

Clinical sites will be trained on Good Clinical Practices (GCPs), including the process of obtaining informed consent (for parents or guardians) and assent (for children). The potential known risks of participating in the study, including potential loss of future fertility and alternative treatment options, are described in the consent/assent documents clearly and objectively. The consent form also states that there may be unknown risks to the treatment. The consents/assents are discussed with the families at the

clinical sites, and ample time is provided for parents and children to ask questions and meet with the clinical staff to make an educated decision about enrolling in the trial, taking into account the potential risks, benefits, and time commitments.

The investigators provided the most recent integration site analysis data available in subjects treated with LentiGlobin BB305 Drug Product in the response memo and in Appendix 4 to the protocol. They state that to date, there has been a highly polyclonal repopulation of peripheral blood leukocytes at all time points checked and no clonal dominance observed.

The absence of expression of β A-T87Q-globin in the non-erythroid lineages has not been tested on patient cells specifically treated with the LentiGlobin BB305 lentiviral vector; however, expression in erythroid cells has been investigated with the previous vector, LentiGlobin HPV569, which has an identical expression cassette to the LentiGlobin BB305 vector. As described in a published paper (Cavazzana-Calvo et al., 2010), when the expression of HMGA2 in nucleated blood cells was analyzed, HMGA2 was expressed in erythroblasts only, which is consistent with β -locus control region erythroid specificity. In addition, HMGA2 messenger RNA was undetectable in granulocytes and monocytes. There is no evidence that LentiGlobin BB305 lentiviral vector-derived DNA will be present in any untreated cells, since it is used in an ex vivo gene transfer protocol. Furthermore, based on published data from in vivo animal studies, germline transmission of the vector sequence is also highly unlikely.

Bluebird bio, Inc. considers some of the information requested under Appendix M to be commercially confidential, including the detailed plasmid and vector maps and details of nonclinical studies. The response memo includes clinical efficacy data marked as confidential; this information is not presented in these minutes. Additional information was provided in response to the issues and questions delineated in this appendix and per the reviewers' comments, as follows.

The investigators confirmed that the vector described in the 2015 paper by Negre et al. (per Dr. Hammarskjöld's review and query) is the same vector (LentiGlobin BB305) referenced in Appendix M. LentiGlobin BB305 lentiviral vector is an HIV-1-based lentiviral vector expressing the human β A-T87Q-globin gene. The major functional element of the LentiGlobin BB305 lentiviral vector is the β A-T87Q-globin transgene; its *cis*-regulatory elements are cloned from the β -globin locus control region (LCR), which activates expression specifically in the erythroid lineage. The native vector system derives from HIV-1. All of the accessory viral genes that are dispensable were removed from the system. These include HIV envelope, *tat*, *vpr*, *vpu*, and *nef*.

Further detail about the LentiGlobin BB305 lentiviral vector was provided. This lentiviral vector is a replication-deficient nonpathogenic third-generation lentiviral vector carrying an extensive deletion (400 nucleotides) of the U3 region in the 3' LTR, including deletion of the TATA box, creating a SIN vector to minimize the risk of vector mobilization and transactivation of neighboring genes via insertional mutagenesis. The investigators noted that the LentiGlobin BB305 lentiviral vector is structurally and functionally similar to other lentiviral vectors (including LentiGlobin HPV569) used in previous preclinical studies and in a clinical study conducted in France. No lentiviral vector-related toxicity has been observed in any of the preclinical animal studies or the completed or ongoing clinical studies. Data show that LentiGlobin BB305 lentiviral vector has a strongly reduced oncogenic potential compared to positive control lentiviral vectors with known mutagenic potential. In addition, the LentiGlobin BB305 vector has been tested prospectively for RCL formation in a large-scale assay with a detection limit of one 50 percent tissue culture-infective dose.

A general summary of the nonclinical studies performed to support the clinical use of LentiGlobin BB305 Drug Product was provided in tabular form. Release criteria are not considered to be commercially confidential information, and details regarding these criteria were provided with the response to the reviewers' comments.

The animal model used for the nonclinical pharmacology and toxicology study is a mouse model of β -thalassemia (Hbbth1/th1 mice). These mice were selected for this study because they represent a relevant model of human β -thalassemia. Adult Hbbth1/th1 mice can be studied, since this natural model

of β -thalassemia is not lethal in utero. Multiple gene transfer and expression studies were carried out in CD34+ cells in vitro. In addition, numerous in vitro and in vivo experiments assessing the effectiveness of the delivery system in achieving the minimally required level of gene transfer and expression have been conducted. One of the most relevant studies with previous LentiGlobin lentiviral vectors is a study on permanent and panerythroid correction of murine β -thalassemia by multiple lentiviral integration in HSCs following transplantation of syngeneic bone marrow transduced with LentiGlobin HPV524 (a similar lentiviral vector to LentiGlobin BB305). The viral titers produced achieved transduction of virtually all the hematopoietic stem cells in the graft with an average of three integrated proviral copies per genome in transplanted mice. The transduction was sustained for more than 7 months in both primary and secondary transplants, at which time approximately 95 percent of the RBCs in all mice contained human β -globin contributing to 32 percent \pm 4 percent of all β -like globin chains (Imren et al., 2002). In addition to these preclinical studies, the LentiGlobin BB305 lentiviral vector has been used in ongoing clinical studies (HGB-204 and HGB-205). Preliminary clinical data demonstrate a rapid and significant production of HbAT87Q, resulting in minimal to no transfusion support for otherwise transfusion-dependent thalassemia subjects.

The response memo provides detailed descriptions of risks to and the status of individuals with thalassemia, with a focus on age-related complications. The investigators note that iron accumulation begins at a young age, even with modern chelation therapy, which leads to important clinical consequences such as cirrhosis of the liver, cardiomyopathy, and diabetes mellitus. While severe end organ damage is more common in adults, damage begins to occur in childhood. The prevalence of iron-induced liver complications in older patients (median age 16, range 6 to 47 years of age) has been found to be 39 percent with hemosiderosis of at least grade III, 39 percent liver fibrosis, and 2 percent liver cirrhosis. Among younger patients (median age 12, range 2 to 27 years of age), 44 percent have hemosiderosis of at least grade III, 30 percent have liver fibrosis, and 0 percent have liver cirrhosis. Cumulative liver damage is associated with an increased risk of myeloablative conditioning and worsens transplant outcomes. Cumulative tissue iron levels also increase in organs other than the liver after 2 years of age, which is the usual age of starting chelation. By 18 years of age, 20 percent of patients also have evidence of cardiac disease, 27 percent have significant growth delays, and 33 percent have evidence of endocrinopathies.

A progression of a Pesaro risk class 1 to 3 is not expected for patients aged 5 to 12 years. All eligible patients may be enrolled in the study irrespective of Pesaro risk class. The study will exclude all subjects with advanced liver disease defined per the protocol based on persistently elevated liver function test (LFT) values; elevated prothrombin or partial thromboplastin times at baseline; or liver biopsy demonstrating cirrhosis, evidence of bridging fibrosis, or active hepatitis. Iron chelation history will be assessed given that subjects must be treated and followed in a specialized center that maintained detailed medical records for a minimum of 2 years before enrollment in the proposed study.

A significant reduction in a patient's post-transplant RBC transfusion requirements will not only affect quality of life but significantly reduce iron intake, which is expected to lead to clinically meaningful benefit by slowing the progression of or preventing further organ damage associated with iron overload. Furthermore, although adequate chelation therapy delays the development of iron overload complications, optimal treatment responses and compliance remain problematic for many patients, even in the United States.

The response memo provides detailed descriptions of each stage of the proposed experimental treatment plan. The investigators note that the experimental procedures described in the memo and the revised protocol are the same as those used in studies HGB-204 (OBA Protocol #1204-1164) and HGB-207 (OBA Protocol #1504-1395). The process for preparation of the modified cells was described in detail. Cells will be removed from the subjects and treated ex vivo. Each subject will undergo HSC mobilization with filgrastim and plerixafor. The hemoglobin level for the subject should be at least 11 g/dL before undergoing mobilization. Peripheral blood mononuclear cells will be collected by apheresis. A total of two mobilization cycles may be performed if needed, and each mobilization cycle may include up to three apheresis procedure days. No more than two consecutive apheresis procedure products may be sent for each transduction (each transduction produces an individual drug product lot). If two mobilization cycles

are needed to collect sufficient HSCs to meet the requirement of a total dose of at least 3.0×10^6 CD34+ cells/kg, then mobilization cycles must be separated by at least 2 weeks (i.e., from the last day of cell collection in cycle 1 to the first dose of granulocyte-CSF in cycle 2). Apheresis procedure products can also be used for rescue cells. Alternatively, a bone marrow harvest is allowed to procure cells for rescue. The harvested cells to be used for transduction will be selected for the CD34+ marker to enrich for HSCs, transduced with LentiGlobin BB305 lentiviral vector, and stored under the vapor phase of liquid nitrogen while release testing is ongoing.

Backup rescue cells (minimum 1.5×10^6 CD34+/kg) will be collected for all subjects. These rescue cells can be collected from apheresis procedure products. Alternatively, a bone marrow harvest is allowed to procure cells for rescue. A subject may be infused with their rescue cells if he or she fails to engraft with transduced cells or is unable to receive LentiGlobin BB305 Drug Product after conditioning has commenced.

The investigators did not agree with the suggestion to include a statement in the ICD to indicate risk of infertility with introduction of the modified gene product, citing the lack of any published data indicating this to be a risk for LentiGlobin BB305. Patients who undergo transplantation in the proposed trial will undergo myeloablative conditioning with busulfan before transplant. The investigators note that use of busulfan alone is safer in the short term than co-administration of busulfan and cyclophosphamide or busulfan and fludarabine, which are standard myeloablation conditioning agents for allogeneic sibling hematopoietic cell transplants conducted in the United States. Pediatric subjects and their families will be counseled and consented and assented, if applicable, on the risks of myeloablation. The staged procedures (screening, mobilization, and lentiviral transduction) will provide additional opportunities to further review and discuss the risks of myeloablation and gene therapy and are subsequently opportunities for subjects to leave the study before myeloablation has occurred should the subject and/or family decide to withdraw or if there are newer toxicity data that may alter the safety profile of LentiGlobin BB305 Drug Product. The investigators pointed out that many study sites have experimental approaches to fertility preservation in which subjects may participate while enrolled in study HGB-208.

The investigators accepted the reviewers' other recommendations and have revised the draft consent and assent documents accordingly.

2. Responses to RAC Discussion Questions

Dr. Mark Walters, Oakland Children's Hospital, commented that children can go through a myeloablative conditioning regimen more than once and that use of busulfan in the proposed trial does not preclude a second or even third transplantation. In the team's experience with hemoglobin disorders, second transplantations, often with a repeated myeloablation are needed when a rejection to the first transplantation has occurred. Thus, there is nothing inherently life threatening about exposure to a subsequent conditioning regimen for participants in this study.

Dr. Ross noted that the fundamental question for the study team regarding inclusion of younger children in a LentiGlobin BB305 trial is determining the point at which the risk-benefit ratio is appropriate to begin to enroll pediatric patients. The investigators have proceeded with an incremental suite of clinical trials, starting in adults and then moving to adolescents and finally to children. Based on the safety and efficacy data available to date, the investigators consider the risk-benefit ratio for a child participating in the proposed study to be more favorable than for an adult. By the time that the trial starts, some long-term follow-up data will be available for more than 20 subjects treated with the product. Dr. Thompson added that many families familiar with the other trials have stressed the importance of providing affected children with the same opportunities available to adults. The consent process for the proposed in young children is extensive and thorough. Dr. Thompson anticipates that parents will understand the risks of the study, including possible infertility, as well as the uncertainties about future trials that may offer more effective treatments and/or fewer risks to make a competent, informed decision for their child. Some families will decline participation based on the information presented through the consent process.

Patients with an HLA-matched sibling will not be eligible for the proposed study. Patients without a matched sibling and those with an unrelated donor will be eligible to participate. Dr. Walters noted that in North America, transplantations using an unrelated donor are considered experimental for thalassemia. Such transplantations carry risk of graft rejection and other serious complications. Reports on transplants using unrelated donors usually include only a small number of patients, and the outcomes for this group vary widely, making it difficult to draw clear conclusions on how patients fare. One analysis of available data found an estimated survival rate of about 75 percent in patients using unrelated donors, which is markedly lower than survival rates of about 90 percent for matched-sibling donations. In a recently completed unrelated-donor transplant study of 25 children with thalassemia, the disease-free survival rate was in the upper 70 percent range, while overall survival was about 80 percent. Several children in the study, which Dr. Thompson co-chaired, had significant comorbidity including extensive GVHD. Dr. Thompson noted that transplantation is considered for all children with thalassemia major. If they have siblings, the siblings are HLA-tested for matching. Possible enrollment in an active clinical trial is also discussed. Families interested in learning more are referred to a transplant specialist for an extensive discussion of the risks and benefits of transplantation, options for each identical sibling, and participation in a research protocol. For those without a sibling or an HLA-matched sibling, the family might consider waiting until another sibling is born or pursue an unrelated-donor transplantation.

The investigators clarified that while some information and data needs to remain confidential, including more significant clinical data that have not been publicly presented and are under embargo, other information provided for the RAC review was mistakenly redacted.

Drs. Ross and Thompson reviewed the factors considered in assessing the risk and benefit of the proposed study. The investigators acknowledged the reviewers' concerns regarding the long-term potential efficacy and the documented and potential short- and long-term risks of the study agent, particularly in relation to younger patients. They further acknowledged that there are few to no data on this product in children under age 12, which can be clarified in the consents. The team consulted bioethicists regarding the issue of how to approach approval of enrollment of pediatric patients, and the advice was to base the determination on a risk-benefit assessment in children. The team agreed that once a total of 20 patients, including four to seven adolescents, have been treated, there would be sufficient data within the context of a well-controlled clinical trial to proceed with expansion to younger children. A key question for the investigators is whether children would be at increased risk of insertional mutagenesis because of their age. The investigators agree with the reviewers that a risk of insertional oncogenesis cannot be discounted. It would be a rare event, however, and could take years to manifest, as understood through evidence across gene therapy studies and experiments. The investigators determined that there is no increased risk of insertional oncogenesis for the proposed pediatric population and that the investigational procedure likely involves decreased risk for children as compared to adults.

Concerns about risks associated with available treatments for thalassemia and risk to fertility under the proposed study were also taken into consideration. Dr. Thompson noted that many families choose to forego sibling transplants because of the potential impact on fertility. Some families did not want their children to be given the chelators until those agents were fully tested and commercially available; others were okay with use of chelators while they were still being tested. Similarly, some families and patients accept randomization and others do not. These points underscore the obligation of the investigators and the importance of the consent process in ensuring that all risks and potential benefits are clearly conveyed to and understood by candidate participants and their family members. Ongoing efforts across disciplines and institutions are underway to examine ways to offer fertility preservation for younger children and across the lifespan. Subjects in the proposed study and their families will be counseled on existing options (or the lack thereof based on patient age) and will not be precluded from pursuing current methods available for preserving ova and sperm or participating in experimental trials exploring other preservation methods.

As for the broader question of delaying enrollment of children, the investigators noted the significant gap in curative therapies available for thalassemia and related hematological disorders. The ultimate goal is to offer a curative therapy to children so that their lifespan can be normalized and they will have the benefit of being cured before disease- and treatment-related complications develop. Participation by children is

necessary to achieve this goal. The investigators hope to deliver more efficacious agents to patients when those agents become available, but they do not believe that enrollment of adults and children in studies of currently available products should be deferred while waiting for other trials to be in place.

There have been approximately 20 lentiviral gene transfer trials conducted to date, with more than 60 subjects treated across all studies. The majority of these studies were conducted in pediatric subjects. A trial in Italy followed approximately 20 subjects with metachromatic leukodystrophy for more than 3 years. Another trial in Italy followed more than 10 subjects age 1 to 5 with Wiskott-Aldrich syndrome for more than 3 years, while a trial in France includes more than 6 years of follow-up in four patients with adrenoleukodystrophy. No events of insertional oncogenesis have been reported for these studies. Dr. Ross noted that while the results to date on lentiviruses are encouraging and better than for AAV studies, the information presented does not capture all results and represents only a snapshot of findings. There could be higher rates of toxicities, but short-term toxicity is similar to that of autologous transplants. The investigators acknowledged the reviewers' concerns regarding the importance of assessing each vector and disease condition or patient population separately.

Dr. Walters commented that the criteria for assessing whether a pediatric patient should be considered for a trial include the child's condition and status in addition to the family's motivation. For the proposed study, children must be otherwise healthy. If the family is hesitant, the investigators would suggest discussing possible enrollment at a later date.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The RAC made additional observations and recommendations during the in-depth review and public discussion. Ultimately, the RAC concluded that while preliminary results from these trials seem promising, there are additional concerns associated with expansion of this research to pediatric participants. The proposed study will expose these children to serious risks (outlined below), and at this point, there are other options for treatment—albeit, burdensome—that enable participants 5 to 12 years of age to live a relatively long life. Potential risks include:

- Insertional mutagenesis into the human genome and potential for oncogenesis. There have been no reports of genotoxicity with LentiGlobin BB305 as of yet, but it has been used in only 11 participants to date with limited long-term follow-up. Data show that dominant clones or evidence for clonal expansion sometimes takes 1 to 5.5 years, and this strongly suggests the need for a longer follow-up period in the currently treated patients to evaluate oncogenic potential of LentiGlobin BB305 prior to enrollment of children.
- Busulfan conditioning will most likely result in sterility. In adult populations, efforts to preserve the sperm or eggs are undertaken, but this is not possible in the proposed study population given the pre-pubertal age (5–12 years). While acknowledged in the consent document, this concern in combination with the limited follow-up period in currently treated patients warrants consideration of delaying enrollment until participants' future reproductive options may be expanded.
- Children aged 5 to 12 may have limited comprehension of the risks of the research or the ability to make informed decisions about their future independent of their parents. These children are not facing serious health threats and this study will expose them to serious risks.

Based on the rationale outlined above, the RAC thinks that it is reasonable to delay the enrollment of children for an additional 1 to 2 years to obtain more safety and efficacy data with the adults/adolescents showing a higher prospect of benefit with LentiGlobin BB305 as compared to the alternative treatments available.

G. Committee Motion 3

Dr. Kiem summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kiem requested a vote, and the RAC endorsed these summarized recommendations by a vote of 12 in favor, 0 opposed, 0 abstentions, and 3 recusals (Drs. Kohn, Sadelain, and Zoloth).

VIII. Update on Protocol #0810-946: Phase I Trial of Intratumoral Injection of Vesicular Stomatitis Virus Expressing Human Interferon Beta in Patients with Sorafenib Refractory/Intolerant Hepatocellular Carcinoma and Advanced Solid Tumors with Liver Predominant Locally Advanced/Metastatic Disease

Presenter: Mitesh Borad, M.D., Mayo Clinic, Scottsdale (*via teleconference*)

A. Presentation by Dr. Borad

The focus of this update is a serious adverse event that occurred on this ongoing Phase I trial of a human interferon-beta-expressing vesicular stomatitis virus (VSV-hIFN- β) as a possible treatment of primary hepatocellular carcinoma (HCC). The trial is also open to patients with other cancers with liver metastases. Dr. Borad provided background and history on the viral vector and the trial, which has enrolled 14 patients to date, and a detailed report on the affected patient.

Approximately 1 million people worldwide are diagnosed each year with HCC, and an equivalent number of patients die annually from this disease. Although the United States historically has been considered a low-incidence region, a recent increase in the prevalence of HCC in this country has been observed. This increase is largely attributable to infections caused hepatitis virus types B and C. Current therapies for inoperable HCC are sparse and do not significantly increase response or survival rates. In an effort to address this unmet medical need, the investigators developed a vesicular stomatitis virus (VSV) as an oncolytic vector for the treatment of cancer.

VSV, a Rhabdovirus, is a negative strand RNA virus that replicates in the cytoplasm and is highly lytic, with no known transforming capabilities. VSV is arthropod-borne, and the primary hosts are rodents, cattle, horses, and swine. The virus affects the mucous membranes and can involve the CNS, resulting in neurotoxicity. Most human exposures have been from farm animals. While infection in animals can be fatal, human infection typically results in very mild, flu-like illnesses. One case of pediatric encephalitis possibly associated with VSV has been previously reported. VSV has been shown to be a potent oncolytic agent against a variety of both human and murine tumors of several different histological types via intratumoral or systemic routes. The VSV vector used in this protocol has been engineered to overexpress the IFN- β protein. IFN- β produced by cells that are infected with the VSV-IFN- β virus act to restrict virus replication in normal cells, thereby increasing the safety of the virus. However, IFN- β produced by infected cancer cells should have little effect in blocking viral replication because the cancer cells respond very poorly to IFN- β . Given that transformed cells in malignancies have defective interferon pathways, an objective of this research is to determine whether IFN- β can modulate anti-tumor effects with this vector.

The current protocol is open to adults with refractory disease and a life expectancy of at least 3 months. Patients for whom surgery or transplant is feasible are not eligible to enroll. Concurrent systemic therapies are exclusionary. Subjects must be able to provide informed consent. Under this trial, participants receive a single intratumoral injection (with ultrasound guidance) into a lesion site. The volume injected is based on tumor dimensions, up to a maximum of 15 mL. The starting dose is two orders of magnitude lower than the MTD determined in rat studies (5×10^4). Per the planned dosing regimen, the dose of the virus is then escalated at levels of one-half log per level, up to a final dose of 5×10^8 TCID₅₀, for a total of eight dose cohorts including the starting dose. Each dose is tested in two to three patients. The primary endpoints are determination of the MTD, DLT, and overall safety profile of VSV-hIFN β and assessment of the potential for viral transmission of VSV-hIFN β . Secondary endpoints

include determining the pharmacokinetic and pharmacodynamic profiles of the investigational agent, determining the recommended Phase II dose and schedule for multiple administrations of VSV-hIFN β , and assessing for evidence of anti-tumor activity by imaging and Afp response (>50% reduction from baseline). Translational endpoints include time to antibody response. Anticipated toxicities include mouth sores, flu-like illness (fever, chills, myalgias), neurotoxicity, cytokine release syndrome (CRS), liver toxicity (increased transaminases, cholangitis), and injection-related AEs (bleeding, bruising, pain, infection). DLTs are defined as any Grade 3 or higher non-hematologic toxicity, any Grade 4 or higher hematologic toxicity, and any other toxicity that does not meet these criteria but in opinion of the investigators is dose limiting and would constitute a safety signal. The DLT observation period is 28 days post-injection.

Five doses including the starting dose have been tested to date. Of the 14 subjects enrolled, two were found to be ineligible to receive the investigational agent. The highest dose administered thus far, 1.8×10^7 , has been given to two participants. The second patient treated at this dose is the subject of this update. Prior to enrollment, the patient, a 67-year-old white male with refractory colon cancer, underwent therapy with multiple agents and drug combinations including FLOX, bevacizumab, irinotecan, cetuximab, and regorafenib. He also received radioembolization. The subject was treated under this protocol with VSV-hIFN β at 1.8×10^7 dose (TCID₅₀) on February 27, 2015. In the first 2 to 3 days following the injection, he had dyspnea, chills, and chest pain. It was not clear if these initial side effects were related to the study intervention, because some symptoms were present prior to dosing. Upon further evaluation, the patient was found to have bilateral pleural effusions and a drop in oxygen saturation with recovery that was considered secondary to aspiration. His symptoms improved after receiving steroids on day 3 post-dosing. Thoracentesis on day 2 and day 6 were unremarkable. His liver enzymes started trending up on day 5 and remained elevated through day 8. Ascites and paracentesis on day 8 were unremarkable. Encephalopathy with elevated ammonia was observed on day 8; the patient had a seizure later the same day, but a CT scan was uninformative. Antiepileptic medication was started, and an LP was scheduled for the next day. By day 10, the patient's condition deteriorated to the point where he was unable to follow simple commands or to verbalize. Systemic viremia was not clear at this point, but antiviral therapy (Ribavirin) was started on day 10. In the next few days, the patient became hypotensive, tachycardic, required pressors, and developed hepatorenal syndrome. He died on day 13 after withdrawal of aggressive care at the request of the family.

Post-mortem analyses indicated that some of the patient's complications were due to the investigational product. Biological samples (blood, saliva, urine) and tumor biopsy tissue were assayed for VSV-N RNA (determined via VSV-N qRT-PCR). VSV-N RNA was detected in all specimens, although shedding in urine was minimal. Viral RNA levels were two to three logs higher in tumor tissue compared with non-tumor tissue. Blood levels paralleled tumor levels. Interferon expression and VSV-neutralizing antibody serum levels steadily increased in the initial days following dosing but began to decline after antivirals were initiated. In contrast with results from this patient, viral RNA, interferon, and VSV-neutralizing antibody levels in samples from all other participants, including the other patient dosed at 1.8×10^7 , were below the limits of detection. Further, comparison of levels of 10 peripheral cytokines measured for all subjects prior to injection and over the post-dosing period has not been informative. Liver pathology (tissue specimen from day 8) in the affected patient was consistent with acute drug injury. Viral recovery assays indicate two coding mutations in isolated virus from normal liver tissue compared with virus isolated from tumor tissue injected with the vector and virus from non-injected tumor tissue.

The reason for the markedly different response in this patient is not clear. Possible contributory factors include a significant tumor burden; pre-trial radioembolization, which might have suppressed the patient's immune response; and administration of steroids at 3 days post-injection. The investigators continue to explore and analyze the complement of study data in an effort to better understand this case. The team is considering whether to enroll additional patients at lower doses to determine if there is any relationship between the dose and adverse effects of the viral vector.

B. RAC Discussion

Dr. Wooley noted the difficulty with assaying for RNA in saliva and urine samples in particular and stressed the importance of running proper controls to yield the most accurate results with respect to viral shedding and virus copy number. The RNA assay kit used by the investigators includes purified RNA for use as a control. In addition to diluting out the RNA by itself to determine the limit of detection (LOD) for the purposes of the research, positive (RNA-spiked) controls should also be run for each type of specimen being tested to assure that the LOD is known for each specimen. The data presented with this update indicate a negative result or, at most, a very small RNA signal in urine, further supporting the need to run rigorous controls in exactly the same way as the biospecimen. Dr. Borad noted that the team tests positive controls using the same fluids collected for analysis (e.g., saliva, urine). RNA sequencing of the collected samples is ongoing. The full analysis involves extensive bioinformatics and will take about a month to complete.

Dr. Russell, a member of the Mayo clinical team, noted the complexity of studies involving viral vectors and the challenge in interpreting results of assays designed to assess virus infectivity and detect RNA in biological specimens. To date, intact viral genomes/infectious virus have not been recovered from urine, saliva, or feces from any experimental animal. Thus, there is no evidence from any animal species infected with VSV that urine or saliva is the mode of transmission. Limited recovery has been seen in dogs given 10^{10} infectious units intravenously; in these studies, virus was detected for the first 2 hours post-administration but zeroed out within 3 hours, indicating a rapid loss of viral infectivity in blood. RNA was still detected after 3 hours, but, according to Dr. Russell, the RNA detected in the animal samples as well as the specimens collected from the current study is most likely messenger RNA inside a small number of infected cells. In response to a follow-up question from Dr. Hammarskjöld on whether the team measures both minus and plus mRNA, Dr. Borad stated that the assay can detect both because it uses both primers at the same time. However, the assay hasn't been validated yet, nor has it been used on clinical samples. In sum, it is important to clarify that there is no evidence from any animal species infected with VSV that urine or saliva is the mode of transmission.

Dr. Hammarskjöld inquired about data to support the possibility that immune suppression may have contributed to the patient's outcome, given the viral expansion seen after administration of the investigational product. To assess this possibility, the team is comparing the type 1 interferon responses at baseline and subsequent time points in the patient who died vs. the other patients. Other factors, such as PD-1 and PDL-1 expression, will also be explored to identify any differences or aberrations in this patient.

Dr. Sadelain noted that the data presented suggest that the affected patient was not immunocompromised, or at least had a comparable antibody response to the other patients. Dr. Sadelain asked about any evidence of known T-cell responses to VSV and if there are any mutations that could facilitate VSV replication (e.g., in the interferon pathway). Dr. Kohn noted that some oncolytic viruses grow in tumor cells because they are *p53* deficient and asked whether a different type of host factor, such as response to DNA damage, could play a role in controlling VSV, or if germline heterozygosity for factors such as *p53* could make patients more susceptible to viral replication.

To date, no evidence of significant immunosuppression has been found. Dr. Russell explained that because of the relatively comparable VSV antibody levels across all patients, it is reasonable to surmise at least a portion of the B-cell response in this subject was quite similar to that of the other patients. The T-cell response could differ, however, but when the team analyzed the patient's tumor tissue, there was no evidence of overt infiltration of CD3+ cells or other cells such as natural killer (NK) cells or neutrophils to suggest that any immune elements had been actively engaged. Other elements, such as tumor-associated macrophages, and markers can be investigated. The antibody response in this patient on day 8 was the highest we have seen for that day among all patients. Typically we would see the highest antibody level at day 15. Thus, the patient appeared to have a robust antibody response, which would be expected given the level of viral replication at that point. Immunohistochemical staining of the tumor biopsies were strongly positive for VSV antigen, indicating a large number of infected cells in the injected lesion. Dr. Russell commented that the questions of immune T-cell response and immune cell infiltration are yet to be fully determined. While the team is very interested in exploring these questions further, there currently are no good assays in place for VSV-specific immune responses. Propagation of the virus in this

patient's tumor but not in others is similar to what is seen in different mouse models. For the majority of tumor models, there is little virus propagation, but occasionally, the virus spreads rapidly through the tumor. Preclinical data suggest that this activity is a consequence of a weakened innate immune response in most tumors.

The team is doing RNA sequencing to identify any mutations or deletions that could be responsible for or contribute to this process. Dr. Borad noted that there is a known relationship between the *p53* gene and VSV, which will be explored in this case to try to determine if there is something different between the patient that died and the other participants. Genes involved in the Type 1 interferon pathway are other candidates to consider. These genes are found in a region where other tumor suppressor genes (e.g., *p16*) are located, and one scenario could be that the genes for the interferon pathway are co-deleted with a loss of the tumor suppressor genes.

In response to a question from Dr. Kohn about other currently active clinical trials of the VSV vector, Dr. Borad noted one trial of attenuated VSV studied as an HIV vaccine that reported no safety issues in a cohort of more than 50 patients. Another trial in development is planning to inject the same VSV vector as a potential treatment for refractory recurrent head and neck cancers. A related oncolytic virus (Maraba virus) is being studied in a trial conducted in Canada.

Dr. Zoloth requested further elaboration of the investigators' response to the decline and death of the patient following the family's decision to withdraw treatments. She also inquired about the informed consent discussion with the patient and what his wishes were. Dr. Borad explained that given the type of agent administered under this current study, the response seen in this case should probably be reviewed similarly to studies of CAR T-cell therapies, where some patients experience significant toxicities but pull through this period to subsequently demonstrate an anti-tumor response. Per the investigators' perspective, with this patient's history of metastatic refractory cancer and the leanings of the physicians who were taking care of him in the ICU, the family felt that survival from this event was unlikely. The family understandably did not want the patient to undergo any further suffering. Once the presser-supported ephedrine was withdrawn, the patient had very little chance of overcoming his situation. The informed consent discussion included the possibility of a strong cytokine response but not this specific event. The patient was aware of the type of treatment he was given and that some adverse events were possible. However, the severity and nature of these toxicities only came to light with this case. Following this experience, the investigators modified the consent document and consent process to include death and acute intensive care over a prolonged period of time.

The patient's advanced directive indicated his preference to forego aggressive treatment, including use of a ventilator. The investigators have since discussed whether other patients with similar directives would not be suited for this trial because their ability to derive benefit from the study would become limited. Dr. Zoloth cautioned against excluding patients with an advance directive that limits interventions. Instead, the investigators should assure that patients are informed about and understand the possible risks of participation, including death. Similarly, it would be helpful for the investigators to know the patient's wishes in case of a life-threatening event.

The investigators will incorporate the RAC's suggestions into the protocol and consent in a future amendment.

C. Public Comments

No comments from the public were offered.

IX. Vector Design Influences Hepatic Genotoxicity after Adeno-Associated Virus Gene Therapy

Presenter: Charles Venditti, M.D., Ph.D., National Human Genome Research Institute, NIH

A. Presentation by Dr. Venditti

Dr. Venditti's presentation focused on the development and study of a recombinant AAV viral vector expressing the mitochondrial enzyme methylmalonyl-CoA mutase (MUT) for use as a potential treatment for methylmalonic acidemia (MMA), a group of inborn errors of metabolism that derive primarily from the oxidation of amino acids (AA) inside mitochondria. For most patients, the final enzyme in this AA oxidation pathway, the MUT enzyme, is defective, resulting in the accumulation of methylmalonic acid leading to the clinical MMA phenotype. Due to its impact on the metabolism of amino acids, MMA affects multiple organ systems leading to organ failure and death associated with hyperammonemia and keto- and lactic acidosis. The condition manifests through poor growth and developmental delay. As MMA progresses, patients may be diagnosed with pancreatitis, cytopenias, optic nerve atrophy, lipodystrophy, stroke syndrome, and kidney failure. The immune response is suppressed, placing patients at increased risk of fungal and gram-negative infections. The quality of life of patients and their families is also impacted by MMA. A combined liver-kidney transplant or an orthotopic liver replacement can be done to stabilize patients against fatal metabolic morbidity by providing a depot of the enzymes needed for the AA oxidation pathway.

The research team has been studying the natural history of MMA for the past decade. Overall survival is poor but varies depending in part on whether the patient is homozygous or heterozygous for the *Mut* gene. Patients with the most severe type of MMA are vitamin B12-nonresponsive and constitute a nonresponsive *Mut*⁰ serial subtype. Despite the implementation of newborn screening to assess B12-responsiveness, outcomes for patients with MMA are very poor. Data from multiple studies show overall survival rates between 39 and 60 percent. Among B12-nonresponsive patients with early-onset disease, a mortality rate as high as 87 percent with a mean survival of 1.5 years has been reported.

The search for alternatives to transplantation has focused on different viral vectors (i.e., lentiviral, adenoviral, AAV) to treat mouse models and cell culture models of MMA. The murine model of *Mut* MMA has been instrumental in these investigations. This model is created by removing exon 3 of the murine *Mut* gene. The murine model of MMA is a complete null mutation for MUT enzyme with no detectable RNA or protein. These mutant mice have elevated levels of methylmalonic acid in the plasma and urine. The mutant mice appear normal at birth, but most die within the first few days of life. As with affected humans, the rare surviving *Mut*^{-/-} mice exhibit growth retardation and short lifespan. Dr. Venditti noted that, to date, the best outcomes have been seen with AAV vector gene transfer. *Mut*^{-/-} mice given a single hepatic injection of recombinant AAV (rAAV) in the neonatal period are rescued from lethality through correction of the metabolic error caused by the genetic mutation. The same outcome is seen with a variety of rAAV serotype MUT vectors with different promoters and enhancers. Whether a single treatment is sufficient to effect this change over the lifespan or if multiple treatments are needed is not currently known and warrants further study.

While the treated animals seemed to fare very well in the short term, a large number developed serious long-term sequelae following rAAV MUT gene transfer in the neonatal period. Specifically, as the treated MMA mice aged, they began to lose weight, stopped eating and their bellies became distended. Necropsy revealed multiple abdominal tumors in these mice, which histopathology showed to be hepatocellular carcinomas (HCC). For the last several years, the NIH trans-disciplinary research team has been investigating the question of why mice treated with a non-pathogenic AAV develop HCC. The team's efforts, results, and conclusions to date were recently published (Chandler et al., *J Clin Invest*, Feb 125(2):870-880, 2015) and were summarized during the meeting.

As part of this analysis, the investigators not only considered the overall outcomes and survival for the *Mut* mice, they also followed a large group of control *Mut* MMA mice (that had not been administered rAAV MUT vector) for 26 months to determine the inherent tumor and survival rate for untreated animals. Results showed that only 3 of 51 untreated control mice developed HCC. In contrast with the untreated mice, about 50 percent of heterozygote (*Mut*^{+/-}) animals, which are biochemically unaffected, and 75 percent of mutant animals (*Mut*^{-/-}) with a therapeutic transgene, developed HCC after receiving the rAAV MUT vector, as did 50 percent of heterozygous (*Mut*^{+/-}) mice that received a rAAV vector expressing green fluorescent protein (GFP). These data suggest that the AAV vector itself, and not the genotype of

the animal or the administered transgene, is causing the cancer. Further analysis indicated that this response appears to be dose-related. When the dose of the vector injected into the mouse during the newborn period is dropped by one log (from 10^{11} to 10^{10} genome copies per mouse), the cancer rate also goes down significantly, to a rate that is similar to that seen in the untreated mice. In considering these outcomes in the *Mut* MMA mice, the investigators turned to early reports of cancer in rAAV-treated mice, which occurred in a model for another metabolic disorder, mucopolysaccharidosis type VII (MPSVII). Although the initial study was small, three of five MPSVII mice treated with high-dose rAAV in the neonatal period developed HCC when they were older (between 35 and 72 weeks old).

What was confusing about the findings in the MPSVII mice is that the AAV genome copy number in the tumors was low, suggesting that cancers did not result from a clonal event. The caveat, however, is that if the recombinant vector product has a transgene that is embedded in the vector and the vector is rearranged or deleted, a one-to-one ratio of genomic DNA to vector is not seen because the part of the vector that would be detected is gone. Some trials were put on hold following publication of the study on MPSVII mice.

Dr. Venditti reviewed the findings of several publications that reported similar or conflicting results. One analysis involving nearly 700 normal mice given AAV vectors found that only one mouse developed cancer, yielding a tumor frequency of 0.14 percent. A symposium titled "Safety considerations in the use of AAV vectors in gene transfer clinical trials", jointly sponsored by the NIH and the FDA, was convened in March 2001 to review available data on AAV vectors. The conclusion reached at the symposium was that there was "no evidence to suggest that the vector caused cancer" based on data from hundreds of mice. The large study had several critical differences when compared to the MPSVII mouse studies that could explain the marked difference in cancer rate. The doses of the vectors given to the mice in the large study were considerably lower than the dose given to the MPSVII mice. The vectors had different promoters, enhancers, and transgenes. In addition, mice were treated in the juvenile stage, not when they were newborns. The duration of follow-up is also critical. An HCC takes 16 months to be clinically significant in the mouse, and if the animals are sacrificed at 6, 9, or even 12 months, it is unlikely that any HCCs would be seen. Thus, generalized statements about AAV vectors based on studies that did not control for factors that contributed to tumorigenesis do not reflect accurate comparisons.

A subsequent study investigating the therapeutic treatment of another metabolic mouse model (i.e., deficient in ornithine transcarbamylase (*Otc*)) found that mice treated with an AAV vector that expressed *LacZ* had an increased risk of developing HCCs compared to both untreated animals and animals given vectors that expressed *Otc*. Based on these findings, the authors concluded that AAV vectors alone do not contribute to tumor development. Several additional studies have looked at development of hepatic tumors and tumors in other organs over time using different routes of administration of AAV vectors in various animal models with varying results. In one study, mice given rAAV vectors intracranially at birth developed HCC between 13 and 19 months of age. Dr. Russell and colleagues took a different approach to this problem by making a vector to target a specific gene locus. In this case, an integrating AAV vector was designed to activate genes in the region that causes HCC; the integration was successful, and mice developed and died from HCC. Results of another study using a targeted integrating AAV vector were paradoxical. The study used juvenile mice prone to HCC that underwent a partial hepatectomy early after gene delivery, which was expected to reduce the tumor rate. Although a low number of integrations was found, 80 percent of the mice had liver tumors.

To better understand how a non-pathogenic AAV can cause cancer in these animal models, several teams, including Dr. Venditti and his colleagues, considered various factors such as vector serotype and expression cassettes, in relation to the full integration profile of the AAV and the cancer risk the vector confers. Collaborating with Dr. Burgess's group in the Translational and Functional Genomics Branch, NHGRI, NIH, Dr. Venditti and colleagues proceeded to map and sequence AAV ITR integration sites in hepatic tumors of treated animals and the liver of matched controls. Analysis of 33 cancers and 31 matched control liver samples revealed nearly 2,900 unique integrations at a number of chromosomal locations. Many of the integrations map to the *albumin* (*Alb*) and *alpha-fetoprotein* (*Afp*) loci, which would be expected to be highly expressed in the neonatal liver and thus responsible for the high number of integrations seen in HCC DNA. Upon closer examination, the *Rian* murine genomic locus, which includes

a number of microRNAs (miRNA), emerged as a significant locus because it exhibited rare integration events in HCC DNA that correlated with an elevated incidence of liver cancer and associated clonal events. The *Rian* locus was also identified as the main locus in another study with using different strains of mice. The team validated each integration junction by sequencing and cloning out all junctions from every tumor with an integration event to prove that these events were unique and clonal. Expression analysis was done for the AAV-associated HCCs and miRNAs, showing that integration events in the chromosome 12 *Rian* locus correlated with significant dysregulation of miRNAs in that region (*cis* effects) as well as dysregulation of miRNAs on chromosome 4 (*trans* effects). The results of these analyses have been published, and the data sets are available to the community.

A similar integration pattern was observed with another AAV vector expressing a synthetic codon optimized *MUT* gene driven by the human alpha-1-antitrypsin (hAAT) promoter. Equally high integration frequencies were observed in both the *Afp* and *Alb* loci as well as rare integrations in the *Rian* locus that mapped identically to those seen with other AAV vector constructs. However, the incidence of HCC using the hAAT AAV construct was lower, leading to the corroboration of a previously formulated hypothesis that the strength of AAV-driven promoter-enhancer transactivation at distal regulatory sites predisposes cells to becoming malignant.

Analysis of the integration events identified specific locations in the *Rian-Mirg* region, specifically the miRNA *Mir341* locus where rare AAV vector integrations predominantly occurred. In mice and rats, the integration sites occur within a repeat sequence that is absent in other mammals. The sequences surrounding the AAV integration site are highly conserved. The basis for integration preference into a specific locus is not yet known and to date, efforts to locate the other side of the ITR integration site have been unsuccessful.

A primary task going forward is determining how to balance the risk of HCC and the factors contributing to this risk against the therapeutic benefit of the experimental treatment for a research participant. Questions warranting further investigation include whether development of HCC is a mouse-specific phenomenon, why *Mir341* is a preferred integration target, which genes and miRNAs are causative vs. associated to tumorigenesis, and whether distribution of AAV integration in primates would be similar in subjects treated with rAAV. In addition, studies are needed to explore other promoter/enhancer cassettes that could integrate into the *Mir341* locus and not cause HCC but show therapeutic efficacy.

B. RAC Discussion

Dr. Chatterjee noted that some factors such as dose/genome number and age at the time of injection were similar in studies that reported different outcomes, including the study involving hundreds of mice. The group at Ohio State University has injected large doses of vector genomes (e.g., 10^{13} to 10^{14}), and several other groups have followed treated mice for long periods of time without seeing any HCCs. Given these findings, it isn't clear that dose is a major contributing factor to tumorigenesis in animals administered rAAV vectors. Dr. Venditti acknowledged that results vary across studies. However, the absence of HCC in treated mice does not necessarily mean there are no integrations. If different methods and platforms are used, it becomes difficult to compare outcomes. Further, unless a comprehensive integration analysis is done to find, validate, and map integration events, then the results may be more "anecdotal" than evidentiary. Regarding specific studies, Dr. Venditti pointed out that the mega-mouse study was not a dedicated study to assess HCC formation; rather, it was a compilation of many different studies with different vectors that were combined into a cohort of nearly 700 mice. Some of the toxicity studies may have ended at 1 year, which would not have been a sufficient amount of time for development of HCCs.

Given the inconsistent findings regarding HCC association with AAV, Dr. Chatterjee asked whether contamination during vector preparation could account for any of these outcomes. Dr. Venditti noted that contamination cannot be completely ruled out, but he considered contamination unlikely given that the fundamental mechanism and locus of the integration appear to be conserved across different mouse models and different platforms (i.e., different promoters and enhancers, different vectors). An experiment involving preparation of AAVs using different techniques and transfection methods could be done to test

for contamination. The same result across all preparations and platforms would indicate that the process and vectors are not contaminated.

Dr. Chatterjee inquired about whether there is a human counterpart for the putative “hot spot” that causes HCC in mice and if so, how the frequency of integration will be determined at that locus in clinical studies (e.g., via primers that flank the integration site). Use of appropriate primers could be incorporated into new trials and perhaps retrospectively in ongoing studies where applicable. Dr. Venditti noted that there is a counterpart for the *Rian* locus in humans, but it does not contain one of the repeat sequences found in mice and rats. Whether this sequence is critical for HCC formation is not known.

Dr. Chatterjee also noted that most of the rearrangements seen with AAV integration usually occur at the end of a sequence, near the ITRs. If the ITRs are rearranged, deleted, or otherwise modified, use of ITR primers would likely underestimate integration events. Dr. Chatterjee also asked if the investigators have ever taken the primers to the center of the vector, which doesn't frequently rearrange, as a control to compare with ITR primer data. Dr. Venditti recognized the potential drawbacks with ITR primers given ITR rearrangements, including micro-rearrangements at the end of the ITR at the integration junction and gross rearrangements that remove an entire ITR. Dr. Venditti noted that in his studies, he is unable to detect the presence of a transgene in HCC DNA suggesting rearrangements are occurring at the integration sites such that only one AAV ITR can be detected. As noted during the presentation, it has not been possible for his team and others to march along the vector genome using PCR primers to identify the integration site of the second ITR, suggesting again that gross rearrangements of the vector genome may also be occurring. However, if the enhancer-mediated transactivation activation model is correct, enhancer sequences should be present at the expected one-to-one frequency.

Oncogenes and tumor suppressor genes are typically highly conserved, making it difficult to translate results from animals to humans. Dr. Hammarskjöld noted that dysregulation of the genes associated with the locus for AAV HCC in mice further complicates the translational aspect of this research because of the significant evolutionary differences in non-coding RNA between mice and humans.

In response to a question from Dr. Hearing, Dr. Venditti noted that about 75 percent of HCCs have an AAV integration at the *Rian* locus. There is no nucleotide sequence homology with the AAV vector in that region. In response to Dr. Kohn's question regarding the possibility that the *Mir341* integration site is fragile, Dr. Venditti responded that the *Mir341* RNA is transcribed at a high level in the mouse liver early in life (i.e., in the neonatal period), but it is not known if it is in a fragile site. The investigators will consider Dr. Kohn's suggestion to model this in cell lines.

Dr. Zoloth asked whether testing should proceed in humans based on results in animals. Dr. Venditti commented that data suggesting that AAV can cause HCC in animal models remain controversial. How the research proceeds will depend on decisions by investigators and institutions based on interpretation of available data. Per results of mouse studies by the NIH team and others, the genomic profile suggests that a specific locus is associated with HCC formation and that there might be other factors within the locus that accentuate the integration phenomenon in mice. Whether this phenomenon translates to humans and/or other species is unknown at this point because the locus-specific sequences in mice do not appear to be present in the genomes of humans or other animals. Consent documents for AAV gene therapy trials should include information about the mouse studies, the safety profile of the vector, and why the risk of HCC in humans is not known.

C. Public Comments

No comments from the public were offered.

X. Closing Remarks and Adjournment

Dr. Kohn thanked the RAC members and the OBA staff and adjourned the June 2015 RAC meeting at 5:47 p.m. on June 9, 2015.

(Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, they are not considered final until approved by the NIH Director.)

Lyric Jorgenson, Ph.D.
RAC Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and the following Attachments are accurate and complete.

This Minutes document will be considered formally by the RAC at a subsequent meeting; any corrections or notations will be incorporated into the Minutes after that meeting.

Date: _____

Donald B. Kohn, M.D.
Chair, Recombinant DNA Advisory Committee

**Attachment I:
Recombinant DNA Advisory Committee Roster**

Chair

KOHN, Donald B., M.D.
Professor
Microbiology, Immunology, and Molecular
Genetics and Pediatrics
David Geffen School of Medicine
University of California, Los Angeles
Los Angeles, CA 90095

Members

ATKINS, Michael, M.D.
Deputy Director
Georgetown-Lombardi Comprehensive Cancer
Center
Professor of Oncology and Medicine
Georgetown University School of Medicine
Washington, DC 20057

CAI, Tianxi, Sc.D.
Associate Professor
Department of Biostatistics
Harvard University
Boston, MA 02115

CANNON, Paula M., Ph.D.
Associate Professor
Department of Pediatrics and Biochemistry
Keck School of Medicine
University of Southern California
Los Angeles, CA 90027

CHATTERJEE, Saswati, Ph.D.
Professor
Division of Virology
City of Hope National Medical Center
Beckman Research Institute
Duarte, CA 91010

CURRY, William, M.D.
Director
Department of Neurosurgical Oncology
Massachusetts General Hospital
Associate Professor of Surgery
Harvard Medical School
Boston, MA 02114

DONAHUE, Kevin, M.D.
Professor
Cardiovascular Medicine
University of Massachusetts Medical School
Worcester, MA 01655

DRESSER, Rebecca, J.D.
Daniel Noyes Kirby Professor of Law
Professor of Law and Ethics in Medicine
Washington University School of Law
St. Louis, MO 63130

HAMMARSKJÖLD, Marie-Louise, M.D., Ph.D.
Professor of Microbiology
Associate Director, Myles H. Thaler Center for
AIDS and Human Retrovirus Research
Charles H. Ross, Jr., Professor of Multiple
Sclerosis Research
University of Virginia School of Medicine
Charlottesville, VA 22903

HARDISON, Angelica, M.B.A.
Compliance Analyst
Office of Compliance and Enterprise Risk
Management
Georgia Regents Health System
Georgia Regents University
Augusta, GA 30912

HEARING, Patrick, Ph.D.
Professor
Department of Molecular Genetics and
Microbiology
Stony Brook University
Stony Brook, NY 11794

KAUFMAN, Howard, M.D., F.A.C.S.
Professor
Department of Surgery
Robert Wood Johnson Medical School
Rutgers, The State University of New Jersey
New Brunswick, NJ 08854

KIEM, Hans-Peter, M.D., Ph.D.
Professor of Medicine
Adjunct Professor of Pathology
University of Washington School of Medicine
Jose Carreras/E. Donnall Thomas Endowed
Chair for Cancer Research
Fred Hutchinson Cancer Research Center
Seattle, WA 98109

PILEWSKI, Joseph, M.D.
Associate Professor of Medicine, Pediatrics,
and Cell Biology and Physiology
Pulmonary, Allergy, and Critical Care Division
University of Pittsburgh
Pittsburgh, PA 15213

ROSS, Lainie, M.D., Ph.D.
Professor
Departments of Pediatric, Medicine, and Surgery
University of Chicago
Chicago, IL 60637

SADELAIN, Michel, M.D., Ph.D.
Director
Center for Cell Engineering and Gene Transfer
and Gene Expression Laboratory
Stephen and Barbara Friedman Center
Memorial Sloan-Kettering Cancer Center
New York, NY 10065

WHITLEY, Richard, M.D.
Distinguished Professor of Pediatrics,
Microbiology, Medicine, and Neurosurgery
Loeb Eminent Scholar Chair in Pediatrics
Department of Pediatrics
School of Medicine
University of Alabama, Birmingham
Birmingham, AL 35233

WOOLEY, Dawn P., Ph.D.
Associate Professor
Department of Neuroscience, Cell Biology, and
Physiology
School of Medicine
Wright State University
Dayton, OH 45435

ZOLOTH, Laurie, Ph.D.
Professor of Medical Humanities & Bioethics
and Religion
Director, Center for Bioethics, Science and
Society
Feinberg School of Medicine
Professor of Religious Studies
Director, Brady Program in Ethics and Civic Life
Weinberg College of Arts and Sciences
Northwestern University
Evanston, IL 60208

Executive Secretary

JORGENSON, Lyric, Ph.D.
Acting Director
Office of Biotechnology Activities
Office of Science Policy
Office of the Director
National Institutes of Health
U.S. Department of Health and Human Services
Bethesda, MD 20892

Ad Hoc Reviewers and Participants

GALPERN, Wendy, M.D., Ph.D.

Program Director

Office of Clinical Research

National Institutes of Neurological Disorders
and Stroke

National Institutes of Health

U.S. Department of Health and Human Services

Bethesda, MD 20892

LUKSENBURG, Harvey, M.D.

Special Advisor to the Director

Division of Blood Diseases and Resources

National Heart, Lung, and Blood Institute

National Institutes of Health

U.S. Department of Health and Human Services

Bethesda, MD 20892

Ad Hoc Presenters and Speakers

BORAD, Mitesh, M.D.
Assistant Professor of Medicine
Mayo Clinic
Scottsdale, AZ 85259

NOLTA, Jan, Ph.D.
Professor and Director
Stem Cell Program
Institute for Regenerative Cures
University of California, Davis
Sacramento, CA 95817

ROSS, Robert, M.D.
Senior Vice President
Clinical Development
Bluebird bio, Inc.
Cambridge, MA 02141

TABAK, Lawrence, D.D.S., Ph.D.
Principal Deputy Director
Office of the Director
National Institutes of Health
U.S. Department of Health and Human Services
Bethesda, MD 20892

TARAPOREWALA, Zenobia, Ph.D.
Division of Cellular and Gene Therapies
Office of Cellular, Tissue, and Gene Therapies
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
U.S. Department of Health and Human Services
Rockville, MD 20852

THOMPSON, Alexis, M.D., M.P.H.
Professor of Pediatrics
Feinberg School of Medicine, Northwestern
University
Section Head
Ann and Robert H. Lurie Children's Hospital of
Chicago
Chicago, IL 60611

VENDITTI, Charles, M.D., Ph.D.
National Human Genome Research Institute
National Institutes of Health
U.S. Department of Health and Human Services
Bethesda, MD 20892

WALTERS, Mark, M.D.
Director
Blood and Marrow Transplantation
UCSF Benioff Oakland Children's Hospital
San Francisco, CA 94609

WHEELOCK, Vicki, M.D.
Clinical Professor
Department of Neurology
Davis Medical Center
University of California, Davis
Sacramento, CA 95817

Nonvoting Agency and Liaison Representatives

***U.S. Department of Health and Human
Services***
Office for Human Research Protections

BORROR, Kristina, Ph.D.
Director
Division of Compliance Oversight
Office for Human Research Protections
U.S. Department of Health and Human Services
Rockville, MD 20852

U.S. Food and Drug Administration
Office of Cellular, Tissue, and Gene Therapies

GAVIN, Denise, Ph.D.
Product Specialist
Division of Cellular and Gene Therapies
Office of Cellular, Tissue, and Gene Therapies
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
U.S. Department of Health and Human Services
Rockville, MD 20852

**Attachment II:
Public Attendees**

(This list includes only individuals who are not identified elsewhere in this document. It does not include two individuals whose names are illegible on the sign-in sheets.)

Geralyn Annett, University of California, Davis
Khandan Boradoran, Dimension Therapeutics
Gerhard Bauer, University of California, Davis
Jim DeTore, bluebird bio, Inc.
Jennifer Dittman, bluebird bio, Inc.
Anne-Virginie Eggimann, bluebird bio, Inc.
Jeff Ellsworth
Narin Hussan, Novartis
Kyle Fink, Ph.D., University of California, Davis
William Gruenloh, University of California, Davis
Oliver Nagne, bluebird bio, Inc.
Manisna Patel, Novartis
Alexandria Petrusich, bluebird bio, Inc.
Robert Ross, M.D., bluebird bio, Inc.
Jennifer Sloan, National Human Genome Research Institute
Sandeep Soni, bluebird bio, Inc.
Teresa Tempkin, University of California, Davis
Gerald Schwarz
Mark Walters, M.D., Oakland Children's Hospital
Eleanor Yu, bluebird bio, Inc.

Attachment III: Abbreviations and Acronyms

AA	amino acids
AAV	adeno-associated virus
AE	adverse event
Afp	alpha-fetoprotein
allo-HSCT	allogeneic hematopoietic stem cell transplant
ALS	amyotrophic lateral sclerosis
BDNF	brain-derived neurotrophic factor
BLA	Biologics License Application
BMT	bone marrow transplant
CBER	Center for Biologics Evaluation and Research, FDA
CGI	Clinical Global Impression
CNS	central nervous system
CRS	cytokine release syndrome
CSF	cerebrospinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose-limiting toxicity
DMC	data monitoring committee
DSMB	data and safety monitoring board
ECog	Everyday Cognition scales
FDA	U.S. Food and Drug Administration
FLOX	5-fluorouracil/folinic acid/oxaliplatin
GCP(s)	good clinical practice(s)
G-CSF	granulocyte-colony stimulating factor
GeMCRIS	NIH Genetic Modification Clinical Research Information System
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
hAAT	human alpha-1 antitrypsin
HbA	hemoglobin A
HCC	hepatocellular carcinoma
HD	Huntington's disease
HDSA	Huntington's Disease Society of America
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hMSCs	human MSC/BDNF
HSCT	hematopoietic stem cell transplant
HSV	herpes simplex virus
HSV1	herpes simplex virus type 1
HTT, mHTT	huntingtin protein, mutant huntingtin protein
IB	Investigator Brochure
ICD	informed consent document
ICH	International Conference of Harmonization
IFN β	interferon-beta
iMRI	interventional MRI
IND	investigational new drug
IRB	institutional review board
ITR	inverted terminal repeat
LCR	locus control region
LFT	liver function test
LP	lumbar puncture
LTR	long terminal repeat
mHTT	mutant huntingtin protein
MMA	methylmalonic acidemia
MoCA	Montreal Cognitive Assessment
MOI	multiplicity of infection
MPSVII	mucopolysaccharidosis type VII

MRI	magnetic resonance imaging
MSC	mesenchymal stem cells
MSC/BDNF	mesenchymal stem cells engineered to produce BDNF
MTD	maximum tolerated dose
NCI	National Cancer Institute
NHGRI	National Human Genome Research Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities, NIH
OCTGT	Office of Cellular Tissue and Gene Therapies, FDA
OD	Office of the Director, NIH
<i>Otc</i>	ornithine transcarbamylase
PCR	polymerase chain reaction
PI	principal investigator
PBMCs	peripheral blood mononuclear cells
rAAV	recombinant AAV
RAC	Recombinant DNA Advisory Committee
RBCs	red blood cells
RBD	radial branched deployment
RCL	replication-competent lentivirus
SAE	serious adverse event
SCID	severe combined immunodeficiency
SIN	self-inactivating
TCID ₅₀	50 percent tissue culture infective dose
TFC	Total Functional Capacity
TFCs	TFC Scale
UC	University of California
UHDRS	Unified Huntington's Disease Rating Scale
VBGT	virus- and bacteria-based gene therapy
VCN	vector copy number
VSV	vesicular stomatitis virus
VSV-HuIFN- β	interferon-beta-expressing vesicular stomatitis virus

**Appendix A:
Public Comments**

[No public testimony was provided at the June 2015 RAC meeting.]