
RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 9, 2015

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

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[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>].

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

September 9, 2015

The Recombinant DNA Advisory Committee (RAC) convened for its 143rd meeting at 10:00 a.m. on September 9, 2015, at the National Institutes of Health (NIH), Building 35, Conference Room 620/630, Bethesda, Maryland. Dr. Hans-Peter Kiem (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 10:00 a.m. until 3:42 p.m. on September 9, 2015. The following individuals were present, either in person or by teleconference, for all or part of the September 2015 RAC meeting.

Committee Members

Michael Atkins, Georgetown University School of Medicine
Paula Cannon, University of Southern California
Saswati Chatterjee, City of Hope National Medical Center
Mildred Cho, Stanford University School of Medicine
David DiGiusto, City of Hope National Medical Center
Kevin Donahue, University of Massachusetts Medical School
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 (RAC Chair), University of Washington School of Medicine/Fred Hutchinson Cancer Research Center
Dean Lee, University of Texas MD Anderson Cancer Center
Douglas McCarty, Ohio State University College of Medicine
Joseph Pilewski, University of Pittsburgh
Lainie Ross, University of Chicago
Richard Whitley, University of Alabama at Birmingham (*via teleconference*)
Dawn Wooley, Wright State University
Laurie Zoloth, Northwestern University (*via webcast*)

NIH Office of Biotechnology Activities (OBA)

Lyric Jorgenson, Office of the Director (OD), NIH

Nonvoting Agency Representatives

Lisa Buchanan, Office for Human Research Protection, NIH
Denise Gavin, U.S. Food and Drug Administration (FDA)
Carrie Wolinetz, Office of Science Policy, NIH

NIH/OD/OBA Staff Members

Linda Gargiulo
Morad Hassani
Robert Jambou
Maureen Montgomery
Chris Nice

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.

Marina O'Reilly
Gene Rosenthal
Aparna Singh

Attendees

There were 48 attendees at this 1-day RAC meeting.

Attachments

Attachment I contains a list of RAC members, nonvoting agency and liaison representatives, and attendees present for the bioethics discussions. 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. Kiem, the RAC Chair, called the meeting to order at 10:00 a.m. on September 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 August 19, 2015 (80 FR 50299). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC) and public review and discussion of three gene transfer protocols.

RAC members introduced themselves by name, affiliation, and research interests. The committee welcomed the following new members: Dr. Cho, Dr. DiGiusto, Dr. Lee, and Dr. McCarty. Dr. Kiem, who has been a member of the RAC since 2011, is the incoming RAC Chair with the departure of Dr. Donald Kohn, who served as the previous RAC Chair.

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, June 9, 2015

RAC Reviewers: Drs. Curry and Wooley

Because Dr. Curry was not able to attend the meeting, consideration and approval of the minutes of the June 9, 2015, RAC meeting were deferred.

III. Review and Discussion of Human Gene Transfer Protocol #1507-1446: A Phase I, Open-Label, Ascending Dose Study to Assess the Safety and Tolerability of AAV2/6 Factor IX Gene Therapy via Zinc Finger Nuclease (ZFN)-Mediated Targeted Integration of SB-FIX in Adult Subjects with Severe Hemophilia B

Co-Principal Investigators (Co-PIs): Nadia Ewing, M.D., and John Zaia, M.D., City of Hope
National Medical Center

Sponsor: Sangamo BioSciences, Inc.

RAC Reviewers: Drs. Donahue, Hammarskjöld, and Hearing

Drs. Cannon, Chatterjee, and Kiem were recused from consideration of this protocol due to conflicts of interest. As a result of Dr. Kiem's recusal, Dr. Hammarskjöld chaired this section of the September 2015 RAC meeting.

A. Protocol Summary

Severe hemophilia B is an inherited blood disease that affects boys starting in the first two years of life. Boys with this condition are born with very low levels (<1 percent) of one of the proteins that are needed to make their blood clot, called Factor IX (FIX). Without treatment, patients are at risk for spontaneous and post-traumatic hemorrhages, typically in joints or muscles but also in the brain (as intracranial hemorrhage), at frequencies of two to five times per month. Current optimal treatment entails a lifetime of repeated infusions of recombinant FIX, up to twice a week, as prophylactic treatment, supplemented by additional infusions at the time of trauma or bleeding. While factor replacement therapy is generally effective at preventing and treating bleeds, breakthrough bleeding can occur in between doses of replacement factor.

The goal of the proposed clinical trial is to enable permanently, in a single treatment, hepatic production of therapeutic levels of human FIX (hFIX) activity to decrease or potentially to eliminate the need for prophylactic treatment. To accomplish this aim, a “genetic editing” technology will be used to disrupt the endogenous albumin gene in hepatocytes cells by using zinc finger nucleases (ZFNs), which causes a double-strand DNA break and subsequent repair by the natural DNA repair mechanisms of the cell to insert FIX complementary DNA (cDNA) by homologous recombination. The *in vivo* genome editing method will use two ZFNs targeting intron 1 of the albumin locus (ZFN1 and ZFN2) and a human FIX donor template construct (cDNA donor). The ZFNs and donor template will be encoded on separate adeno-associated virus (AAV) 2/6 vectors; the ZFNs will be used to specifically insert the AAV2/6 FIX cDNA (SB-FIX) in the liver albumin genome locus. The SB-FIX construct will be injected intravenously and transduce hepatocytes, resulting in targeted insertion of a corrected copy of hFIX gene into the albumin locus in a proportion of liver hepatocytes. Instead of producing albumin, these “corrected” hepatocytes will then produce hFIX in therapeutic quantities, driven by the highly active albumin enhancer or promoter, to treat hemophilia B. *In vivo* experiments in mice and non-human primates (NHPs) using AAVs to encode the appropriate species-specific ZFNs and hFIX donor template show measurable evidence of gene modification of hepatocytes at the albumin locus and sustained levels of circulating hFIX in both species that would be therapeutic if achieved in human subjects with hemophilia B.

The proposed trial is a Phase I, open-label, ascending dose study to assess the safety of a single intravenous (IV) infusion of SB-FIX in adult research participants with severe hemophilia B. Up to 18 adult males with severe hemophilia B without inhibitors to FIX and no hypersensitivity to recombinant FIX will be enrolled. There will at least three research participants enrolled into each of the three dose cohorts. Enrollment within a cohort may be increased to six research participants if a Grade 3 adverse event (AE) related to the study drug occurs in one of the first three research participants. Within each cohort, treatment will be staggered so that each subsequent research participant cannot be infused until at least four weeks after the preceding research participant. Dose escalation to the next cohort cannot occur until at least four weeks after the last research participant in the preceding cohort has been dosed and safety data from the entire prior cohort has been reviewed by the Safety Monitoring Committee (SMC). A single dose of each of the three components of SB-FIX (ZFN1, ZFN2, and cDNA donor) will be mixed in phosphate-buffered saline containing 0.25 percent human serum albumin and administered via intravenous infusion while the research participant is in the hospital. After a 24-hour observation period, research participants will be discharged from the hospital and then evaluated weekly at the study center for the first three months and monthly between months three and 12. At each study visit, a clinical history and physical examination will be carried out, samples for routine laboratory tests and study specific assays will be collected, and the frequency of bleeding episodes and FIX protein concentrate use will be assessed.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because it involves the first-in-human use of genomic editing to introduce a gene rather than disrupt gene expression.

Three RAC members provided written reviews of this proposed Phase I trial.

Dr. Donahue found the proposed strategy to be a novel and interesting idea. He noted, however, that the planned vector dose is considerably higher than prior AAV hemophilia clinical trials. Since those trials showed toxicity at their highest dose (lower than this trial's starting dose), it might be prudent to start at a lower dose. Dr. Donahue requested further information on how the differences in the proposed strategy will prevent AAV dose-related toxicity. In addition, he found the immunotherapy plan to be somewhat vague and asked whether it is possible to design the plan specifically to prevent vector-related toxicity or to make sure research participants are effectively treated before significant loss of transduced hepatocytes occurs.

Potential toxicity from gene editing is a novel component of this trial, since it is the first-in-human use of ZFNs to introduce a gene with *in vivo* delivery. This puts the proposed trial in a different risk category than prior hemophilia clinical trials. Even for a Phase I trial, this is a small study. Given the additional potential risk with a first-in-human intervention, instead of using the typical "a few in each dose group" Phase I design, the study should be powered to be sufficient to determine reasonable safety for progression within the trial and for progression to a Phase II study after completion of the Phase I trial.

In the sample size discussion in the protocol, the replacement of research participants that are not dosed seems reasonable. The replacement of research participants lost to follow-up could add bias to the results, however, and handling of these research participants should be reconsidered so that potential study toxicity is not missed.

Dr. Donahue had the following additional comments and questions about the study protocol and preclinical data:

- The protocol states that liver function will be monitored weekly "for four weeks until normalization of liver enzymes...." The parameters for monitoring need to be clarified; that is, will liver function be monitored weekly for four weeks total or weekly until four weeks after normalization of liver enzymes?
- AAV6 is reported to have tropism to a variety of organs. What is the biodistribution of the planned vector in preclinical studies? What are the risks of off-target integration and how will these be treated (e.g., if tumor activity or other toxicity is seen with the anticipated very low-level disruption of *Smchd1*)?
- The scientific abstract and appendix M discussion cite an interval of two weeks between enrollments within each dosing group, but the study protocol cites a four-week interval within each cohort. This interval needs to be clarified and made consistent across and throughout all documents. Dr. Hammarskjöld also noted this discrepancy and commented that it would be prudent to wait four weeks between research participants, since potential side effects due to immune responses would not be seen right away.
- Long-term study follow-up will likely be required and should be considered given the gene editing component of this work. The investigators should check FDA guidance on this issue.

The language used throughout the informed consent document (ICD) is beyond what the typical fifth-grader would be able to understand. The investigators should review the entire document and simplify the language for improved comprehension. In addition, all abbreviations should be defined and explained. Dr. Donahue also suggested rephrasing the description of the role of the institutional review board in the ICD.

Dr. Hammarskjöld considered the protocol to be generally very well written and designed to achieve the goal of insertion of a FIX cDNA into the albumin gene. She noted that previous strategies using AAV vectors for hemophilia therapy have generally not achieved the required levels of FIX expression for prolonged periods. While the proposed strategy is a logical next step, there are several known and unknown risks, as described in the protocol.

Dr. Hammarskjöld had the following comments and questions regarding Appendix M:

- The description of the AAV2/6 donor vector containing the hFIX donor is somewhat unclear. The cDNA would not contain the splice acceptor site, meaning that the “cDNA” contains some intron 1 sequences. Further detail is needed on how much of intron 1 was.
- It also would be helpful to include a schematic drawing of what the complete “pre-mRNA” that will be expressed from the hybrid albumin (hALB)/FIX “locus” will look like. Details such as whether there a 5’ splice site at the end of the FIX “transgene” and the location of the start site AUG in the spliced messenger RNA (mRNA) product should be provided. Nucleotides 1890-2114 appear to represent a bovine growth hormone polyA, but it is not clear whether these sequences are supposed to result in efficient termination or polyA addition.
- Preclinical studies were performed both in mice and monkeys, and the investigators also used human tissue culture cells to look at transduction efficiency and off-target and other events. Was an RNA sequencing analysis ever performed to look at the “hybrid” mRNA that was expressed from the recombined albumin locus? If so, what did the mRNA look like, and was there any evidence of mis-splicing and “exon” skipping that could possibly result in aberrant albumin proteins being synthesized?

The following additional comments and questions focused on the clinical protocol:

- The statement, “The zinc finger nucleases induce a double strand break in the albumin genome, which is repaired by homologous recombination with Factor IX cDNA,” probably means to say “the albumin gene” instead of “the albumin genome.” It also would be more correct to state that the repair is a result of homologous recombination with albumin sequences flanking the FIX coding region.
- It is not clear how the investigators will determine whether potential research participants have developed inhibitors to FIX and whether this is an exclusion criterion for the proposed study. The stated exclusion criterion, “history of hypersensitivity response to FIX,” is vague, and it is not clear how this parameter will be assessed.
- The proposed three-year follow-up period may not be sufficient. Considering that this protocol is designed to insert a heterologous gene fragment into the genome by homologous recombination, resulting in permanent changes, the investigators may want to consider a longer long-term follow-up period that takes potential long-term risks into account (e.g., a malignancy may take many years to develop).
- References to “peak levels of ZFN activity” following administration of the vector in mice need to be more clearly defined, including how ZFN activity was measured.
- It is not clear how long after vector administration levels of hFIX peaked in NHPs. Any additional data on long-term FIX production in these animals should be provided.
- The protocol states that follow-up analysis for nine of the ten potential off-target sites is still in progress. Dr. Hammarskjöld asked whether this analysis been completed and whether there are any additional data on possible off-target sites.

Dr. Hammarskjöld suggested the following revisions to the ICD:

- The statement, “You do not have to be in this study to get help for your hemophilia B illness,” is potentially misleading, because it sounds as though participating in this study is anticipated to “help” patients with this disease. This is inappropriate for a Phase I study and should be rephrased.
- The document also says, “...There is no guarantee that being in this study will help you.” As with the above statement, this language is misleading for a Phase I study, for which there are inherent unknown risks and potential benefits. The investigators should delete this wording.
- Statements regarding “withdrawal” need to explain clearly that because this is a gene therapy study, research participants cannot withdraw from the study in the same way as with other types of trials. The ICD should emphasize that for this reason, research participants may want to continue to be monitored for possible side effects even after they decide to “leave” the study. The current statement, “You can decide not to be in the study and you can change your mind about being in the study at any time,” needs to be modified to reflect these points.

Dr. Hearing found the proposed trial to be a sophisticated and well-developed first-in-human protocol based on extensive preclinical studies. He had the following questions and comments:

- Have AAV vectors that were produced by using the baculovirus system been used in previous clinical trials? Were the AAV 2/6 vectors that were used for preclinical studies, particularly with mouse and NHP experiments, produced by using the baculovirus system?
- In a prior Phase I and II trial (Nathwani et al., *NEJM* 2014), both research participants treated with the highest AAV8 dose (2.0×10^{12} vector genomes [vg]/kg) experienced symptoms that required prednisolone treatment for six to nine weeks. The total starting dose in the proposed protocol is 5.0×10^{12} vg/kg, with planned dose escalations to a total of 1.0×10^{13} vg/kg and 5.0×10^{13} vg/kg. There does not appear to be any reason to expect that the AAV2/6 vector will have any fewer complications than the previous AAV8 vector. Should a lower dose of AAV2/6 vectors be used with the starting dose based on the results with the AAV8 trial?
- In the responses to Appendix M, it states that 90 percent of liver hepatocytes are expected to be transduced. To what dose of AAV2/6 vectors does this pertain? If this is at one of the lower doses, what is the expected benefit of increasing the dose?
- Consistent with Dr. Hammarskjöld's comment and query, Dr. Hearing noted that considerable effort has been undertaken by Sangamo to predict and examine potential off-target effects of the ZFN used for Factor IX gene integration, since this appears to be the most important potential adverse side effect of the trial. Off-target integration events were identified, but only one of ten has been characterized, with nine of ten potential off-target sites still under investigation. Is there any new information about these nine uncharacterized, potential off-target sites? Are the investigators planning to wait until more of the potential off-target sites have been investigated before initiating the trial?
- The *in vitro* experiments using primary human hepatocytes and HepG2 (hepatoma cell line) cells to examine on-target and potential off-target effects of the zinc finger nuclease found very high on-target specificity (6.1 percent, 27.9 percent, and 30.0 percent at the low, middle, and high doses, respectively). However, the anticipation in the current study is that while 90 percent of liver hepatocytes will be transduced, only a fraction of these cells (<1 percent) will have the FIX gene integrated in the albumin locus. This estimate appears to be based on the NHP studies. To better relate NHP (or other results) with expectations in humans, what percentages of liver hepatocytes were transduced with the AAV vectors at the low, mid, and high doses used in the NHP studies?
- One would anticipate that the AAV2/6 vectors that express ZFN would persist in transduced liver for an extended period. Do the *in vitro* analyses that have examined potential off-target effects of ZFN take this into account? Were these short-term assays? Would additional potential off-target effects of the ZFNs be revealed if long-term assays were employed with persistent ZFN expression?
- Is there any indication that human albumin intron 1 contains any regulatory sequences (e.g., transcriptional) that may influence the expression of neighboring genes? Per the proposed experimental design, human albumin exon 1 will be spliced to the exon of the inserted FIX gene, and human albumin exon 1 is coding. Will an albumin-FIX fusion protein be made, or will FIX be expressed from its own initiation codon?
- As pointed out by Dr. Hammarskjöld, the ICD should state that while the participant may withdraw from the study or be asked to withdraw, the treatment cannot be reversed.

C. RAC Discussion

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

- Dr. Donahue focused on identifying unanticipated toxicities and other potential problems that might be able to be addressed through refinement or modification of the study design. The proposed starting dose is considerably higher than the highest dose tested to date in other hemophilia trials. Further, in those trials, liver toxicity was seen in a high percentage of patients at the highest dose. Thus, the starting dose in the planned trial presumably would have at least a similar level of hepatotoxicity as in prior trials. The rationale for the proposed starting dose makes sense, given the objective of wanting to have some chance of benefit, in contrast with starting at a lower dose that would have no chance of benefit but might potentially expose participants

unnecessarily to risk. Within the context of the probability of hepatotoxicity, however, the investigators should either consider prophylaxis against this adverse effect, or at the very least, have a strict, upfront screening and ongoing monitoring in place to address this concern. The proposed monitoring plan to be reasonable, but it needs to be clearly incorporated into the protocol. The timing of liver function test (LFT) monitoring has been clarified, but another factor that needs to be addressed in the protocol, perhaps in how steroids are tapered, is the increase in LFTs seen after stopping steroids. Including such provisions should reduce the risk of liver toxicity and, in turn, potential loss of efficacy with the study intervention.

- AAV6 has broad tropism, and the biodistribution data presented by the investigators are reassuring. Although the vector transduce the heart more efficiently than AAV2, AAV6 is expected to have very low levels of transduction of heart compared to the liver. Preliminary calculations of the percentage of heart cells that are going to be transduced, assuming equal efficacy in heart and liver cells, suggest that only a small number of heart cells will have zinc finger activity and insertion. Even with a low rate of transduction, however, as many as $1e3$ or $1e4$ cells in any of the off-target organs are likely to have some level of transduction and, potentially, insertion. The liver-specific promoter that will be used in the construct for the proposed trial and the absence of toxicity in preclinical studies are reassuring, but they do not preclude a low probability of off-target events that could be problematic or harmful.
- In response to concerns about long-term follow-up of research participants in the proposed trial, the investigators noted that they will encourage research participants to enroll in a long-term study. In the meeting presentation, the investigators said that research participants would be followed for an additional three years, but it wasn't clear if this follow-up is part of the primary protocol or a secondary study. Drs. Donahue and Hammarskjöld suggested putting the entire follow-up period into a single study given the importance of following these research participants, who will be given a novel intervention, and the limited long-term experience in the preclinical setting. In addition, including long-term follow-up under the primary study may improve the ability to collect data over time to counter loss of interest and retention problems with a secondary study. Although it may not be possible to avoid all drop-outs and losses to follow-up, every reasonable attempt to follow research participants as long as possible should be made. Research participants who withdraw before the study ends or who are lost to follow-up, in turn, should be considered part of the trial and should be handled in statistically appropriate ways to remove any bias of research participants dropping out because of toxicity.
- Dr. Hammarskjöld noted the failure of previous gene transfer approaches to achieve levels of FIX expression that are necessary for any therapeutic benefit but pointed to the potential of this new strategy to overcome this problem. The investigators presented a clear description of what the modified albumin gene will look like after successful homologous recombination of the AAV6 vector. Further clarification was requested, however, regarding how benefit from insertion of the whole vector might be achieved given the projected relatively low frequency of homologous recombination versus non-homologous end joining. Dr. Hammarskjöld also asked about any mis-splicing events and any fusion proteins identified with this approach.
- Dr. McCarty inquired about the estimated rate of homologous recombination versus non-homologous end joining and the minimum per-cell vector copy number that would be needed so that all three constructs work together as expected. He asked whether the minimum copy number could be determined based on, for example, Poisson distributions and probabilities for the three constructs together.
- Dr. Hammarskjöld acknowledged the investigators' point that doing biopsies on all research participants would be difficult but questioned how the outcomes seen in preclinical studies will be verified in human research participants.
- Drs. Hammarskjöld and Cho raised concerns and questions about the Smchd1 off-target site. The Smchd1 gene is a chromatin modifier and has been implicated as a tumor suppressor. Although it is unlikely that the function of this gene will be lost with the dosing regimen under the proposed trial, this possibility (i.e., turning the gene on in even a few cells, particularly in research participants who have cancer) needs to be taken into consideration. Dr. Cho also asked whether the target sequence in the Smchd1 off-target site was identified.

- The gene therapy product Glybera is a recombinant AAV produced with baculovirus that is approved for use in the European Union. Dr. Hearing asked whether the investigators used the same baculovirus system to produce the AAV used in their preclinical studies.
- Dr. McCarty asked about the minimum per-cell vector copy number that would be needed so that all three constructs work together as expected. He also inquired about the estimated rate of homologous recombination versus non-homologous end joining.
- Dr. Whitley also supported the recommendation to include research participants who withdraw from the trial in the analysis and to encourage research participants to continue with long-term follow-up.
- Dr. Lee noted that the dose escalation schema and the stopping rules are all based on toxicity assessments. Because this disease has a very clear and well-established biomarker for efficacy (i.e., FIX levels), the investigators should consider incorporating into the stopping rules and dose escalation a measure of FIX correction, since there is no need to continue escalating if the primary aim of the study has been achieved.

D. Investigator Response

1. Written Responses to RAC Reviews

The gene therapy product, Glybera, from uniQure (Netherlands), is produced using AAV vectors manufactured in baculovirus and is approved for use in the European Union for the treatment of lipoprotein lipase deficiency. Other investigational products in production for clinical trials have been discussed with FDA in preclinical meetings, as has the one that will be used in the proposed trial.

The relationship between total AAV vg/kg shows an NHP minimal effective dose of 1×10^{13} vg/kg to achieve a one percent hFIX level. The proposed starting dose of 5×10^{12} vg/kg is half this minimal effective dose in NHP. Because the virus titer may differ twofold using the quantitative polymerase chain reaction (qPCR) assay, the investigators have chosen to start at the lower dose. For AAV immunogenicity risk mitigation, LFTs will be measured three times per week for ten weeks post-injection of AAV (as corrected in the protocol); methylprednisolone will be started at 1 mg/kg if there is a two-fold increase in aspartate aminotransferase (AST) or alanine aminotransferase (ALT). Methylprednisolone will be adjusted to effectively suppress such transaminitis, and a slow taper will occur once the LFT elevation begins to resolve. In the Nathwani study, six research participants were treated at 2×10^{12} vg/kg with the AAV8 vector; four of the six research participants developed increasing liver enzyme elevations with peak ALT occurring between seven and nine weeks post-infusion. With institution of methylprednisolone at two to seven days after the increase in ALT, all research participants responded to treatment with normalization of ALT. Frequent sampling of liver enzymes and a rapid response to initial elevation in transaminase levels with administration of methylprednisolone should provide both the kinetics and treatment for AAV-induced hepatic immunotoxicity for these dose levels in this Phase I study and with a treatment method that will minimize exposure to methylprednisolone.

AAV6 primarily targets the liver in mice and NHPs, as measured by qPCR; levels of AAV in heart are about two orders of magnitude less than levels in liver. The investigators have not seen evidence of hyperplasia or tumorigenicity in the mice or NHP toxicology studies.

The data cited in the Appendix M on the proportion of liver hepatocytes expected to be transduced are from the published literature; the studies cited used AAV2/8 and analysis of proviral AAV DNA by fluorescent *in situ* hybridization (FISH). The investigators do not have the same type of FISH data for AAV2/6. Major differences between the two vector serotypes are not expected based on these other studies. Data generated in preclinical studies at Sangamo indicate similar results on vector biodistribution by polymerase chain reaction (PCR) by using AAV/8 and AAV2/6 in NHPs. The expected benefit of the higher doses is based on ZFN cutting levels and FIX efficacy. Dose selection is based not as much on percentage of transduction as on gene expression and copy number to drive integration and achieving therapeutic FIX levels (per dose response in NHPs).

The investigators have not evaluated the percentage of transduction in the liver cells in NHPs. They are proposing to use the gene modification and hFIX levels seen in NHPs to select the human dose, since there is a dose relationship with these parameters.

As requested, a schematic drawing of the albumin locus and a description of the construct and how the construct works was provided. The unmodified albumin locus produces the wild-type mRNA of 2.3 kilobases (kb). The hFIX construct contains the exon 2-8 cDNA with the hFIX splice acceptor site of exon 2 retained at the 5' end. The exon 1 of hFIX contains the signal peptide of hFIX and was omitted since the signal peptide of human albumin is used for processing. After ZFN-mediated integration of the hFIX construct into intron 1 of the albumin gene, a hALB/hFIX fusion mRNA (1.7 kb) is transcribed driven by the albumin promoter. The translation of this mRNA is started at the AUG provided by the albumin exon 1 leading to a hALB/hFIX fusion protein. The hALB exon 1 is encoding the signal and pro-peptide from albumin while the hFIX exons 2 through 8 encode an additional pro-peptide. After processing in both the endoplasmic reticulum and trans-Golgi network, these peptides have been removed by using the indicated cleavage sites. Three HepG2 (hepatoma cell line) subclones, which are heterozygous for the hFIX donor integrated in albumin intron 1, have been used to further characterize the expression and splicing events by using the indicated PCR primers. In these subclones, RT-PCR detected both the unmodified hALB wild-type 2.3-kb transcript and the predicted hALB/hFIX fusion 1.7-kb transcript (by using primers shown in the provided figure). The PCR product representing the hALB/hFIX mRNA was further subcloned and sequencing confirmed the predicted splice event.

The levels of ZFN activity at the albumin locus will be measured by deep sequencing using the next-generation sequencer MiSeq (Illumina). This assay allows the quantification of insertion and deletion mutations (indels) caused by non-homologous end joining, an error-prone DNA repair pathway, relative to wild-type alleles (percentage of indels). Our studies have shown that the percentage of indels is a good marker for ZFN expression and activity in a dose dependent manner. The peak levels of both percentages of indels and plasma hFIX levels respectively represent the highest values measured during study duration (six months).

In NHPs, gene modification analysis was typically assessed for two to three months. For a subset of animals in these studies, analysis was conducted for up to one month. In mice, the study duration was up to six months.

The investigators have developed an *in vitro* human hepatocyte system that uses systematic evolution of ligands by exponential enrichment (SELEX)-, AAV capture-, and oligonucleotide capture-based methods to identify and verify potential off-target sites of their lab's ZFN reagents. While SELEX- and AAV capture-based methods could be used to identify off-target sites of mouse surrogate ZFN reagents, no off-target activity has been detected for the human ZFN reagents by using these methods. For the identification of off-target sites by oligonucleotide capture, the initial analysis was expended from ten to 47 potential off-target sites for verification. Of these 47 sites, only Smchd1 showed significant modification.

For *in vitro* analysis of off-target sites, the use of human primary hepatocytes precludes studies longer than seven to ten days. Longer studies are theoretically possible in stable hepatoma cells like HepG2, but because these cells divide frequently, episomal AAV2/6 virus will be diluted out. Thus, there is no appropriate *in vitro* system to study long-term effects of ZFN expression. Analysis of AAV-driven ZFN expression in NHP studies shows that, after peak expression around day 14, very little ZFN protein can be detected at day 28 by Western blot. Thereafter, the signal is below the level of quantification. MiSeq analysis shows that on-target ZFN activity in these studies continues and reaches a maximum around day 42, with no further significant increase. Should unanticipated signs or symptoms of occult off-target effects occur, the treatment plan is designed to capture these findings using history, physical exam, liver function tests, and biliary enzymes. Structural analysis will be added at baseline and every six months with noninvasive liver elastography followed by MRI if liver elastography is abnormal.

Regarding the sample size and replacement of research participants, the PI explained that the research participants enrolled in this study are hemophilia B patients who are chronically treated at national hemophilia treatment centers throughout their lifetime. As such, there is minimal expectation of loss to

follow-up. In addition, Dr. Ewing plans to enroll research participants who have historically exhibited a positive record of therapeutic adherence to their FIX treatment regimen.

Only research participants who have mutations that are not associated with formation of inhibitory antibodies in the database and who have received multiple FIX infusion treatments without developing inhibitors will be selected for the study. The Bethesda assay will be used to determine if inhibitors are present in the research participants. FIX inhibitors will be measured at screening as exclusion criteria and during visits during the trial on at least a monthly basis.

Research participants who develop an inhibitor can be treated with activated Factor VIIa on a prophylactic or on-demand schedule depending on their bleeding propensity. Plasma-derived prothrombin complex concentrates can also be used but may have further immunogenicity due to the FIX content. The concentrate may also cause a raise in the inhibitor titer. Immune tolerance induction using recombinant or plasma-derived FIX concentrates is a potential strategy to eradicate inhibitors, but the success rate is low, and about 20 percent of patients who have had an allergic reaction at the time of inhibitor formation are at increased risk of developing nephrotic syndrome.

The protocol has a provision to expand the sample each dose-level cohort in the event of a safety issue. The study stopping rules (as delineated in the response memo and the protocol) specify when dosing should be curtailed to investigate a safety finding. In addition, the investigators are trying to minimize participant exposure to ineffective doses or toxicity by using a dose-escalation scheme with small cohorts of research participants at each dose. Once an effective and safe dose is achieved, the trial will be amended to include a larger number of research participants to better characterize safety and effects on coagulation studies and bleeding episodes.

Appendix M has been corrected to state that there will be an interval of four weeks within each cohort, not two weeks.

Research participants will be asked to enroll in a separate long-term follow-up (LTFU) protocol at the end of the study; per FDA guidelines, the duration of LTFU will be discussed and implemented with FDA consultation.

The investigators agreed with the recommendations regarding the ICD and have modified the proposed ICD accordingly.

2. Responses to RAC Discussion Questions

Dr. Kathleen Meyer, Senior Director of Toxicology at Sangamo BioSciences, explained that while the AAV6 vector may be in off-target tissues, because the promoter is specific for the liver, the chance of transduction and insertion of FIX in heart cells is very small. Dr. Michael Holmes, Vice President of research at Sangamo BioSciences, added that biodistribution studies in both mice and NHPs, which looked at the potential for impact on organs other than the liver, found that when AAV6 is delivered systemically, most of it goes to the liver. Other studies of various AAV serotypes, including AAV6, show that AAV6 is distributed to other tissues. The proposed trial has added safeguards to regulate the expression of ZFN to avoid having any type of impact on heart tissue. The investigators will take the reviewers' additional concerns and suggestions regarding the potential of off-target expression into consideration for the clinical setting.

By extrapolating from biodistribution data in NHPs, the investigators estimate that ten copies of the ZFN and about 100 copies of the vector are needed for the construct to work as planned. The numbers are similar for the mouse model. The exact number of copies per cell is not known, because of limitations with the mathematical model. One of the key factors in the proposed trial, in contrast with other AAV trials, is that appropriate levels of all three components of the SB-FIX construct are needed to assess potential efficacy of the product. The investigators may consult a mathematician to discuss these issues and to obtain a better estimate of the number of copies of each component needed for the proposed intervention.

Estimating the rate of homologous recombination versus non-homologous end joining has been challenging. Initial studies in neonatal mice suggested that a majority of the events occurred via homologous recombination. In experiments in adult mice and adult NHPs, the mechanism switches over to being primarily non-homologous end joining, possibly in part because of the reduced rate of growth in the liver in adults versus neonates. Dr. Holmes noted that the cleanest experiments have been in mice, when the homology arms were either on or off. In these studies, about two-thirds of the activity was through non-homologous end joining, and about one-third of the activity was through homologous recombination. The planned doses take into account that essentially half of the non-homologous end joining will be in the inverse orientation and will be nonfunctional.

With homologous recombination, FIX inserts without the inverted terminal repeat (ITR) sequences being present; in this scenario, the splice acceptor allows the FIX gene to be essentially spliced into the first exon of the albumin gene. With non-homologous end joining, the whole vector ligates in, including the ITR sequence; transcription of the locus includes the splice acceptor. In this approach, all of the ITR and homology arms get spliced out of the transcript. In both cases, the sites are ablated by separating them by several kilobases so that the two ZFNs can't dimerize together and bind to and cut the DNA. Thus, even though the ITR and the full AAV vector genome are present, the FIX gene still gets spliced into the albumin locus. These methods have been verified in transformed hepatocytes. In subclones that had integrated the vector by either recombination or non-homologous end joining, the transcripts were identical and showed splicing into the first exon of the albumin gene. This type of splicing event has been documented in *in vitro* experiments by looking at the mRNA transcripts. In *in vivo* studies, the homology arms are completely removed so that the only way the donor sequence can be integrated into the genome is through non-homologous end joining. Nearly identical levels of FIX expression are seen with and without the homology arm, indicating that this non-homologous end joining approach is successful.

To address the question about mis-splicing, the investigators used three modified HepG2 cell lines that had integration via both homologous recombination and non-homologous end joining; the cells were heterozygous in that one copy on the locus was functional and the other copy of the locus had FIX inserted into it. RT-PCR analysis identified only two transcripts, either the full-length albumin or the FIX-albumin fusion. Results of these *in vitro* experiments did not find any alternative splicing, mis-splicing, or read-through in these cell lines, indicating that the splicing occurred as intended. In the proposed trial, expression levels will be based on FIX levels in the blood, which will serve as a surrogate of the findings from the mouse and NHP studies.

Dr. Holmes noted that the target sequence in the *Smchd1* off-target site is on the edge of one of the last exons and just inside one of the coding sequences, which is why the team investigated off-target levels of expression of that sequence in the NHP setting.

Dr. Meyer noted that the one-year NHP study included only two monkeys and a control that was given the ZFNs alone. The investigators did not measure levels of FIX in that study. The study continued for a year to allow for monitoring for potential effects of the high-dose ZFNs without immunosuppression. The duration of Factor IX expression in NHPs has not been measured, but results of Western blots showed the indels to be stable over time. Levels of FIX were relatively stable for about 9 months. ZFN levels declined during the post-dosing period, and no cells expressing ZFNs were present at the end of the one-year NHP study.

The study team used the baculovirus system for producing the AAV used in their preclinical studies.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

Clinical and Trial Design Issues

- The starting dose of vector proposed in this protocol is considerably higher than the doses of AAV vectors administered in previous hemophilia clinical trials in which toxicities and loss of transduced hepatocytes were reported. The protocol should clearly state the monitoring, prophylactic, or mitigation strategies planned to address potential hepatotoxicities.
- The protocol should also include stopping rules for the dose escalation in the event that therapeutic levels of Factor IX are observed in research participants who received lower doses.
- Given the possibility of off-target effects resulting from the genomic editing approach, the trial should include a plan for long-term follow-up to detect these effects, which may not develop until late after vector administration.
- Research participants should be encouraged to participate in the long-term follow-up study. The data from participants who withdraw from the trial should be incorporated into the study analysis, as available, so as not to inadvertently bias the findings.
- The language of the informed consent document should be revised to be more easily understandable by potential research participants and should include:
 - Acknowledgement of the unknown nature of some of the risks.
 - An explanation that while a research participant may withdraw from the trial, the administration of the gene transfer product is not reversible.

G. Committee Motion 1

Dr. Hammarskjöld summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Hammarskjöld requested a vote, and the RAC approved these summarized recommendations by a vote of 11 in favor, 0 opposed, 0 abstentions, and three recusals (Drs. Cannon, Chatterjee, and Kiem).

IV. Review and Discussion of Human Gene Transfer Protocol #1507-1449: A Phase I/II Multicenter, Open-Label Study to Evaluate the Safety, Tolerability, and Pharmacodynamics of RGX-111 in Subjects with MPS I (IDUA Deficiency)

PI: Stephen Yoo, M.D., Regenxbio, Inc.

Investigators/Presenters: Raymond Wang, M.D., Children's Hospital of Orange County
Christian Hinderer, Ph.D., University of Pennsylvania

Sponsor: Regenxbio, Inc.

RAC Reviewers: Drs. Atkins, Chatterjee, and Hardison

A. Protocol Summary

Mucopolysaccharidosis type I (MPS I), also known as Hurler or Hurler-Scheie syndrome, is a rare genetic lysosomal storage disorder caused by an autosomal recessive inherited deficiency of the enzyme, α -L-iduronidase (IDUA). The disease pathology is attributed to the loss of function of IDUA, which is required for the lysosomal catabolism of the complex polysaccharides heparan sulfate and dermatan sulfate. These polysaccharides, called glycosaminoglycans (GAGs), accumulate in the tissue of MPS I patients, resulting in characteristic storage lesions and diverse disease sequelae. Most significantly, many patients

develop symptoms related to GAG storage in the central nervous system (CNS), which may include hydrocephalus, spinal cord compression, and cognitive impairment.

Although treatments exist for the systemic effects, including enzyme replacement therapy (ERT) and hematopoietic stem cell transplant (HSCT), these interventions have limited effect on the neurological symptoms associated with MPS I. HSCT can have a delayed effect on the CNS, but survival rates for Hurler patients have been reported to vary between 50 percent and 85 percent, and engraftment failure is frequently reported. Intravenous ERT does not cross the blood–brain barrier and therefore has limited potential to treat CNS symptoms.

Data generated from preclinical studies show that AAV-mediated gene transfer of IDUA into the CNS can increase IDUA expression in a robust and rapid manner. Within 7 days of transfer, animals treated with the AAV vector product expressed IDUA at levels significantly greater than untreated wild-type animals, resulting in rapid and near complete correction of IDUA deficiency. Intrathecal delivery of AAV9.CB7.hIDUA (RGX-111) offers a unique approach to providing clinical benefit to those with CNS manifestations of MPS I.

The proposed Phase I study will deliver the clinical candidate RGX-111 to adults with MPS I who have evidence of early stage neurocognitive deficit in a single administration intrathecally (suborbital) enabling direct access to the CNS. The study population will include participants with the more attenuated condition: those with Hurler-Scheie syndrome. The IDUA-expressing capsid AAV serotype 9 proposed for use in this study is the same capsid used in three recent protocols evaluated by the RAC. The study will include two cohorts (n = 3–6 adult participants per cohort), with each cohort receiving a dose based on findings from preclinical dose escalation studies and further informed by the GLP toxicology study. The primary endpoint assessed at 24 weeks will be safety. Participants will be monitored closely to assess physical condition and laboratory abnormalities. Participants will also be evaluated for enzyme activity and biomarkers to assess GAG storage correction. Safety assessment will continue for 5 years.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because this is the first gene transfer trial to target Hurler-Scheie syndrome (MPS I) and the AAV construct will be delivered by intrathecal administration.

Three RAC members provided written reviews of this proposed Phase I/II trial.

The reviewers found the study to be well designed and the proposed intervention to be appropriate for the target population.

Dr. Atkins inquired about the number of sites that will be participating in this trial, the names of those sites, and how the investigators will ensure that all sites are aware of issues in patients at other sites. He had the following additional comments and questions:

- How long is IDUA production expected to last in humans, and how will the investigators assess the extent and location of CNS transduction?
- To what extent did antibodies against human IDUA (and its earlier elimination) in animal models contribute to the perceived safety? Could the planned dose be too high in humans who are less likely to develop such antibodies?
- What is the likelihood of anti-IDUA antibodies hastening neurologic deterioration in patients with partial IDUA deficiency?
- Participants are ineligible if they have had a HSCT. Are participants prohibited from having a future HSCT if this becomes a safer procedure?
- Serum biomarkers are being tested. Do the investigators expect systemic vector circulation and transduction or is this just being done as a precaution?
- Has the NHP (rhesus macaque) study that is supposed to determine the safe dose to be studied in human trials been completed?

Dr. Chatterjee noted that in the dog model, the dose-limiting toxicity (DLT) associated with hind limb weakness and pain was observed at the highest dose of vector used. While IDUA antibodies were observed, the levels were comparable to that seen at lower doses. However, a T-cell response to transduced motor neurons was observed. She asked the investigators to comment on the specificity of the T-cell response and whether an anti-capsid T-cell response (in addition to an anti-IDUA response) is possible. She also asked whether participants will be screened for the presence of anti-AAV9 antibodies.

Dr. Chatterjee had the following additional comments and questions:

- How long is IDUA production expected to last in humans, and how will the investigators assess the extent and location of CNS transduction?
- What is the incidence of anti-IDUA antibodies in patients receiving ERT? Is it known whether a viral infection could accelerate the onset of immune responses to IDUA or capsid proteins by adjuvant type effect?
- What recourse would patients have if they failed RGX-111, presumably by developing anti-IDUA antibodies? How would they be treated under those circumstances?
- The data on the correction of the biochemical markers are impressive and correlate with the dose. Is it possible to tolerize patients, as in the dog model, and then administer higher doses?
- The data on genome copies per cell in the CNS justifies the doses chosen. However, the level of transduction with 1×10^8 vg is overall comparable or even a bit higher than that seen with 1×10^9 vg, which warrants further comment.
- If the proposed approach successfully corrects the neurodegenerative defects of MPS I, what is the plan to address the systemic effects? For example, would intrathecal or IV injections of RGX-111 be used in conjunction with ERT?

Ms. Hardison's comments focused on the ICD. She found the ICD overall to be satisfactory and to include all of the relevant information needed for potential participants to consider whether to participate in this research.

Ms. Hardison suggested reorganizing the section of the ICD that describes what research participants undergo during the study to improve readability to include a brief description of the screening visit; a bulleted list of the tests done to determine eligibility; and sections on pre-study drug administration, administration of the gene therapy agent, post-administration of the gene therapy agent, and follow-up. The description of the procedure (i.e., the cisternal puncture) in which the needle is inserted into the back of the head to deliver the study agent is very technical and may need to be simplified for participants to make an informed decision. An illustration would also improve the ability of potential participants to understand what is entailed in this procedure.

The paragraphs and information on possible risk of being in this study may seem long and overwhelming to potential participants. Participants should be encouraged to consider all risks associated with tests and procedures associated with participation in this study in order to appropriately analyze risks and benefits. Ms. Hardison presented an alternate way to organize this section of the ICD to present the appropriate information so that it is easier to read and understand. In addition, she recommended emphasizing the following statements at the end of this section (e.g., via highlighting or bolding): "There may be risks to being in this study that we do not know about now. You will be told of any changes in the study or if there is new information about the study drug."

Ms. Hardison found the section title, "What Are the Unknown/Unforeseeable Risks and Discomforts?", to be misleading. This section of the ICD describes possible risks to the potential participants and should have a more appropriate title, such as "What Are the Other Possible Risks and Discomforts?"

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. They went through their comments and the investigators' responses to their queries and suggestions.
- The team plans to have at least two study sites and will develop a communication plan that is consistent with GCP standards. Dr. Atkins noted, however, that protocols presented for review to the RAC usually identify the specific sites and investigators who will be participating in the study so that the committee can assess the experience of those institutions and researchers within the context of the proposed trial.
- Dr. Atkins found the preclinical data to be compelling for significant gene transduction into the CNS. The investigators anticipate IDUA production to persist in humans for at least a decade and have clarified how they will assess the extent and location of the CNS transduction, which is expected to be widespread. The specific sites and degree of transduction will not be measured but will be inferred by levels of the IDUA.
- The antibodies against the human IDUA and the demonstrated elimination in animal models contribute to the perceived safety of the study intervention and led the reviewers to ask whether the proposed dose for the clinical trial is too high given that humans are less likely than animals to develop such antibodies. Drs. Atkins, Chatterjee, and Hammarskjöld considered the results in tolerized versus non-tolerized animal models to be informative regarding the safety and potential risks of the experimental agent in humans. The model of the tolerized dog to human IDUA appears to most closely represent the safety profile in humans. Drs. Hammarskjöld and Chatterjee noted the serious consequences of high doses in the dog model (e.g., hind-limb paralysis, pleocytosis, and T cells on autopsy), however, and asked whether the investigators plan to look specifically for T-cell responses. Dr. Hammarskjöld added that the response in the dog model did not reflect a typical T-cell response given intracellular uptake and expression of the vector product. She also inquired about the T cell and overall immune response in the presence of different mutations, given the patient population.
- Dr. Chatterjee also inquired as to the possible reason for the immune response at the low and middle doses, in which the antibody response appeared to resolve on its own. In addition, she asked whether the specificity of these T cells was against the IDUA, the transgene product, and/or the AAV capsid proteins.
- Dr. Atkins noted that the anti-IDUA antibodies are primarily against the extracellular enzyme but that these antibodies diminish over time and do not appear to impact endogenous intracellular levels. He also pointed to the increased risk of anti-IDUA antibodies to hasten neurologic deterioration in those with Hurler-Scheie syndrome who have partial IDUA deficiency. Dr. Lee asked whether the study agent could induce neutralizing antibodies to IDUA and therefore render a participant insensitive to IDUA for further therapy, to the extent that they would not respond to the current standard of care. Given the inflammatory response incited by the virus, the investigators should consider following antibody titers during the course of this trial to monitor for any changes.
- The investigators clarified that other interventions such as stem cell transplant that might be applicable to this adult population that are not part of standard care and would not be prohibited in the future.
- The plan to monitor systemic vector circulation and transduction is based on prior findings indicating peripheral distribution that resulted in detectable serum IDUA activity and some improvement in the systemic disease. Dr. Atkins recognized that the specific vector that will be used in the proposed trial was chosen because it is better at transducing the CNS, but he questioned whether the investigators expect any problems from systemic transduction of this vector.
- The investigators estimate that only about 1 percent transduction is needed to see correction of the disease in the CNS. Dr. Chatterjee asked why amelioration of the disease is seen (or anticipated) if IDUA is primarily an intracellular protein and 99 percent of the cells are not corrected.
- The FDA has granted approval to proceed with the study in the rhesus macaque model. The investigators were asked whether they will wait for the results in this animal model before launching the proposed trial.

- Dr. Ross noted that none of the animals tested to date were on ERT and inquired about the possible risk of an interaction between the intravenous ERT that will be given and intrathecal administration of the study agent.
- Dr. Atkins noted that the protocol does not clearly state whether the investigators plan to stop ERT therapy in the CNS while participants receive RGX-111 and, if so, when ERT will be resumed.
- Drs. Hammarskjöld and Kiem cited the importance of the route of administration and asked about the risk associated with use of intrathecal administration for this population. Dr. Chatterjee inquired whether research participants who receive the AAV construct, express anti-IDUA antibodies, and have a concomitant or subsequent viral infection would develop an adjuvant-type effect that might end up eliminating their transduced cells. She inquired about the mechanism for this response, that is, whether the antibodies to IDUA are not neutralizing. She also noted that the presence of these antibodies does not appear to limit the efficacy of the ERT.
- Dr. DiGuisto pointed to results indicating liver transduction with the vector and absorption of proteins by endocytosis and a class 2 pathway of presentation. However, intracellularly virally expressed proteins usually that through a class 1 pathway and would be much more likely to generate a cellular response if they reach to the liver. He asked whether the investigators have measured liver enzymes or done any histopathology to determine if there are any T cell infiltrates in the liver or any other organ that may have been transduced.
- Dr. Lee noted the favorable risk-benefit ratio for patients who participate in the proposed trial and asked why both Hurler and Hurler-Scheie patients will not be eligible for the study.
- Ms. Hardison found her suggestions on how to reorganize some of the information in the ICD to increase the readability and to better describe the cisternal puncture (with inclusion of an illustration) to be adequately considered by the investigators.
- Dr. Hammarskjöld underscored the importance of explaining to participants that they cannot withdraw from the study in the sense that people usually consider with respect to the word “withdrawal.” The ICD needs to clearly explain that patients can decline or withdraw from further treatment and follow-up but that researchers cannot “withdraw” or remove the modified cells once the cells are injected.

D. Investigator Response

1. Written Responses to RAC Reviews

A minimum of two sites will participate in the proposed trial. The specific sites have not been identified yet, but the team is in the process of selecting and qualifying potential sites. The study will be conducted under good clinical practice guidelines, which state that it is the sponsor’s responsibility to communicate between investigators. Therefore, Regenxbio will ensure that each investigator is aware of any potential issues observed in participants at other sites, through frequent communication (i.e., via regular written and verbal communication), site visits, and monitoring.

Four normal dogs have received an intrathecal injection of an AAV9 vector expressing canine IDUA. These animals have exhibited elevated cerebrospinal fluid (CSF) IDUA activity for more than 2 years, and there has been no decline in expression over the past year. Previous studies using an AAV2 vector demonstrated expression of a transgene in muscle for 15 years in macaques and 10 years in humans. Based on these data, expression of the AAV9 vector is expected to last for at least a decade. Although the team will not be able to directly measure the extent of CNS transduction in research participants, CSF IDUA activity and CSF biomarkers will provide estimates of CNS transduction and correction of tissue storage lesions.

The potential for administration of an exogenous protein to induce immunity against a patient’s residual endogenous protein is an important consideration. However, it is unlikely that antibody responses to IDUA would exacerbate disease. The phenomenon of cross-correction, in which cells secrete IDUA that is taken up by other cells, is critical, since a small number of transduced cells overexpress and secrete the enzyme to supply the rest of the brain. In contrast, during normal IDUA production, enzyme secretion and

uptake is a minor pathway; virtually all intracellular IDUA activity is derived from enzyme produced within the cell. Thus, anti-IDUA antibodies pose a threat to extracellular enzyme generated via gene transfer or ERT but should not have an impact on endogenous intracellular levels. This is supported by clinical experience with ERT, which shows that patients with attenuated MPS I who elicit antibodies to IDUA do not develop a more severe form of the disease.

The inflammatory response observed in the dog model could be explained by immunity against either the vector capsid or the transgene product. To identify the target antigen, the investigators harvested peripheral blood mononuclear cells and splenocytes at the time of necropsy, and performed interferon- γ Enzyme-Linked ImmunoSpot (ELISPOT) assays to detect T-cell responses to both AAV9 capsid and human IDUA peptides. These assays did not detect a response to capsid or transgene antigens. However, the preceding course of steroids may have resulted in false-negative ELISPOTs, or the reactive cells may not have been present at detectable frequencies in the peripheral circulation.

Although the investigators could not directly evaluate the specificity of the immune response, one observation from the MPS I dog studies suggests that it was directed against the transgene product. All naïve MPS I dogs treated with RGX-111 exhibited a lymphocytic pleocytosis 3 weeks after vector administration. This pleocytosis was more pronounced in the two dogs exhibiting neurologic toxicity and was temporally correlated with the onset of symptoms, suggesting that lymphocyte recruitment into the CSF was related to the inflammatory response in the spinal cord. When RGX-111 was administered to MPS I dogs that were tolerized to human IDUA through neonatal liver-directed gene transfer (i.e., in the minimum effective dose [MED] study), the animals did not exhibit the lymphocytic pleocytosis that was observed in the non-tolerized animals. The team subsequently confirmed this result in two MPS I dogs tolerized to human IDUA through neonatal infusion of the recombinant enzyme before intrathecal RGX-111 administration at 1 month of age. Because these animals have a blunted immune response to IDUA but an intact capsid immune response, the lack of CSF pleocytosis in this cohort indicates that lymphocyte recruitment is due to a transgene-specific response. Given the apparent connection between this pleocytosis and immune-mediated toxicity in the high-dose non-tolerized animals, these findings suggest that the toxicity was due to an immune response to human IDUA.

The immune response to the human IDUA protein has previously been shown to be exaggerated in MPS I dogs compared to humans after intravenous or intrathecal delivery. Intravenous delivery results in stronger anti-IDUA antibody responses and more frequent anaphylactic reactions in MPS I dogs relative to humans. Intrathecal human IDUA injections in dogs resulted in antibody induction and lymphocytic infiltration of the meninges, whereas intrathecal iduronidase has been well tolerated in humans and elicited CSF antibodies in only one of five participants tested to date. It therefore appears that the destructive immune response against the transgene product is likely a function of the animal model and not representative of the immune response that would be observed in humans.

Neutralizing antibodies against AAV9 will be measured in research participants before and after treatment to evaluate any potential relationship between AAV9 antibodies and the safety and efficacy of RGX-111. However, research participants will not be excluded on the basis of preexisting antibodies to the capsid. The team's studies in NHPs have shown no loss of transduction or increased toxicity in animals with preexisting AAV9 antibodies. Another group has demonstrated that intrathecal AAV9 delivery is safe and achieves significant brain transduction in dogs that have extremely elevated AAV9 titers following immunization against the capsid. Based on the available data, preexisting capsid antibodies are not expected to put participants at increased risk of AEs or preclude potential benefit.

Although there is no direct evidence for such an occurrence in the literature, immunological principles would suggest that a coincident infection could promote immunity to an exogenous protein, such as a vector capsid or transgene product, by providing an innate inflammatory signal that could contribute to initiation of an adaptive immune response to the newly encountered antigen. The investigators do not believe that such a phenomenon, if it does occur, would pose any additional risk to gene therapy compared to other methods for chronic delivery of an exogenous protein (e.g. ERT). Furthermore, the intrathecal AAV-mediated gene transfer approach the team plans to employ has two important advantages for the avoidance of transgene specific immunity. First, AAV-mediated expression of a protein

has been repeatedly demonstrated in multiple species to be less immunogenic than direct infusion of the protein and, in some cases, even induces immunological tolerance that can prevent or reverse immunity to a protein. The intrathecal delivery method offers a second advantage over previous approaches to CNS gene therapy; specifically, this route appears to be less prone to induce transgene-specific immunity than direct injection of the vector into the brain parenchyma. Presumably, this difference is related to the local inflammation induced by direct brain injections, which, like a coincident infection, may provide an inflammatory signal that promotes adaptive immune responses to newly expressed proteins.

The group's preclinical studies of intrathecal AAV9 delivery in MPS I cats and MPS I dogs indicate that substantial correction of brain storage pathology occurs in animals that develop antibodies to IDUA, albeit to a lesser degree than in antibody-negative animals. The investigators therefore do not believe that the development of antibodies in a research participant would preclude therapeutic benefit. In the MED study of RGX-111 in MPS I dogs, prevention of antibody responses to human IDUA was attempted not because antibodies completely abrogate response to therapy, but because the initial clinical experience with intrathecal ERT suggests that CSF antibody responses to IDUA are uncommon in humans. Thus, an effective dose selected in dogs with a strong antibody response against human IDUA may overestimate the MED in humans, potentially leading to a selection of an unnecessarily high starting dose and exposure of research participants to unnecessary risk. Currently, there is no treatment for neurologic disease in patients with attenuated MPS I. Induction of antibodies against IDUA in the CSF would therefore not prevent administration of any other medication.

The initial clinical experience with repeated intrathecal administration of recombinant IDUA in infants and adults indicates that CSF IDUA is well tolerated and that anti-IDUA immune responses are rare. The immune response observed against human IDUA in MPS I dogs are therefore not thought to be representative of the likely outcome in humans. Instead, the tolerized dogs, which exhibited minimal anti-IDUA immunity and robust correction of storage pathology, are expected to better represent potential therapeutic effects of intrathecal RGX-111 administration.

The correlation between CSF IDUA expression and vector dose in animal studies demonstrates that transduction was dose dependent; however, among the animals treated at the lowest two vector doses, there was no clear relationship between vector dose and the number of vector genomes detected in the brain by quantitative PCR. The investigators believe that this is most likely due to the large regional variation in brain transduction, coupled with the sampling error inherent in measuring vector genomes in small pieces collected from the whole brain. Using larger numbers of animals in each group would allow for more reliable evaluation of quantitative vector distribution in the brain; the planned biodistribution study in 12 NHPs, which has not yet started, will be useful to this end. The investigators are currently awaiting final recommendations from the FDA before initiating the NHP study.

While the proposed study is intended to evaluate the safety of intrathecal RGX-111 administration for targeting the CNS, vector distribution to peripheral organs, especially the liver, has been observed in some animal models. In MPS I cat studies, for example, this peripheral distribution resulted in detectable serum IDUA activity and some improvement in systemic disease. More than 90 percent of MPS I patients treated with intravenous ERT develop serum antibodies to IDUA. Among the five MPS I patients treated with intrathecal ERT who have been tested for CSF antibodies, only one has been positive. The proposed clinical trial will include assessments for any potential effect of intrathecal RGX-111 on serum biomarkers.

Patients enrolled in the proposed trial will be maintained on systemic ERT, which is the current standard of care for the treatment of peripheral symptoms of MPS I. Preclinical data indicate that RGX-111 may ultimately have utility in the treatment of systemic disease. However, it is more appropriate to initially evaluate this approach for the treatment of CNS disease manifestations for which there is no safe and effective standard of care.

Due to the morbidity and mortality associated with the procedure, HSCT is currently recommended only for MPS I patients under 2 years of age who are believed to have the severe form of MPS I. Patients in this study would therefore not be candidates for HSCT. However, patients would not be prohibited from having HSCT or any other future therapies except repeat dosing with RGX-111.

The investigators agreed with the recommendations regarding the ICD and have modified the proposed consent document accordingly.

2. Responses to RAC Discussion Questions

The types of sites that the team is targeting are highly specialized in MPS I disease and have well-known experts in the field and highly trained neuropsychiatrists to do the clinical assessments and evaluations.

Regarding systemic transduction with the chosen vector, Dr. Hinderer noted that based on preclinical studies, it depends on the animal model. For example, in cats, there is significant liver transduction after intrathecal injection, less in primates, and almost none in the dog model. Thus, there is potential for peripheral transduction. However, although the expression product is a ubiquitously expressed protein, safety issues have not been observed with respect to systemic transduction. In contrast, some beneficial effect might result from this activity. Formal toxicology studies have not yet been conducted, but results of the animal studies to date have not identified safety concerns. Clinical chemistry labs are closely monitored across studies. Although increased levels of transaminases have been observed in trials in which AAV8 has been delivered systemically, thus far, the investigators have not seen significant increases in liver enzymes or any other evidence of liver pathology. As an added safeguard, the proposed study will include intensive monitoring of LFTs and any increases in transaminases.

The investigators noted that the T-cell response is primarily against the transgene product rather than the capsid proteins. In one study, the team tried to directly evaluate the T-cell response using ELISPOTs to the AAV9 capsid as well as to the IDUA gene, which is the definitive way to discern the specificity of the immune response. There were no positive results to either agent, which most likely was an artifact of the preceding course of steroids that likely blunted the response. Given this lack of response, the investigators had to infer what the target of the response was. They posit that in this phenomenon (CSF pleocytosis), the lymphocytes are being trafficked to the CNS. The observed toxicity was in the location of the trafficked lymphocytes, that is, the spinal cord; death of motor neurons at this site, clinical signs of injury, and pleocytosis are believed to be directly correlated with that response. Pleocytosis is seen in the non-tolerized animals, which have an intact immune response to the human IDUA enzyme, but not in the tolerized animals, which have intact AAV immune responses. The tolerized animals are not tolerant to AAV, but they are tolerant to the enzyme. The tolerized animals were not given the same high doses as the non-tolerized animals, and no CSF pleocytosis was seen at the doses given to the tolerized animals. In similar studies in macaques and other animals, no AAV9 capsid-directed response in the CNS has been observed within the range of doses administered. Per these results, the investigators hypothesize that the cell trafficking and ultimately the cellular immune response were directed against the human transgene. Whether the rate of clearance of AAV9 capsid proteins from cells contributes to the presence or absence of anti-capsid responses is not clear. Results from hemophilia clinical trials indicate that transaminase elevations in some patients may correlate with ELISPOTs to the capsid. Interpretation of these findings is difficult, however, in part because of capsid cross-presentation. The remaining toxicology studies will validate T-cell responses to the capsid and the transgene; the proposed clinical trial will include ELISPOTs to both antigens.

The starting dose of RGX-111 is the lowest dose evaluated in the toxicity study completed to date, and it was well tolerated. Even in the worst case, a response in humans would be more similar to what was seen in the non-tolerized dogs with the human vector. The antibody response at the low and middle doses is reproducible and seems to occur at the peak of enzyme expression. In these cases, there likely is an immune response that initiates limited production of chemokines that, in turn, attract cells. In the absence of any strong inflammatory event, however, this initial activity is probably not sufficient to sustain the response. In cases involving muscle gene transfer, T cells initially traffic to that tissue but there is no

subsequent elimination of cells. The cells eventually become tolerized or anergize and do not respond; the reason for this lack of a response is not clear, however.

Dr. Hinderer noted that although there is a theoretical increased risk of T-cell responses to transduced cells because of intracellular presentation of antigen (i.e., via cellular intake of the enzyme with ERT), no data to date support this response. The reviewers' assessments of the intracellular and extracellular impacts of the anti-IDUA antibodies and the potential risk of anti-IDUA antibodies in a subset of patients with Hurler-Scheie syndrome (i.e., those with partial IDUA deficiency) are correct.

The impact of the treatment with versus without ERT is not known. Some studies have included both gene transfer and enzyme replacement, but these interventions were not given simultaneously. In preclinical experiments conducted as a sort of a neonatal tolerance induction protocol, animals were given sequential doses of enzyme at 1 and 2 weeks of age and were then given the vector at 4 weeks of age. No adverse outcomes were observed in these experiments. Although these studies did not use the same design as for the proposed trial, no toxicity due to an interaction between the two agents is anticipated.

Regarding the potential impact of the proposed intervention on future therapies, Dr. Wang noted that some patients given intravenous ERT develop very high anti-IDUA antibody titers (e.g., in the one to one million range) but they often for not have any clinical symptoms. Pharmacodynamic parameters, such as urinary glycosaminoglycans, might be a somewhat higher than in patients with a lower antibody titer, suggesting that a high titer might have an inhibitive response. Dr. Wang pointed out that the target population for the proposed trial has likely been receiving ERT for more than 10 years. It is therefore likely that these patients are tolerized, serum-wise, so that if they are re-challenged with IDUA, it is unlikely that they will mount a very pronounced response with subsequent exposure(s). The protocol includes assays to monitor antibody titers over the course of the study.

Participants who develop a viral infection concomitant with or subsequent to the study intervention are not expected to have an adjuvant-type effect that would eliminate their transduced cells based on results of prior trials, in which only one of five participants who received intrathecal ERT mounted an immune response. Evidence from animal studies show that antibody titers decrease efficacy with both intravenous and intrathecal ERT, which may be what happened in the clinical setting. In MPS I, most antibodies aren't actually enzyme inhibitors because they don't directly block activity. This difference is probably due to blocking cross-correction, by blocking the uptake by cells, enhancing clearance of the enzyme, or another mechanism.

Although IDUA functions intracellularly in the lysosome, enzyme replacement works in MPS I even with a very low rate of transduced cells because of cross-correction. IDUA carries a mannose 6-phosphate residue that allows for recognition of surface receptors on the cell so it can be taken up and trafficked to the lysosome. In the preclinical models, a small percentage of cells in the brain of an in an animal that has no endogenous expression are transduced. Those cells overproduce and secrete large amounts of the enzyme, which acts as the enzyme replacement. The surrounding cells pick up enough of the enzyme to correct storage pathology. Antibodies that develop with normal IDUA production are not expected to interfere with any endogenous residual levels of activity in an attenuated MPS I patient because the intracellular pathway that picks up IDUA from the extracellular space is probably very minor compared to endogenous expression within the cell.

Dr. Hinderer noted that intrathecal ERT is not an approved method of treatment and is currently considered investigative only. There are two clinical trials, one in Hurler patients and one in Hurler-Scheie patients, using this intervention and route of administration. Because intrathecal ERT is still investigational, those patients given ERT via this route will not be eligible to receive another investigational agent. However, the investigators expect patients on intravenous ERT to be able to remain on that treatment for the duration of the trial. Dr. Hinderer added that the proposed trial seeks to address to address the CNS symptoms of intrathecal ERT, which intravenous ERT does not.

The risks of the intrathecal route of administration are similar to those of a lumbar puncture. Dosing (i.e., needle insertion) is done using a suboccipital puncture with imaging guidance after an angiogram to identify blood vessels; these additional steps are included as added safety measures to minimize the risk to research participants. In the past, when needle insertion and placement were done as a blind procedure, patients with a low-lying cerebellar artery had an increased risk of damaging that blood vessel during the needle insertion. A review of the safety of suboccipital puncture under imaging guidance reported five severe bleeding events in 2,000 procedures due to hitting that vessel. Dr. Yoo noted that experts at the University of Minnesota, in collaboration with neuroradiologists at the University of Pennsylvania, looked extensively at MRIs and the anatomy of patients with MPS I and found that the intracisternal space in these patients actually tend to be larger than patients without this condition. Furthermore, the team opts for a suboccipital puncture instead of the more common lateral C1–C2 puncture, because patients with MPS I have a narrowing of the C1–C2 space, which, if accessed for this procedure, would increase risk to participants.

The investigators recognize the potential benefit of gene transfer for both Hurler and Hurler-Scheie patients and agree that the intervention should be studied in those with each syndrome. As Dr. Yoo explained, however, since this will be a first-in-human investigation of RGX-111, the team determined, based on the potential immune response that it would be better from a safety perspective to proceed initially in patients with the more attenuated condition (i.e., those Hurler-Scheie syndrome). Discussions with the FDA supported this position and with the decision to study adults before children.

The investigators confirmed that they will wait for the results of the study in the rhesus macaque model before starting the proposed trial.

The investigators will revise the ICD regarding the meaning of the term “withdrawal” within the context of this gene therapy trial as recommended.

E. Public Comment

Public comments from two parents of adult children with MPS I, Mark Dant and Eric Merrell, were offered in support of this clinical trial. Their testimony is provided verbatim in Appendix A.

Dr. Kiem thanked Mr. Dant and Mr. Merrell for attending this RAC meeting and for sharing their stories. It is always helpful for members of the RAC to hear from patients and family members to better understand the patients’ perspective and needs.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC’s in-depth review and public discussion:

Preclinical Issues

- In some animal models, distribution to peripheral organs of the intrathecally administered study agent has been observed. Systemic monitoring of toxicity should be considered.
- A non-human primate toxicology study is being planned and should be completed prior to enrolling any human participants. The clinical protocol should be revised as necessary based upon the results of the non-human primate toxicology study.

Clinical and Trial Design Issues

- Intrathecal iduronidase replacement in humans has been tolerated fairly well. However, in this protocol, the transgene product (iduronidase) will be expressed intracellularly, which will lead to processing and presentation in the context of MHC class I. This is different from presentation of an extracellular antigen and may induce cytotoxic T-cell responses not induced by replacement

therapy. In addition, AAV vector proteins (capsid) will also be presented in transduced cells. The potential T-cell response to both the transgene and the vector should thus be evaluated.

- Enzyme replacement therapy is the current standard of care for the peripheral symptoms of this disorder, however it is unknown how administration of the study agent will affect ERT. Participants in this trial who receive ERT should be tested to determine whether an ERT immune response is generated following the intrathecal administration of the study agent.
- Once more than one site for this study is identified, a management plan should be developed to ensure that all investigators will be informed of any potential issues.
- An explanation should be part of the informed consent document that indicates that while a research participant may withdraw from the trial, the administration of the gene transfer product is not reversible.

G. Committee Motion 2

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 approved these summarized recommendations by a vote of 13 in favor, 0 opposed, 0 abstentions, and 0 recusals.

V. Review and Discussion of Human Gene Transfer Protocol #1507-1445: Phase I/II Study Administering Peripheral Blood Lymphocytes Transduced with a CD70-binding Chimeric T-Cell Receptor to Patients with CD70 Expressing Cancers

PI: James Yang, M.D., National Cancer Institute (NCI), NIH

RAC Reviewers: Drs. Kiem, Pilewski, and Ross

A. Protocol Summary

Studies in experimental animals have demonstrated that the cellular rather than the humoral arm of the immune response plays the major role in the elimination of murine tumors. Much of this evidence was derived from studies in which the adoptive transfer of T lymphocytes from immune animals could transfer resistance to tumor challenge or in some experiments, result in the elimination of established cancer. Thus, most strategies for the immunotherapy of patients with cancer have been directed at stimulating strong T-cell immune reactions against tumor-associated antigens.

Adoptive T-cell therapy (ACT), which involves the administration of autologous tumor-specific T cells, can mediate durable, complete regression of certain advanced-stage malignancies. The CD70 molecule was initially described as the ligand for the T-cell co-stimulatory receptor, CD27. When T cells are initially stimulated via their TCR ("signal 1"), they require an additional co-stimulus to be activated ("signal 2"), or they can be rendered anergic. The most common co-receptors mediating signal 2 are CD28 and CD27. CD70 was found to be the natural ligand for CD27 and, to date, appears to be the only ligand for this co-receptor. Although T cells can express both CD27 and CD70, they typically are not co-expressed by the same cell. Naïve and resting memory T cells tend to express CD27, while a subset of T cells expresses CD70 transiently after activation. Gene expression databases indicate that only a subset of normal T cells, B cells, and dendritic cell express CD70. A number of human tumors can also express CD70. Most consistently, it is found on nearly all renal cell carcinomas (RCCs) as well as on a proportion of lymphomas, thymomas, and CNS tumors. The rationale for the proposed trial is to use the natural ability of CD27 to bind CD70 to construct a chimeric TCR where the binding domain of CD27 is fused to the intracellular signal transduction apparatus of a T cell to redirect it to recognize CD70. This approach has been successfully demonstrated in vitro and in preclinical murine models. For the proposed clinical study, peripheral blood lymphocytes (PBLs) will be transduced with a retroviral vector that expresses a chimeric antigen receptor (CAR) that targets CD70.

The proposed Phase I/II trial will test a treatment that targets CD70-expressing cancers using T cells engineered with a CD27-based CAR. The study will be conducted by using a Phase I/II optimal design, in which the Phase II component will have two separate cohorts, including one cohort with renal cell carcinoma patients and one cohort with non-renal cell cancer patients. A total of up to 111 evaluable patients may receive the experimental treatment. Approximately 25 patients enrolled in the Phase I arm of the trial, and the rest enrolled in the Phase II arm. The accrual ceiling of 111 participants allows for only a very limited number of patients who are evaluable. The primary objectives of this study are to evaluate the safety of administering PBLs transduced with the anti-CD70 CAR in concert with preparative lymphodepletion and high-dose interleukin-2 (IL-2; aldesleukin) and to determine whether these anti-CD70 CAR-transduced PBLs can mediate the regression of CD70-expressing tumors. The secondary objectives of this study are to determine the in vivo survival of the anti-CD70 CAR transduced cells and to determine the toxicity of this treatment regimen. Patients who have a partial response to the study intervention may be re-treated.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because it is a first-in-human study using CAR T cells targeting CD70.

Three RAC members provided written reviews of this proposed Phase I/II trial.

The reviewers found the study to be well designed and the proposed intervention to be appropriate for the target patient population. In addition, they found the Appendix M section to be well written with appropriate references to the protocol.

Dr. Kiem had the following comments and questions:

- How well can off-target effects be evaluated in the mouse model(s)?
- In the Phase I arm of the study, different types of cancers will be treated, which also means that these patients will have had different types of chemotherapy regimens. As a result, it is likely that the side effects will vary significantly from patient to patient and may thereby influence the dose escalation schema.
- The rationale for excluding myelosuppression from the DLT definition needs to be clarified, since it is also expected to be a side effect of the CD70 CAR T cells. In the immunotoxin studies referenced, SGN-75 seemed to have caused thrombocytopenia.
- How can the investigators separate conditioning-related toxicities, IL-2-related toxicities, and toxicities related to the CD70 CAR T cells?
- Because the Phase II arm of the trial focuses on only two cohorts (patients with clear cell or RCCs and those with non-RCC cancers), wouldn't it be better to establish the dosing and conditions in those patients?
- The ICD says that the experimental intervention might be able to shrink the patient's tumor and also states that it is possible that the modified CD70 CAR T cells may not have this effect. It probably is preferable to state that the effect of the CD70 CAR T cells is not known.
- The consent document discusses gene therapy-related insertional mutagenesis but does not include a discussion on cytokine release syndrome, which is a concern in this setting with moderate- or high-dose conditioning chemotherapy and high-dose IL-2.

Dr. Pilewski noted that in one figure in the protocol (Figure 5), the response to CAR T cell infusion in mouse melanoma studies appears very transient, implying limited duration of effect and that retreatment would be necessary to have a clinically meaningful response. He inquired whether the investigators have any data in animals on the safety and efficacy of retreatment.

As also noted by Dr. Kiem, Dr. Pilewski commented that the protocol and Appendix M section do not thoroughly discuss the potential immunosuppressive effects of a CD70-binding CAR. The protocol includes 6 months of prophylaxis against infections caused by pneumocystis and herpes viruses, implying

significant concerns for prolonged immunosuppression. In addition, long-term immunosuppression is mentioned in the consent form. Given reports of an important role for CD27-CD70 co-stimulation in control of cytomegalovirus (CMV), this concern seems reasonable. However, the protocol does not explicitly discuss this issue, and there appear to be no long-term safety studies in an animal model to provide guidance. Presumably, the transient expression of CD70 during T-cell activation will minimize risk, and there were few reported infectious complications in patients treated with CD70-targeted antibody-drug conjugate SGN-75, but this risk should be clearly delineated in the protocol and/or Appendix M.

The safety monitoring period between dose escalation cohorts is 2 weeks following treatment of the first patient in each cohort. This provision seems to provide a very limited time period of monitoring for possible adverse effects in patients at each dose level. The investigators should consider 2 weeks following T cell infusion of the last patient in each cohort or, ideally, a longer interval to allow for emergence of adverse events and infectious complications.

Viral prophylaxis is described using either acyclovir or valacyclovir, which are suboptimal for preventing reactivation of CMV. The investigators should consider use of valganciclovir as alternative in patients who have evidence of prior CMV exposure. In addition, if there is significant concern for viral reactivation, periodic monitoring of CMV via PCR assays should be considered.

Dr. Pilewski inquired about the criteria that will be used to determine which patients undergo leukapheresis rather than peripheral blood cell collection at 4 to 6 weeks after infusion for analysis of CAR T cell persistence and effects on lymphocyte subsets. With respect to the inclusion and exclusion criteria, the rationale for setting the upper age limit at 70 was requested. Dr. Pilewski noted that given significant variability in functional status based on age, this criterion seems arbitrary. Additional information is needed regarding how patients with CD70-expressing cancers will be identified and whether CD70 staining is in widespread use at the NIH and cancer centers for all malignancies.

There was a question as to whether the screening procedures for pulmonary assessment, which include pulmonary function testing for patients with a prolonged history of cigarette smoking, should specify “more than 20 pack-years of smoking continuing to within the last 2 years” instead of “20 pack-years of smoking within the last 2 years.” Dr. Pilewski suggested omitting the last 2-year constraint and simplifying to significant smoking history to “more than 20 pack-years of tobacco use” or symptoms of respiratory disease.

The ICD includes the statement, “This experimental treatment can lead to long-term decrease in your immune function,” which does not translate into meaningful terms from a patient’s perspective. The investigators should consider providing simpler explanations and more detail with specific mention of infection as a potential side effect of the intervention.

Dr. Pilewski also asked whether the safety monitoring committee (SMC) is independent of the investigative team.

Dr. Ross found the ICD easy to read but raised the following issues and questions for further clarification:

- The researchers make an important point in the ICD: “This is the first time these cells will be given to humans.” They also state, “Since this is a Phase I/II trial, it is unlikely that you will receive any benefit.” The ICD needs to clearly convey how early in the human testing stage this research is to assure that participants understand this point.
- The protocol and ICD state that participation is voluntary and that participants can choose to stop taking part in the study or decline treatment at any time without adverse consequences. Such statements are not completely accurate, however, and these aspects of research participation need to be carefully and clearly conveyed. These documents need to be revised to clarify that once the modified cells have been infused, they cannot be removed and that although participants can withdraw from further treatment and stop being followed, the intervention itself cannot be withdrawn (i.e., the cells cannot be taken out). This information needs to be consistent within and throughout the documents.

- The section of the ICD that discusses cell infusion states that because the specific gene-modified cells that will be used in this study have not been given to patients yet, the investigators “do not have much information about their side effects.” Dr. Ross noted that because this is a first-in-human Phase I study, there is “no information” about the use of the product in humans. This language should be revised to state something such as, “These specific gene-modified cells have not been given to patients; thus, we do not have any information about their specific side effects.” The investigators might want to add that they have some information about similar gene-modified cells.
- The benefits section of the ICD continues this theme by stating, “Because there is not much information about the effect of this treatment on cancer, we do not know if you will benefit from taking part in this study, although the knowledge gained from this study may help others in the future who have cancer.” In this case, it would be better to omit the first clause (“Because there is not much information”) and simply use the rest of the statement (“We do not know if you will benefit from taking part in this study, although the knowledge gained from this study may help others in the future who have cancer”).

Drs. Pilewski and Ross noted that the consent document includes boxed references to a “minor patient’s assent to participate” at the top of each page and signature boxes for a parent’s permission for a minor patient and for a child’s verbal assent (if applicable). However, because the proposed study will enroll only adults, these references are not relevant and should be deleted.

C. RAC Discussion

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

- The reviewers found the presentation to be clear and their concerns and questions to be well addressed. They went through their comments and the investigators’ responses to their queries and suggestions.
- Dr. Kiem asked about evaluation of off-target effects of the investigational product in the mouse model. He also inquired about the possible impact of prior chemotherapy regimens and variation in the patients’ side effects on the proposed dose escalation plan, especially with a complex regimen that includes cyclophosphamide, fludarabine, and high dose IL-2. Dr. Kiem suggested that a plan to distinguish conditioning-related toxicities, IL-2-related toxicities, and potential toxicities associated with the CD70 CAR T cells needs to be delineated.
- Dr. Pilewski inquired about the duration of the response in the mouse melanoma model and a possible option for re-treatment, including how often re-treatment might be necessary and whether the investigators plan to include re-treatment under the proposed trial.
- Dr. Kiem questioned the decision to exclude DLT myelosuppression, which is an expected side effect of the CD70 CAR T cells. In addition, results of the immunotoxin studies seemed to indicate that SGN-75 may have caused thrombocytopenia.
- Dr. Pilewski expressed concern regarding the immunosuppressive effects in some of the animal models. The proposed trial includes prophylaxis as if the patients enrolled in the study are going to have prolonged T cell immunosuppression and are at risk for pneumocystis, herpes, and CMV reactivation, as well as other potential infections. Dr. Pilewski questioned the rationale for the choice and duration of the prophylaxis and whether the protocol addresses the theoretical risk of these other infections adequately.
- The rationale for not including the same cohorts (or cohort) in both the Phase I and Phase II arms of the protocol was requested.
- The safety monitoring period between dose escalation cohorts was revised to provide for a minimum of a 2-week safety assessment period after treatment of the last person in each cohort to allow sufficient time for potential toxicities to emerge. The reviewers found this change to be appropriate.
- Dr. Pilewski inquired about the rationale for the upper age limit and how the investigators would identify patients with CD70-expressing cancers, particularly for patients referred from outside the NIH.

- The eligibility criteria for smoking history have been clarified, and the investigators confirmed that the NCI Data and Safety Monitoring Committee is independent of the investigative team.
- Dr. Ross noted that the reviewers' questions about and suggested changes to the ICD have been addressed, including those related to statements about potential benefit, known and unknown risks, and what it means for a research participant to "withdraw" from the study.
- Dr. Atkins asked whether IL-2 is needed, given that many other CAR T cell protocols do not use this agent, and whether the antigen on the T cells could induce some degree of cell proliferation, similar to what has been reported in other studies. Dr. Kiem had a follow-up question for the PI to clarify what is meant by a "robust" versus "non-robust" dose of IL-2, including the duration and number of doses of IL-2 given in T-cell transfer trials. Dr. DiGiusto further questioned the plan to base dosing of IL-2 on prior therapy. He noted that patients who underwent irradiation and received ipilimumab had the best response across groups and that with the introduction of the co-stimulatory molecules in CARs, the doses of IL-2 have dropped considerably.
- Dr. Atkins also inquired about the approval status of anti-PD-1 drugs, whether patients enrolled in the proposed trial will be allowed to take these agents, and the long-term impact of this class of drugs, including whether any resulting damage to the patients' T cells from this treatment could interfere with their ability to benefit from future use of an anti-PD-1 agent.
- An additional question from Dr. Atkins was whether patients with prolonged T cell depletion who have problems associated with that condition could be given more T cells as a way of reconstituting their immune system. He asked whether generalizing results from adoptive T cell studies is applicable to the proposed trial, given, for example, that in the CD19 CAR T studies, the B cell precursors are permanently eliminated.
- Dr. DiGiusto noted data from animal studies that showed not only class 1 reactivity but class 2 responses that were about two logs higher in treated cells than in control cells, suggesting the possibility of an uncontrolled response to the antigen/peptide. Given these results, he asked whether a similar response is possible for an immune cell expressing CD70 and whether such a response could, in turn, destroy the patient's immune response.
- Dr. Hammar skjöld inquired about potential off-target reactions to CD70. While the investigators have focused on hematopoietic cells, CD70 can be expressed in brain tumors, which raises concerns about possible neurological effects. In addition, there were a lot of complications, including some very severe events, in the MAGE-A3 trial, in sites where there was not supposed to be any expression. Upon further investigation, the researchers confirmed that expression in those cases did occur.

D. Investigator Response

1. Written Responses to RAC Reviews

The PI acknowledged that there are always uncertainties regarding off-target effects across species. He pointed out that to date, no other ligand for CD27 has been found in the mouse or human and that in the mouse, knocking out CD27 obviates the phenotype of a CD70 transgenic mouse.

All patients must have recovered their blood counts from prior therapy to be eligible for the proposed study, which will reduce the variability in side effects. To further address this issue, patients with lymphoma will not be included in this initial trial. Investigators in the NCI Surgery Branch have experience treating patients not only with melanoma and renal cancer but with common epithelial cancers, lung cancer, and human papillomavirus (HPV)-associated cancers, all of whom have been heavily pretreated with a variety of chemotherapeutic regimens. Before enrollment, patients undergo extensive screening for cardiac, pulmonary, and renal function as well as overall performance status. The team anticipates that, as with all ACT protocols, the majority of side effects will be a result of the conditioning chemotherapy regimen. The investigators have attempted to make the dose escalation schema independent of the expected toxicities seen with the chemo-preparative regimen.

Regarding the suggestion to increase the time between dose cohorts as an added safeguard, the investigators will revise the study design to allow for a 2-week safety assessment period following treatment of the last patient in each cohort.

Regarding exclusion of myelosuppression from the DLT definition, the PI explained that this exclusion would apply only if myelosuppression recovers within the expected time frame for the cyclophosphamide and fludarabine (Cy-Flu) conditioning.

With more than 500 patients treated with Cy-Flu and IL-2 across many cell transfer protocols, the toxicities from those components of cell transfer are well known and largely reversible, typically before patient discharge. Some specific toxicities related to the CD70 CAR T cells that will be used in the proposed trial are expected, such as the deletion of the CD70+ T-cell population. As with anti-CD19 CAR and B-cell aplasia, an additional risk-benefit consideration will be needed if any toxicities result in clear consequences that were not seen in the mouse model. Consistent with all of the team's ACT protocols, all intubations, uses of vasoactive agents, and requirements for hemofiltration are tracked and reported in an effort to monitor any increase in these serious events over historical expectations.

Given the team's experience with a variety of solid tumors, the toxicities are expected to be similar in both the RCC and non-RCC cancer cohorts. The investigators have decided not to treat patients with lymphomas or other hematopoietic cancers based on the experience indicating that the toxicity profile is vastly different in this patient population. The focus of this research will therefore be on solid tumors in both the Phase I and Phase II portions of this protocol.

Most patients will not be retreated based on the experience in other T-cell transfer protocols, in which retreatment typically has not been needed, in contrast with what has been observed in many murine models, which have failed to achieve eradication of tumors. The murine models have been poor predictors of the quantitative effects of T-cell transfer in part because the number of cells that can be given to a mouse on a weight basis is significantly less than in humans. The NCI team's experience with T-cell retreatment in humans indicates that retreatment is safe but typically not more efficacious than the initial treatment. Some participants have a very dramatic response and have limited therapeutic options, who could be retreated. As a result, retreatment is reserved for those who relapse after an initial beneficial outcome.

The investigators agreed with the reviewers that there is a theoretical risk of infection due to potential immunosuppressive effects of a CD70-binding CAR (e.g., as reported with use of a CD70-targeted antibody-drug conjugate). Although there is little evidence of a demonstrated risk in immunotoxin studies, the investigators will add this possible risk to the ICD.

The viral prophylaxis regimens (e.g., use of acyclovir or valacyclovir) are based on the outcomes for the approximately 500 patients treated with Cy-Flu within the setting of T-cell therapies at NCI. Some patients on these regimens have a prolonged CD4 deficiency, but with the use of standard prophylactic measures for CD4 deficiencies, there have been few opportunistic infections and essentially no major problems related to post-treatment CMV. Since it can take months or even years for CD4 counts to increase, the protocol will include a 6-month prophylaxis regimen. The PI noted that the proposed strategy and regimens are consistent with the institutional standards of the NIH Clinical Center on advice from infectious disease consultants.

The PI noted that the upper age limit has gradually increased as the team has gained more experience with elderly patients receiving this type of regimen with T-cell transfer. The consensus of the senior staff is that the likelihood of patients over age 70 receiving this regimen safely is very low. Having an upper age limit allows for more efficient screening of candidates and, in turn, enrollment of patients on a protocol for patients with aggressive metastatic disease. The proposed trial includes extensive screening evaluations to ensure that all patients have a functional status that will enable them to recover from the treatment.

The NCI Pathology Department is currently working on a CLIA-certified immunohistochemistry test that will be used to identify CD70-expressing cancers to determine whether patients are eligible for the proposed trial.

Determining whether patients undergo leukapheresis rather than peripheral blood cell collection will be based solely on vascular access and patient consent.

The PI clarified that the NCI SMC is independent of the investigative team.

The inclusion of references to minor assent in the ICD is per the NCI consent template and requirements. Similarly, the boxes for parental permission and the child's assent are part of the NIH Clinical Center ICD template and cannot be changed at this time.

The investigators agreed with the other recommendations and suggestions regarding the ICD and have modified or will revise the proposed consent document accordingly.

2. Responses to RAC Discussion Questions

No major differences in CD70 expression between humans and mice have been observed to date. A lot of the data cited regarding expression of the unique ligand was established in the mouse. Review of information in databases and several other sources reveals only lymphoid and antigen-presenting cells as expression sources, which is consistent across databases. No expression of CD70 in normal brain cells has been reported in the available databases. Histology of all organs of mice that received high-dose cell transfers found no expression of CD70 in the brain, and no abnormal behaviors were noted in the exposed animals.

The investigators have experience with the use of the regimen for the proposed trial in more than a dozen different tumor types. To date, T-cell immunotherapy has been conducted in approximately 500 cancer patients in NCI Surgery Branch protocols. Pre-study chemotherapy treatments for many of these patients have included doxorubicin or anthracyclines. Differences in disease type and therapies can result in important clinical differences across a cohort. The proposed study includes added safeguards to assure that patients on certain regimens or with other risk factors will be carefully evaluated and monitored for, for example, cardiac risks and other potential complications. The type of cancer and chemotherapy regimen is not expected to influence the dose escalation scheme because patients with long-term hematopoietic suppression from prior therapies will not be eligible to enroll in the proposed trial. All participants will be required to have normal counts and normal recovery from their prior therapies. The patient's treatment history is more likely to affect the preparative regimen, which will be modified to accommodate those differences.

The study team has a consistent record of recovery from the proposed Cy-Flu regimen, which is similar to that given in other trials. To date, only a small number of patients have had a prolonged delayed recovery in their counts. Patients who proceed to the study intervention are selected based on recovery to normal counts. Most patients recover within 10 days, and any longer term hematopoietic suppression will be identified per the proposed monitoring plan. No patient is discharged from the hospital until their platelet counts have recovered. The two reported cases of thrombocytopenia were in an antibody trial with a unique immunotoxin (SGN-75) that contained components that targeted factors other than CD70. The cause of thrombocytopenia in that study is not clear, and thrombocytopenia has not been consistently found in other protocols of anti-CD70 CAR transduced cells. As an added safeguard, patients in the proposed trial will be monitored daily for thrombocytopenia, and dose escalation will be done only with recovery and normalization of cell counts.

Dr. Yang acknowledged the challenge in being able to distinguish among the toxicities associated with each of the three products given to patients (i.e., Cy-Flu, IL-2, and the modified CAR T cells). The investigators will be guided by careful documentation of toxicities (e.g., hematopoietic suppression, neutropenia) in the 500 previous patients who had high-dose IL-2 with the exact same preparative regimen. These events are similar across protocols and do not differ by cancer type. In addition to these

500 patients, the NCI team has given IL-2 to an additional 3,000 patients. Adverse events in these cohorts were used to develop a solid database of the expected toxicities of IL-2 and Cy-Flu, which have been consistent, are very reproducible, and resolve or reverse quickly.

Dr. Yang pointed out that the proposed prophylaxis regimens are based on experience with the other 500 patients getting Cy-Flu. Some of these patients have a prolonged CD4 deficiency. Few opportunistic infections have been seen because of the use of standard prophylactic measures for CD4 deficiencies. As a result of this strategy, opportunistic infections have been well controlled. CD4 counts can take months to even a year to recover, however, and the team explored ways to better monitor immune surveillance. The investigators determined that a control group would be needed to test for immune responses for any active intervention. If this plan were followed, the study would need to include a population of patients who receive a completely different T cell product with the same preparative regimen and vaccinations, or a separate study with this group would need to be conducted. The investigators concluded that these options were not reasonable for patients who do not undergo a CD70-targeted therapy. Further, there is no clearly established means of monitoring functional immune responses in these patients without a control population. Therefore, the plan to assess and monitor for clinical outcomes will be based on prior experience with other CAR T cell immunotherapies.

The study team has a long history of attempting re-treatment with other T-cell transfers. Dr. Yang noted that re-treatment is rarely done, however, and accounts for only about 5 percent of prior T-cell transfers. Further, re-treatment typically is not more efficacious than the first treatment. The investigators expect some patients will have a very dramatic response to the initial treatment and have no other options; the protocol includes re-treatment options for this group, but routine re-treatment is not planned.

The decision regarding the patient cohorts was based on participant availability; the investigators anticipate that renal cancer is the only group for which a sufficient number of patients will be consistently available. Patients with other types of less common cancers, such as thymic carcinoma, will likely be more challenging to recruit. In addition, running a separate protocol for another histology will be very difficult. Regarding the upper age limit, Dr. Yang explained that the team took an incremental approach to increasing this eligibility criterion. When the team first started T-cell transfer trials, patients over age 60 were excluded; the cohorts were subsequently expanded to include patients up to age 65 and then to age 70. In the absence of a cutoff in the upper age criterion, the team was inundated with screening calls from patients who were not even remotely eligible to participate in these trials. Based on experience to date, the team has concluded that patients over age 70 are highly unlikely to be able to tolerate the required regimens. Without an upper age limit, considerable staff resources would be spent on screening many patients who will not qualify to participate.

Procedures to screen for specific antigens have been standardized and are currently in use in several other protocols. The investigators request unstained paraffin sections, which then undergo a CLIA-certified immunohistochemical analysis. The method for identification of CD70-expressing tumors for the proposed trial is being developed by the NCI's pathology department. Per the pre-specified criteria, at least 50 percent of all the tumor cells must express the target antigen for a patient to be eligible for this study.

Regarding the inclusion of IL-2, Dr. Yang noted that there are very few data on treating solid tumors without IL-2. Among melanoma patients treated with tumor-infiltrating lymphocytes, the overall response rate to T-cell transfer with IL-2 is 50 percent, while the complete response rate is 24 percent; nearly all these patients are cured of metastatic disease after 10 years of follow-up. In contrast, the overall response rate among patients in the same setting who did not receive IL-2 is about 20 percent, with only one or two durable responders over the follow-up period. Data from these studies also show that persistence of T cells with IL-2 is very high, in contrast with zero persistence in melanoma patients who were not given IL-2. While these are not randomized findings, they represent the best information currently available. The PI noted that the IL-2 given to patients in these trials adds very little toxicity, because the doses are low compared to the robust doses given as a monotherapy. The term "robust" refers to the number of doses of IL-2. In T-cell transfer trials, four doses of IL-2 typically are given over 1.5 days, and this regimen has been well tolerated in lymphodepleted patients. In rare cases, up to 12 doses

might be given. The number of doses given depends on clinical judgment and the degree of toxicity that develops. If it appears that a patient is going to need a vasopressor or a lot of fluid resuscitation, the investigators will stop further IL-2 dosing. However, if an IL-2 monotherapy regimen is being used, those additional interventional measures would be taken.

Dr. Yang acknowledged that cell proliferation could be induced, as seen in other CAR T cell protocols. The cell numbers and types of toxicity in the CD19 CAR T cell studies of hematological cancers differ, however, from what has been observed in hundreds of patients with solid tumors. The reason for these differences is not clear. It could be due to the difference between the hematopoietic cancers and solid tumors, or other factors as well. Eligibility for the proposed trial will be limited to patients with solid tumors, which is expected to control potential confounding by cancer type. In addition, if any evidence of cytokine toxicity is seen at the lowest dose, escalation to the next dose will not proceed until a sufficient number of additional patients have been tested.

Preliminary results from Phase III trials assessing the efficacy of anti-PD-1 agents in renal cancer patients are not as encouraging as results from trials in patients with melanoma. However, the investigators need to review the full set of randomized data from these Phase III studies to make a more definitive decision about whether to use an anti-PD-1 drug for renal cancer. Data regarding follow-up and the rate of complete response in renal cancer patients are needed for a more complete assessment of this class of drugs as a potential treatment for renal cancer. If the intervention is not curative, the team will not require it. On the other hand, if PD-1 is effective in treating renal cell cancer, an eligibility criterion for patients to try that drug first would be added to the protocol. Dr. Yang noted that it is unlikely that the immune repertoire would be damaged to the point where a patient could not respond to PD-1 based on experience to date. This type of damage has not been seen in the melanoma patients treated with T-cell transfer who have gone on anti-PD-1 agents; rather, they have had good responses. In addition, no synergistic or unusual toxicities have been observed in melanoma patients who have received anti-PD-1 therapy and subsequently received T-cell immunotherapy. The target differs between T cell protocols, but the response thus far among treated patients has been acceptable.

It is not clear how well the immune repertoire can be reconstituted by simply giving the patient transfused mature T cells. While there usually is a transient improvement, the duration of this effect has been variable. Dr. Yang noted that in the CD19 CAR trials and similar protocols, any transferred T cell population persists at a very low level; further, it is unusual to have more than 1–2 percent of the transferred cells in the peripheral repertoire. Thus, the large number of cells given to patients typically disappears within days. Permanent B cell aplasia in patients treated thus far with CD19 CAR has been extremely rare. For some patients with B cell lymphoma, the response has been excellent, with B cell aplasia lasting for only weeks to months. Many other events, including autoimmune toxicities, are self-limiting, and efforts to reconstitute the T cell repertoire at a later time are unlikely.

In one set of experiments, normal mice given a T-cell transfer with and without irradiation were subsequently vaccinated with vaccinia virus expressing ova or hgp100, another class 1 antigen. In both groups, the class 1 reactivity against ova and hgp100 was preserved in the spleen, but it was slightly better without irradiation than with irradiation. A strong class 2 restricted response was seen in the lymph nodes. Thus, despite depletion of CD70 cells in these mice, the animals were still able to mount responses against both class 1 and class 2 peptides. Dr. Yang pointed out, however, that even among mice given the same regimen, there is considerable variability in the response among different animals. The dataset presented during the meeting compiled various information for class 1 and class 2 responses. Other experiments, for example, have not shown a similar dramatic enhancement of reactivity. The reason for such a large response to CD70-targeted therapy (and a nominal antigen) is not clear, but it might reflect cytokine toxicity.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

Clinical and Trial Design Issues

- If anti-PD-1 is approved for treatment of CD70-expressing malignancies, participants in this trial should first try and fail this therapy prior to enrollment.
- Participants should be closely monitored for the recovery of both B cells and T cells and infectious disease complications given the potential risks for the immunosuppressive effects of anti-CD70 CAR T cells as well as the myelosuppressive effects of various conditioning chemotherapies used for the malignancies targeted in this trial.
- Participants should be closely followed for various IL-2 toxicities, and consideration should be given as to whether IL-2 administration is necessary as most CAR T cell immunotherapy trials do not use this conditioning cytokine.
- Revise the consent language to simplify complex concepts such as the potential effects of the study product on the participants' immune system and function.
- The consent language should also strive to avoid therapeutic misconceptions (e.g., do not imply that these cells have a known cancer killing effect) and clearly outline the potential risks of the study agent such as the occurrence of cytokine release syndrome.

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 approved these summarized recommendations by a vote of 12 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VI. Gene Transfer Safety Assessment Board Report

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

A. GTSAB Report

Dr. Kiem presented the GTSAB report for the third quarter of 2015. Within the past three months, NIH OBA received a total of 30 protocol submissions, 27 of which were not selected for public review at this RAC meeting. Of the 27 protocols not selected for public review, 24 were oncology protocols, one was a monogenic disease protocol, one was a peripheral artery disease protocol, and one was an eye disease protocol. Among these 24 protocols, five used plasmids, five used retroviruses, five used herpes simplex virus, three used lentiviruses, two used adenoviruses, two used RNA, two used attenuated *Listeria monocytogenes*, one used AAV, one used DNA, and one used vaccinia virus.

For the third quarter of 2015, the GTSAB reviewed 22 serious adverse events (SAEs) from 19 protocols, including initial and follow-up reports. (Information about these trials is available on the OBA website and in the future, a web summary of the events reviewed will be available on the OSP website and in the NIH Genetic Modification Clinical Research Information System, also known as GeMCRIS.)

For the third quarter of 2015, OBA received notification that 15 new protocols opened. Six protocols were previously publicly reviewed:

- *OBA Protocol #0810-946, reviewed in December 2008: 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

- OBA Protocol #1007-1050, reviewed in September 2010: Phase I Study of an Active Immunotherapy for Asymptomatic Phase Lymphoplasmacytic Lymphoma with DNA Vaccines Encoding Antigen-Chemokine Fusion
- OBA Protocol #1301-1200, reviewed in March 2013: Phase I/II Gene Transfer Clinical Trial for LGMD2D (Alpha-sarcoglycan Deficiency) Using SC rAAV8.tMCK.hSGCA
- OBA Protocol #1406-1319, reviewed in September 2014: A Phase I Study of Chimeric Antigen Receptor Modified T-cells Targeting NKG2D-Ligands in Patients with Acute Myeloid Leukemia/Advanced Myelodysplastic Syndrome and Multiple Myeloma
- OBA Protocol #1410-135, reviewed in December 2014: Pilot Study of Autologous T-cells Redirected to Mesothelin and CD19 with a Chimeric Antigen Receptor in Patients with Metastatic Pancreatic Cancer
- OBA Protocol #1411-1357, reviewed in March 2015: Phase I, Randomized, Open-Label, Active-Controlled, Dose Escalation Study to Evaluate the Safety, Tolerability, and Immunogenicity of INO-1800 Alone or in Combination With INO-9112 Delivered IM followed by Electroporation in Select Nucleos(t)ide Analogue-Treated, HBeAgPositive, Chronic Hepatitis B Patients

The following protocols opened in the third quarter of 2015 but were not publicly reviewed:

- OBA Protocol #1304-1228: A Phase I Study of Autologous T- Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases SB-728 in HIV Infected Patients Pre-treated or Not with Cyclophosphamide
- OBA Protocol #1310-1267: Pilot Study of Autologous T Cells Redirected to EGFRvIII with a Chimeric Antigen Receptor in Patients with EGFRvIII+ Glioblastoma
- OBA Protocol #1310-1271: Phase I Study of Cellular Immunotherapy Using Central Memory Enriched T Cells Lentivirally Transduced to Express an IL13Ra2-Specific, Hinge-Optimized, 41BB-Costimulatory Chimeric Receptor and a Truncated CD19 for Patients with Recurrent/Refractory Malignant Glioma
- OBA Protocol #1311-1277: Phase I clinical trial of autologous CART-meso cells in patients with mesothelin expressing cancers
- OBA Protocol #1312-1282: Administration of TGF-Beta Resistant Cytotoxic T-Lymphocytes to Patients with EBV-Positive Nasopharyngeal Carcinoma (RESIST-NPC)
- OBA Protocol #1406-1322: A Phase Ib Randomized Clinical Trial to Evaluate the Safety and Immune Response to a Mammaglobin-A DNA Vaccine in Breast Cancer Patients Undergoing Neoadjuvant Endocrine Therapy
- OBA Protocol #1408-1344: A Phase Ib Study Evaluating the Safety, Tolerability and Immunogenicity of CMB305 (Sequentially Administered LV305 and G305) in Patients with Locally Advanced, Relapsed, or Metastatic Cancer Expressing NY-ESO-1
- OBA Protocol #1410-1351: A Phase II, Single Arm, Multicenter Trial to Determine the Efficacy and Safety of CTL019 in Pediatric Patients with Relapsed and Refractory B-Cell Acute Lymphoblastic Leukemia
- OBA Protocol #1502-1388: Phase I/II Study in WT1-Expressing Non-Small Cell Lung Cancer and Mesothelioma, Comparing Cellular Adoptive Immunotherapy with Polyclonal Autologous Central Memory to Naïve CD8+ T Cells that have been Transduced to Express a WT1-Specific T-Cell Receptor

Dr. Kiem presented key findings from two recent publications of NIH OBA gene therapy trials:

- Long et al., 4-1BB co-stimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors, *Nat Med* 21(6): 581-590, 2015. Results of this study found that CD28 co-stimulation augments, whereas 4-1BB co-stimulation reduces, exhaustion induced by persistent CAR signaling. In addition, this study found that CD19 CAR T cells incorporating the 4-1BB co-stimulatory domain are more persistent than those incorporating CD28 in clinical trials.
- Rapoport et al., NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma, *Nat Med* 21(8): 914-921, 2015. Results of this study found that NY-ESO-1 TCR-engineered T cells were safe, trafficked to marrow, and showed extended

persistence that correlated with clinical activity against antigen-positive myeloma. Encouraging clinical responses were observed in 16 of 20 patients (80 percent) with advanced disease, with a median progression-free survival of 19.1 months.

B. RAC Discussion

RAC members had no comments or questions.

C. Public Comment

No public comments were offered.

VII. Closing Remarks and Adjournment

Dr. Kiem thanked the RAC members and the OBA staff and adjourned the September 2015 RAC meeting at 3:42 p.m. on September 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: _____

Hans-Peter Kiem, M.D., Ph.D.
Chair, Recombinant DNA Advisory Committee

Attachment I: Recombinant DNA Advisory Committee Roster

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**Attachment II:
Public Attendees**

(This list includes only individuals who are not identified elsewhere in this document.)

Dale Ando, Sangamo BioSciences, Inc.
Sara Berl, Regenxbio, Inc.
Mark Dant, Ryan Foundation
Robert Fiorentino, Regenxbio, Inc.
Julie Hagan, Regenxbio, Inc.
Michael Holmes, Sangamo BioSciences, Inc.
Arati Kamath, NCI, NIH
Mari Maurer, Regenxbio, Inc.
Marguerite McDonald, University of Pennsylvania
Lynne McGrath, Regenxbio, Inc.
Eric Merrell, Patheon Biologics LLC
Kathleen Meyer, Sangamo BioSciences, Inc.
Debbie Nathan, NCI, NIH
Michael O'Callaghan
Betty Poon, National Institute of Allergy and Infectious Diseases, NIH
Reka Shinkle, Regenxbio, Inc.

Attachment III: Abbreviations and Acronyms

AAV	adeno-associated virus
ACT	adoptive T-cell therapy
AE	adverse event
ALT	alanine aminotransferase
CAR	chimeric antigen receptor
cDNA	complementary DNA
CMV	cytomegalovirus
CNS	central nervous system
CSF	cerebrospinal fluid
Cy-Flu	cyclophosphamide and fludarabine
DLT	dose-limiting toxicity
ELISPOT	Enzyme-Linked ImmunoSpot
ERT	enzyme replacement therapy
FDA	U.S. Food and Drug Administration
FISH	fluorescent in situ hybridization
FIX	Factor IX
GAG	glycosaminoglycan
GTSAB	Gene Transfer Safety Assessment Board
hALB	hybrid albumin
hFIX	human FIX
HSCT	hematopoietic stem cell transplant
ICD	informed consent document
IDUA	α -L-iduronidase
IL-2	human interleukin-2
indel	insertion or deletion mutation
ITR	inverted terminal repeat
IV	intravenous
kb	kilobase(s)
LFT	liver function test
LTFU	long-term follow-up
MED	minimum effective dose
MPS I	mucopolysaccharidosis type I
MRI	magnetic resonance imaging
mRNA	messenger RNA
NCI	National Cancer Institute
NHP	non-human primate
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PBL	peripheral blood lymphocyte
PCR	polymerase chain reaction
PI	principal investigator
qPCR	quantitative polymerase chain reaction
RAC	Recombinant DNA Advisory Committee
RCC	clear cell renal cancers
SAE	serious adverse event
RT-PCR	reverse transcription polymerase chain reaction
SAEs	serious adverse events
SELEX	systematic evolution of ligands by exponential enrichment
SMC	Safety Monitoring Committee
TCR	T-cell receptor
vg	vector genomes
ZFN	zinc finger nuclease

Appendix A: Public Comments on Protocol #1507-1449

[This testimony is provided verbatim, as reported in the transcript of the September 2015 RAC meeting.]

Testimony of Mark Dant

Good afternoon. My name is Mark Dant. I'm a Hurler-Scheie father, also an assistant chief of police in Carrollton, Texas. I am the volunteer executive director of the Ryan Foundation, a nonprofit that supports research science in MPSs. I also, in February, will assume the role of the National MPS Society's executive director.

I'm here today to talk about my family. So I think the most important part of beginning with a family is to show an embarrassing picture. I would like the panel to look behind the main Hurler-Scheie, or attenuated MPS, and see that each of the people we speak of, we all know this. But I want to demonstrate the reality of it all happens behind a family.

My wife and I originally grew up in Louisville, Kentucky, and I was already established as an officer in Texas. So we came back to get married, as my wife was still a student at the University of Louisville. On our long drive back to Dallas, we laid out our future, which included three children, a home, and a long, lengthy career as a police chief one day, my wife in the software business.

April 13, 1988, Ryan Christopher Dant was born, 8 pounds, 13 ounces. Ryan was a healthy, beautiful little baby. His future—I remember, not long ago, a friend of mine retired from the police department and handed out—he found in the back of his locker a cigar. A long time ago, dads used to give away cigars with the birth of their children. And he handed this gift to me back after 27 years in his locker, and it brought back to me all of the thoughts and dreams and hope of what this baby's picture would bring. And we knew at that moment that Ryan would have a huge future, and he may be great in whatever he does, but he would have something to offer to our society. And we knew this would occur because as time went on, Ryan's health continued to improve. He was always a healthy boy.

At the age of three, he was so healthy that he could play baseball in the house and outside and we told him no more baseball in the house. At the age of three-and-a-half, my wife decided to enroll Ryan in a structured preschool. And so, he had a preschool physical.

The pediatrician was very attuned to all things, and he immediately noticed that Ryan's liver and spleen seemed to be too large. He also noticed that Ryan's head was too large. It made me and my wife nervous, because there was something wrong with Ryan, according to the pediatrician. So as Jeanne came home and told me about this, I knew doctors' job was only to find things wrong and not things that were right. I was incorrect.

Three weeks later, we went back for the test results. And as my wife and I walked into the small room, behind us, Dr. Lewis Waber, from Dallas Children's walked in. But he also walked in with a family counselor, another pediatrician, and a genetic counselor. At that moment, all the hopes and dreams I'd handed out that night of April 13, 1988, vanished, because Ryan was diagnosed with MPS I. We were told that Ryan's future was no more, that he would pass away probably in his early teens, if not before. He would be physically handicapped in a wheelchair. His hands would draw up. He would not be able to walk. He would probably be mentally challenged.

At that moment, our life ended. We knew we would have no other children, because Ryan would be with us for a while, and then he would leave, and that would be the end of that.

What we couldn't predict was that great science would be in front of us, and a great scientist by the name of Emil Kakkis, who would surround himself with great scientists—and in a small Quonset hut behind the campus of Harbor-UCLA, Emil Kakkis developed what would later be called Aldurazyme.

And Ryan, who loved baseball as a small child, who had stopped playing baseball by the third grade, stopped playing soccer, because his last game in a soccer field, his inguinal and umbilical hernias presented even worse, and he collapsed, and he never played soccer again. He never played baseball again, because he couldn't grip the bat. And even to this young child, at the age of seven and eight, he no longer talked about the future. Most kids say they're going to be a baseball player or a football player or a great something when they grow up. But Ryan talked nothing about tomorrow, because tomorrow was gone, until February 13, 1998.

Eight weeks before Ryan's tenth birthday, he received his first infusion of Aldurazyme. And to us, his future was back. We were once again able to talk about what would happen tomorrow. Maybe Ryan could be a great scientist, like Dr. Kakkis. Maybe he would be a great attorney or a great doctor or a great police officer. But in the back of my mind, I was often told and often remembered that Aldurazyme doesn't cross the blood-brain barrier. And so, even though we saw his body improve, we saw him play baseball again, we saw him play basketball—I also met a lot of other families with MPS I, attenuated MPS I, Hurler-Scheie.

When Ryan was in the third grade, just before the trial started in fourth grade, I met a professor in East Texas, whose daughter Ginger was 19, and she was attenuated MPS I. Ginger had severe spinal cord stenosis and also had what her mother called the ability to forget everything. Ginger would argue with her mom, the professor, day after day about—"Mom, why did you take my purse? Where did you put my wallet?" They would like to watch—they watched television shows and movies and found that Ginger couldn't remember the end of the movie and make sense out of it from the beginning, because she couldn't recall what happened at the beginning.

Ginger had such severe spinal cord stenosis that, at age 21, she had spinal cord surgery, and 3 hours after the surgery, she passed away. But I never forgot what Ginger's mom told me—was that in Ryan's future, because the drug does not pass the blood-brain barrier, Ryan's attenuated MPS would change his future forever.

Time went forward, and those statements did occur. By eighth grade, we noticed that Ryan couldn't remember what he read two paragraphs before. By ninth grade, he was in different courses in high school. And by tenth grade, he was in content mastery and could never take a test by himself. He did graduate high school, but he didn't graduate at the level of everyone else, because he couldn't remember a test he'd studied for before. But my son has physical courage, and he has intellectual courage. He didn't want to quit. He wanted to continue so he could become something, anything.

So he wanted to go to college. We would travel often to the University of Minnesota, where he was tested constantly for cognitive testing. He would do well and plateau and then decline and do okay, plateau and decline. Over the course from the time he was 4 until the time he was 19 and 20 and 21, that is what we saw.

But Ryan never quit college. He would take one course a semester just to catch up so he could qualify for a college. But time rolled forward, and he still wasn't able to compete in college. He was now 23 and 24 and 25. He couldn't hold a job, because he couldn't remember things that would happen today; now he was supposed to do it in the afternoon.

So in the early spring of 2013, Ryan enrolled in an off-label intrathecal use for enzyme replacement. The remarkable part of the story is, Ryan improved dramatically. He was once again able to take a test and remember. Prior to intrathecal, I saw Ryan study for 3 days hard for a test on geology, memorizing rocks, Friday, Saturday, and Sunday. Sunday night, he knew it. Monday morning, he drove to campus, he took the test, and he called me, and I knew he was going to do well. And he said, "Dad, I failed. I got a 43. I couldn't remember any of it."

Post-intrathecal, things improved, improved so dramatically that he enrolled at the University of Louisville, his dream university. He also works for the football team there in the equipment staff. That's the good part of the story.

Last Sunday, Ryan received his 863rd weekly IV infusion. Next Thursday, the University of Auburn, I believe, travels to—Clemson, actually, travels to the University of Louisville to play a football game. Ryan will work that game. He'll get home to his campus dorm about 1 a.m. The next morning, at 6 a.m., he'll fly to Dallas, and he'll go into the hospital at UT Southwestern, and there he'll receive his 15th lumbar spinal tap to replace the enzyme that helps keep him in college.

Here's the reality of intrathecal: It seems to work for Ryan, but it's not very sustainable. Once every 3 months, he goes back and has a lumbar tap to put back in his system what he can't make. I've spoken to families across the United States and around the world about the importance of some science coming forward for the attenuated MPS I person.

Their lives do matter. But now they're sitting at home waiting. They're waiting, and they're waiting for good science, good, safe, efficacious science that will put them back into the workforce and bring them back to the dreams they once hoped for. They're waiting and they're willing and they are ready. Thank you.

Testimony of Eric Merrell

Good afternoon. My name is Eric Merrell. Like Mark Dant, I'm also an MPS I parent to MPS adults, Sean and Cody, who you see standing next to me there. We're from Festus, Missouri. I'm a validation engineer for Patheon Biologics in St. Louis. That's my background. I'd like to thank you for the opportunity today to talk about my family. I'm very excited about it. Love to talk about my family. I'm sure most parents feel the same.

You know, my family means the world to me. We've been through a lot. The sun rises and sets on those four people right there for me. I love looking at pictures of them. I love this picture. It's from Easter of this year. But I really love to look at any picture where we're all five included, because 15 years ago, I didn't know if we would ever have a picture like this today.

July 14, 2000, 1 week before Sean's eighth birthday, we were in the geneticist's office at Children's Hospital in St. Louis as the doctor explained to us our future. He explained to us the litany of difficulties and challenges and the road that lay before us. He talked about all the symptoms to keep an eye out for as the disease progressed. He talked about carpal tunnel syndrome. He talked about hip dysplasia. He talked about hydrocephalus, spinal cord compression.

These were all pretty dire-sounding words to us. We'd never heard—we'd heard of a few of these, but we never thought that we'd talk about our family with those words. He talked about other words like we've heard today: lysosomes, GAGs, enzymes. Those were words we had never heard before. But we learned quickly.

He said that we should begin to see a panel of specialists, much like Mark explained: cardiologists, pulmonologists, orthopedics, ophthalmologists. He promised us, "We'll treat the symptoms as they present themselves, and we'll do the best we can, and we'll fight with everything we have to give them the best quality of life possible. But as hard as we battle, no matter what we do, MPS is going to win."

I was floored. I looked at them as healthy, perfect children. You know, the symptoms weren't clear to me. I didn't understand. You know, I implored and I asked him, "Doc, there's got to be some kind of treatment. I mean, can you prescribe—there's got to be a pill for this, right? I mean, there's something out there."

I mean, I just couldn't believe that there was no hope. His advice to us that day was that at this time, there is no treatment. He said, "My advice to you: If this was my child, I would take him home. I would love him the best I could and give him the best quality of life possible." So that's what we did.

As we continued to talk to the doctor in the office that day, it didn't dawn on us at first that this was a genetic disorder that my wife and I had passed on to our son. So my immediate thought when I realized this went to our other two children. "Doc, you know, what about Sean and—or what about Cody and Amber? Are they okay?" "Well, we're not sure. We'll have to test them and see." So we did. But we found out Cody also had MPS I but Amber did not. Thank goodness for small miracles.

So I guess that was our diagnosis journey. But my wife, as most mothers—they know when something is wrong with their kids. My wife knew something was different. She knew something was wrong. But despite her constant—not constant, but despite her often observations, I didn't want to believe. I didn't see it. I didn't want to see it.

She would talk to me about how she noticed Sean would have difficulty pulling his shirt on over his head to go to school that day. He really had to struggle. He used to be able to bend over and tie his shoes. Not only could he not bend over to tie his shoes any longer; it was very difficult for him; he had difficulty with the manipulations of his shoestrings. He couldn't tie his shoes. We started buying Velcro shoes. You can see his hands are still curved after years of ERT.

You know, I just downplayed it. I didn't want to believe it. She would say, "What about the constant ear infections?" Sean had four sets of ear tubs as a child. Cody went through three himself. "What about the mitral valve prolapse that we've seen? Those have to be connected." I mean, this is all adding up to a picture that I didn't want to see. I just told her she was being paranoid, because there was nothing wrong with my boy, and that was just the end of it.

But in the doctor's office that day, I couldn't bury my head in the sand any longer. It was obvious that I needed to snap back to reality. So as we sat in the doctor's office that day, probably the most important thing he said to us was that we should call the National MPS Society. He talked about it—was—"a parent support group" was how he phrased it. He gave us a pamphlet and a phone number, and

he said, "Maybe there's somebody there that you can talk to that can help you cope with the feelings you're feeling right now and the road ahead."

So we took his pamphlet. We went home, and basically, we were paralyzed for probably about a week or so—you know, went through the normal why us, why our boys, what did I do in my life that God wanted to inflict this upon my kids. They're innocent. They don't deserve this.

So we kind of went through those feelings, worked through those, and then we decided. We had a choice. We could either accept this, or we could fight. So my wife called the MPS Society about a week, week and a half later. And they gave her some phone numbers of some families with MPS I and asked that we give them a call.

So again, my wife, with her infinite wisdom, knew that this was a good road to take. I was indifferent. I didn't—I'd heard what the doctors said. I heard him say we had no hope. So why are you going to keep -- you know, why are you going to keep pushing this? She said, "You know what? I'm just going to call." I said, "You know what? If that makes you feel better, go ahead." So she did. And thank the Lord that she did, because it led us on the road that we're still on today.

When she called, she was given the name of a family in Texas specifically. That was the first phone call she made, Steve and Amy Holland, live close to Mark in Texas, in Fort Worth. Vicky talked to Steve that night for probably a good 2 hours about the disease, and Steve talked about their experiences and what they had seen with their kids.

But he also gave us a glimmer of hope. He told us about a clinical trial that was upcoming for intravenous enzyme replacement therapy, or Aldurazyme. And the company was actively recruiting patients for that trial. So that was—that was life changing. That was—gave us back what the doctor took away from us back that day in the office, just really a couple weeks' earlier, and that was hope. Like Mark discussed, how that hope just drains away—it just—right out the door, it just goes immediately.

But this—we were so lucky that within this time frame; we had a lifeline. So luckily enough, as fate would have it, just a couple of months later, it was the National MPS Family Conference—Annual Conference. So we decided that we would attend in Los Angeles in September of 2000. And that's where we got to meet Mark for the first time and Dr. Emil Kakkis. And they talked to us more about this exciting therapy that could save our boys' lives. We learned that Dr. Kakkis had found a way to make the enzyme that our boys were deficient in.

More so, he found us a way again to give us something just as valuable, and that was hope, hope that maybe our boys could live past their teens, against the advice or the prognosis that our geneticist had given us that day, hope that maybe they would live to graduate high school, which they both did. And actually, Sean gave a speech at his graduation in 2011, one of the proudest moments of my life. Hope that they could make their dreams come true, not just ours, but whatever they dream. And hope that, like any other parent, they would be healthy and normal kids one day.

So we went home from that conference with this brand new information and made our contacts with the clinical researchers. And just a couple of months later, in January of 2001, less than 7 months after diagnosis, we flew to New York to enroll the boys in the Aldurazyme trial. They did all the normal tests, the 6-minute walk, the blowing into the tube to test their lung capacity. They gave blood and other bodily fluids to be tested.

So we were very hopeful. Unfortunately, while we were in New York, Cody, who had recently been diagnosed with Asperger syndrome at the time, was excluded from the study due to his inability to complete the intricate instructions from the test. So Cody was excluded, but Sean was able to be enrolled. So that was a kind of a double-edge sword, where we were thrilled but heartbroken again that Cody would still continue to suffer from the debilitating effects of MPS and that would continue to progress.

When we asked Sean if he wanted to participate even if Cody couldn't, he bravely stuck his chest out and said, "Yes, Dad," he said, "Because I want to save Cody's life and other MPS kids just like him." He was the bravest, bravest kid I've ever known. And he's ready for this trial, by the way. He is raring to go. He's excited.

So, reenergized and dizzy with the possibility that maybe our boys could have a healthy future, a normal future, that this death sentence was not a death sentence, we dove headfirst in the MPS Society. We became so energized. We made contact with numerous other MPS families. We started having and attending MPS fundraisers to raise money for research and family support. We wrote letters and traveled to Washington, D.C., to lobby for legislation that helped MPS individuals. We ran for and served a term on the board of directors. We were all in. We had a purpose again. We had hope.

One of the fundraisers we attended during that time was one that Mark put on in Austin, Texas, and the Ryan Foundation put on—was a golf tournament. Mark had invited some of the kids from the Aldurazyme trial down to Texas so that the folks attending and contributing their time and money could see the effects of their efforts. They could see that—they could see these kids and put a face to this disease and see why we needed—we needed—we needed their help so that our kids could have hope.

During a black tie dinner at that event, Mark had called and asked—Mark had called all the kids and asked them to come up in front of the room. And one by one, he went down the line and asked them, “What do you want to be when you grow up?” again to drive home that point: These are normal kids. They have dreams and hopes just like every other child across America.

When he came up to Sean, he asked him. Sean knew what he wanted. I knew what he was going to say, too: “I want to be a racecar driver,” just like any other normal kid.

So it was just a really great time for our family, even though we had been—we had dealt with this diagnosis. We had hope again. We were on the right path, we felt. Sean and the other kids continued in the trial. And very quickly into the trial, you could see which kids were on enzyme and which ones were on placebo. They could walk faster. They had more energy. Their bellies were shrinking due to the organs shrinking from the displacement of the GAGs, the reduction in GAGs.

So we went through the trial. We saw the results. In the spring of 2003, after the trial had concluded, our family, along with the Dants and the Hollands, traveled to Bethesda and spoke to the FDA advisory committee and urged them to fast-track approval for Aldurazyme.

Our prayers were answered that day—not that day; our prayers were answered shortly when approval was gained, just a short time later. And then Cody could begin the lifesaving infusions as well. So Sean had set out on his mission to save his brother’s life, and he had. What a miracle. So as time went on, we had this great new treatment now. We could buy some time. It wasn’t a cure, and we knew that. But it was a treatment, something that gave us hope.

We continued to make annual visits to our specialist that our geneticist had recommended in St. Louis. We continued to treat their symptoms as they occurred, and they did occur, but not in the severity that we believe they would have without Aldurazyme.

Sure enough, as the doctor said that day, they both have had carpal tunnel surgery. Sean has had it twice, as a matter of fact. Sean had bilateral hip replacement in 2007. They both had procedures to correct multiple hernias, and they both have also had cervical laminectomies to address their spine issues, just as the doctor predicted that day. So all of his predictions had almost come true, except for one, and that was, MPS had not won. The war wasn’t over, but MPS had not won yet. So far, we’ve been able to withstand the attacks and stand our ground. But there’s still one more battle to wage, and hopefully we will soon be armed for that fight.

When we entered into the Aldurazyme trial, it was explained that the enzyme would not be able to enter the brain due to what we learned was the blood-brain barrier. At the time, I was like, you know, “Man, I don’t care. Just save my kid. We’re ready. We just want to save our boys’ lives.” And we did. Dr. Kakkis did. But now, we have that chance to address that unmet need that Dr. Wang, Dr. Yoo, and Dr. Hinderer have discussed. That unmet need is the cognitive decline of MPS-affected individuals. We can provide the data of the boys’ IQ declined over a 4-year period from Dr. Shapiro’s study that the boys were entered in at the University of Minnesota. And that’s good data. It’s relevant.

But I’m here today to illustrate what that means in real-life terms to my family. I’m here to relay the monumental struggles that these boys had getting through high school. It wasn’t easy. Sean, in his early elementary education years, was a good student. He loved to read. He loved math. He loved going to school every day. But as the years went on and the disease progressed, he stopped reading. He was having more trouble in class. He needed help with tests. Someone would have to read the questions aloud to him, because he was having trouble comprehending. That was hard.

We were treating their bodies, and we could see the effects, but we could also see the decline that was happening in their brain. By the time Sean graduated high school and Cody as well—with his Asperger’s, that’s a little more obvious, but Sean was in LDBD [ph] classes pretty much all day long, except for gym class, and that was really pretty much it. He was in LDBD classes all day. After graduation, Sean tried to continue on and follow Ryan’s example and go to community college, but he could not pass a remedial reading comprehension test. He would study and study and study. He worked hard. And he would read, and we would think he had it. And as Mark—the example Mark provided, he’d go to school the next day and just bomb it, just not a chance.

Sean—as I mentioned, he used to love to read. And one of the perks we gave him for his weekly trips to NYU during the Aldurazyme trial—there was a Barnes & Noble store right around the corner from NYU, and we would always take him as soon as the infusion was done. We'd leave the hospital and go to Barnes & Noble. Sean would pick out a book, and he would pretty much read it cover to cover on the flight home, in a 2-hour flight. It wasn't *War and Peace*, but he loved it. But even that stopped when Sean began to have trouble remembering the stories he had read. He was no longer interested in reading. That was hard to see.

I mentioned earlier Sean's dream of being a racecar driver. Yeah, that's kind of an every-kid dream. You want to be a ballplayer, a racecar driver, sure. So those dreams have subsided. They've been replaced with the dream of simply driving to Walmart. He would be thrilled. After approximately 25 attempts, when he turned 16 to get his—pass a written exam—I've never asked the lady, and I don't know if she would admit it or not.

But Sean would study for that driving test, again, like he did the remedial reading test for college. He would study it so hard. We would go over it with him. We had the guides. And I'm not kidding: It was at least 25 times he went and took that test and never came close to passing. He needed an 80 to pass. Occasionally, he would get in the 60s. You would think after 25 times, he'd have seen every test, and he would remember some of those questions, but he just couldn't. Finally, one—the 26th time, I guess, he passed it. And a lady from our church worked at the DMV, and she administered the test. And I think she had just gotten tired of seeing him there and said, "I'm going to give this kid his permit." I really wish she had not done that. I know he's thrilled, but now he wants to drive every time I turn around.

You know, the Aldurazyme has treated his body well enough that he's physically able to drive. He just—he can't remember the simple instructions that I give him when I do take him out driving. I'll tell him, "Turn your blinker on, but that doesn't mean you have the right of way. You have to let the traffic pass." But he'll—as soon as that blinker goes on, he's ready to go. He's just wanting to cut right in front of traffic. I mean, you can't do that. He just cannot remember those. I'll tell him time and time again, and he just will not remember. He just can't remember.

I've talked a lot about Sean. With respect to Cody, it's difficult to gauge the effect of his decline because of the Asperger's. And I know there's no data to support that that might help. I'm hopeful that it might. That's one of our dreams as well. But we'll see. So I hope, during my talk here, I've demonstrated that we have a suitable treatment for their bodies, even though it is weekly. My hope is still that one day, maybe we could have gene therapy to treat systemic MPS as well. Cody hates—man, he hates needles. Oh my gosh. That's his dream.

So I hope I demonstrated to you that we have a standard of care. But it's not—it's not good enough. It's not what we want. It's not what we need. And I hope that the medical and scientific professionals that have spoken today have shown that the potential benefits are—would be tremendous to our family. So, and I hope you're as excited about that as I am. Thank you very much.