March 6, 2013

Don Kohn, MD
Chair, Recombinant DNA Advisory Committee
Biotechnology Program Advisor
Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750
Bethesda, Maryland 20892

Re: **Protocol 1401-1289, *Phase I/II gene transfer clinical trial of rAAV9.CMV.hNAGLU for Mucopolysacharidosis (MPS) IIIB***

Dear Dr. Kohn,

We wish to thank the members of the Recombinant Advisory Committee for their careful and thoughtful reviews, and for the opportunity to respond to their questions.

We greatly appreciate that the reviewers recognized that mucopolysaccharidosis type IIIB is a devastating and untreatable monogenic illness. We propose treatment with an AAV vector that crosses the blood brain barrier to transfect cells in the central nervous system, with a transgene under the control of the CMV promoter. We are grateful that that they found our approach to be supported by our preclinical efficacy and safety data, and that our proposed trial has modest, safe, and achievable aims.

We respond to the specific questions of each reviewer as follows.

Michael Atkins, MD:

1. **What is the variability of NaGLu levels in the CSF over time in a particular patient? If the answer is undetectable, then this will be no issue. However, if the level is detectable and varies over time, it is possible that changes on a given CSF specimen relative to baseline may be difficult to interpret. Do repeat baseline levels need to be obtained? Does more than 1 follow-up CSF sample within the first 6 months need to be obtained?**

   The variability in CSF NaGlu levels over time in individual patients is unknown. Establishing this variability is one of the goals of our ongoing natural history study, in which we will enroll 15 MPSIIIB patients. Each subject in the natural history study will undergo two lumbar punctures, at enrollment and 12 months later. This will provide intra- and inter-patient data allowing us to assess stability over the year.

Regarding the question of whether more than 1 baseline level needs to be obtained, we do not feel that multiple pre-treatment LPs are justified, for several reasons. First, it is mechanistically unlikely that significant changes in enzyme activity would occur, given that most mutations result in nearly absent enzyme function. Second, data from the non-human primate studies demonstrate that CSF NAGLU rises by fold-change amounts in response to therapy, and variations in baseline CSF levels, if present, would be minor. Finally, additional LPs prior to gene transfer add additional risk without providing additional benefit.

In relation to the question of whether more than one followup CSF sample should be obtained within the first 6 months, we note that within our protocol we actually plan on obtaining LPs at day 30, in addition to 6 months and 12 months, in order to assess safety (including CSF cell count, protein, and opening pressure) and NaGlu expression.

Saswati Chatterjee, PhD:

1. **There are some concerns about the high doses of AAV9 that will be administered systemically in potentially very young patients, particularly in a disease with a lifespan of about 3 decades. Please comment on the justification of the two doses chosen for this study. The differences between the low and high doses are not very marked in the behavioral studies (Figure 9). The mice appeared to perform just as well and perhaps somewhat better at the lower dose as compared to the higher dose in both the water maze and the rotarod tests. Differences in survival were also not marked. Similarly, there did not appear to be a significant difference in the vector genome copy number in the brain between 5e12 and 5e13 vg/kg doses (Table 9), suggesting that this would likely be true of the two proposed doses as well.**

The majority of MPS IIIB patients die in their teens, and few will survive to their third decade. No specific treatment is currently available for this devastating disease.

We wish to point out to the reviewer that data described in pages 55-67 are from experiments treating younger (4-6-week-old) MPS IIIB mice at a pre-symptomatic stage. As the reviewer notes, **Figure 9** describes no significant difference in the water maze and rotarod testing in either of the two doses (5e12 and 1.5e13 vg/kg) used in these younger animals. However, transgene expression levels and genome copy numbers were largely dose-dependent. For example, as noted in **Table 9**, a 3-fold increase in dose (from 5e12 to 1.5e13 vg/kg) leads to a nearly 50% increase in vector genomes in the target tissue (brain) at endpoint (16.1-27.2 months of age).

Our choice of proposed doses was driven by two major factors. First, as seen in **Figure 19**, our preclinical data in MPS IIIB mice demonstrates that 2e13 vg/kg is the minimum effective dose in older animals – specifically, those treated at 4-6 months of age, which is the age at which cognitive defects in the mouse model become measurable. Because the majority of MPSIIIB patients are clinically diagnosed based on cognitive deficiencies, we believe that these

older mice are a better model of the clinical scenario, and we anticipate that a similar dose will be required for a significant therapeutic benefit in patients. Considering the severity of the disease outcome, and the size of the patient population, we cannot justify a sub-therapeutic dose cohort in our trial design. Secondly, the high dose of 5e13 vg/kg is essential to the exploration of a dose range, which is intrinsic to a phase I/II clinical trial. Our pre-clinical data in non-human primates supports the safety of vector delivery at this dose.

2. **How do the two doses compare with heterozygotes? What is the level of NAGLU expression and GAG accumulation at the lower dose (2e13vg/kg) as compared with heterozygotes for MPSIIIB?**

   As in other lysosomal enzyme related diseases and disease models, NAGLU levels in heterozygotes are much lower than in wild type (approximately half), though there is no difference in GAG levels between heterozygotes and wild type. The 2e13vg/kg dose in MPS IIIB mice has yielded NAGLU expression levels above heterozygotes, with the majority of individuals at or above wild type levels (**Table 13**). However, in a corrective therapy, levels higher than those seen in heterozygotes are likely necessary. Although individuals with lifetime expression of this or even lower levels would be expected to be phenotypically normal, neither the mice nor the proposed patient population would be normal at the time of treatment. Both the CNS and somatic tissues will have abundant storage of GAGs, which must be cleared to achieve therapeutic benefit, as well as significant secondary lysosomal dysfunction.

   It must also be kept in mind that not all cells in the CNS will be expressing the recombinant enzyme, and that the majority will be corrected through the bystander effects of secreted enzyme. Thus, the overall expression levels of NAGLU may not accurately reflect the available enzyme for the majority of cells. In any case, the doses proposed are based on our empirical data in mice showing that 2e13 vg/kg is the minimal effective dose at the targeted stages of disease progression.

3. **There are concerns about the overall durability of the treatment and vector persistence. Reductions in NAGLU levels were observed at 12 months post-infusion at the 2e13 vg/kg dose in the brain along with other organs (Figure 21) along with loss of transduction in the liver over time. Is this loss a function of time or the lifespan of the mouse? Have long term studies looking at durability of transduction been done in non-human primates? What are the implications for the durability of the treatment in humans? If there is loss of NAGLU later in life, would disease pathology be attenuated due correction of the defect during early developmental?**

The vector is persistent in the majority of non-dividing tissues, including the brain, until the endpoint of studies in mice (16.1-27.2 months) (**Figure 12; Table 9**). The significant vector loss in the liver is to be expected due to the episomal status of the vast majority of rAAV vector genomes and the turnover of hepatocytes. The durability of the treatment in humans is unclear, because no such information is currently available, but we note the persistence of vector genomes and NAGLU expression in NHP at our longest observation point of 6 months (**Table 15; Figure 29**). As outlined above, there is a rationale wherein the highest levels of enzyme expression may be needed immediately upon treatment to clear accumulated GAG, and lower levels may suffice at later times to maintain clearance. Based on our pre-clinical data, we expect to maintain correction of disease pathology at later time points (**Figure 12a; Figure 14b**). The implication of late loss of NAGLU expression in all tissues is unknown. If that were to happen, we can speculate that disease pathology would probably re-occur, although even this is uncertain because we do not know if these specific GAGs accumulate at constant rates throughout life.

4. **Is anything known about the long-term levels of NAGLU expression required for a cure?**

   We can infer from heterozygote data that levels of approximately one-third to one-half of wild type levels are completely protective (Liem et al, Clinical Genetics, 1976 Nov; 10(5):273-278). However, as noted above, levels of restored enzyme expression may not exactly correlate with the clinical effects of lifelong low level expression. We do note that all FDA-approved recombinant enzyme replacement therapies (ERT) for MPS I, MPS I, MPS VI, Pompe disease, and Gaucher disease require weekly or bi-weekly infusion. This results in very high peak enzyme levels (greatly exceeding normal circulating levels) that decline rapidly following infusion. It is thus very difficult to make an analogy to our gene therapy treatment where enzyme levels may decline very slowly over years.

5. **Is overexpression of NAGLU harmful, particularly since supraphysiologic levels were observed in some cases?**

   We found no evidence that overexpression of NAGLU is harmful. We did not observe any adverse events in our pre-clinical studies in either MPS IIIB mice or in NHP, both of which expressed supraphysiologic levels. Weekly and monthly hematology (**Figure 27**) and blood chemistry (**Figure 28**) studies in NHP did not show abnormal responses to treatment for up to 6 months, and histopathology did not show any treatment-related abnormalities. This safety profile is bolstered by data from enzyme replacement therapies in similar lysosomal storage diseases where peak enzyme levels may be thousands of times above normal without adverse consequences.

6. **Could the immune responses seen against NAGLU in non-human primates (Figure 30) precipitate any autoimmune responses?**

Although an antibody (Ab) response to human NAGLU in NHP was demonstrated, there was no evidence of autoimmune pathology in any tissues examined up to 6 months post-injection. Furthermore, MPS IIIB mice – which are null for NAGLU – did not show evidence of immune-mediated clearance of transduced cells over 2 years, despite demonstrating an Ab response. In addition, ELISPOT did not show cytotoxic T lymphocyte (CTL) responses to either AAV9 or hNAGLU in NHPs, and only a weak Th-1 response to one hNAGLU peptide (aa134-151) at 2wk post-infection in a single NHP (page 86, paragraph 2). Nevertheless, although we feel that an autoimmune response is unlikely, treated patients will be monitored for the development of NAGLU and rAAV9 CTL, as well as for signs and symptoms of autoimmune reactions.

7. **It is important that precautions be in place to avoid inadvertent transduction of caregivers. Although AAV is non-pathogenic and has an excellent safety record, the use of high doses of vector, particularly in very young subjects may lead to shedding and possible bystander transduction. Given the wide tissue tropism of AAV9, this is significant.**

   In mice, AAV9 persists in the blood for longer than most AAV serotypes, with a half-life in circulation of approximately four hours over the first 10 hours post-injection, and approximately 10 hours over the next 3 days post-infection (Kotchey et.al., Mol Ther. 2011 Jun;19(6):1079-89). If this is similar in humans, we expect to go through approximately 9-10 half-lives of vector in circulation over 3 days, during which time the patients can be maintained in the hospital under contact precautions as we previously described under Appendix M-II-B-4-c. No information is currently available regarding human bystander transduction by vector shedding.

<u>Norman Fost, MD, MPH:</u>

1. **Lumbar punctures.**

   Four LPs are planned (at baseline, 1 month, 6 mos, and 12 mos) to assess for enzyme levels and signs of an inflammatory response. Please clarify the scientific rationale for the 6 mos LP. What will be learned from this sample that will not be learned from the 1 month and 12 mos samples?

   This is a phase I trial to assess safety. The LP at 6 months is required to monitor for signs of CNS inflammation or immune response. In addition, this LP will provide an additional data point for CSF NaGLU enzyme activity and GAG levels between early (1 month) and late (12 month) assessments.

2. **Data Safety Monitoring Board. Consider including someone experienced in clinical ethics, preferably research ethics, as a member. This could fulfill the area of expertise described in your 5th bullet at 6.3.3. of the Clinical Protocol; i.e., "Clinical research and clinical trials."**

We intend to include individuals with experience in clinical research and clinical trials in the DSMB. However, it is our practice to rely on our Institutional Review Board to provide a review of the clinical and research ethics related to the study. At least one member of our institution's IRB is an ethicist who participates in full committee reviews, and the IRB receives all DSMB reports and correspondence with the DSMB as part of their ongoing review of the study.

3. **Consent Form**
   a. **Page 1: Introduction**
      i. **The term "treatment" should generally be avoided in Phase-1 studies as it suggests there is a known benefit. Suggest substituting a more descriptive term, such as "intervention" or "gene product."**

We concur that "treatment" might be construed to imply the existence of a known benefit. We have modified the first use of "treatment" to read "potential therapy", and have struck the second reference to "treatment" entirely.

      ii. **Suggest stating in the first or second paragraph that the main purpose of this study is to learn about the safety of the new product, and that clinical benefit is unknown and unlikely (the vast majority of Phase-1 studies do not lead to clinical benefits, and the batting average is worse for gene transfer studies).**

We have modified the introduction to include the following sentence: "The purpose of this study is to learn about the safety of the gene vector, and whether it may have a clinical benefit is unknown." However, although we do not claim a clinical benefit is likely, our clinical equipoise leads us to decline to state that a clinical benefit is *unlikely.* That the majority of Phase-1 studies do not lead to clinical benefits is not germane to the likelihood of *this* study having benefit. In fact, given that the disorder is monogenic, and that non-human primate studies show we can replace the single missing gene at doses to be used in this study, it is reasonable to conclude that there *might* be a benefit to the subject. In fact (as discussed in more detail under question 5, below), if there were no potential for benefit, under 45 CFR 46.405 and 46.406 we would not be able to perform the study in the proposed population.

   b. **Page 2: Why are we doing this research study?**

At the end of the first paragraph you say (italics added):

Scientists have *made a way* to take a copy of the NAGLU gene and get it into cells in the body *so the cells are able to make NAGLU enzyme that works properly and can break down the GAGs that cause MPSIIIB. This special treatment* is called "gene transfer".

This sounds like a report of more knowledge and optimism than is available at this time. Perhaps you are referring to the mouse studies, which are already described in the next paragraph.

If it is intended to refer to what is known about humans with this disorder, suggest something more like, "Scientists *hope to be able* to take a copy of the NAGLU gene, and get it into cells in the body, so the cells are able to make NAGLU enzyme that works properly and can break down the GAGS that cause MPSIIIb. *This approach is called "gene transfer."*

We have modified the language accordingly.

4. **Page 4: Day 7 and 30 (Visits 3 and 4).**

   You state the lumbar puncture at this visit will be under anesthesia, but no MRI is planned for this visit, which was the reason for anesthesia at the other visits. Will you be using anesthesia just for the LP?

   Many MPSIIIB patients exhibit impulsive behaviors that will prevent LPs being performed under local anesthetic alone. We will therefore perform them using sedation delivered under the care of anesthesiologists, who will determine the appropriate level of sedation for the subject. Based upon our experience to date in our ongoing natural history study, this may consist of minimal propofol sedation intravenously.

5. **Page 11: Are there benefits to taking part in this study?**

   Optimism about benefits are understandable, and the study would obviously not be considered without such optimism, but the fact is that clinical benefit is extremely unusual in Phase-1 studies in general, and even less so in gene therapy studies. Therefore, while it is appropriate to say, as you do, that "it is not known if any direct benefit from this vector is possible in humans," a more accurate statement, and one that is more consistent with NIH guidelines on this topic, would say something more like, "Direct medical benefit is not expected from this study (or from the research at this stage) and is unlikely." (Ref: http://oba.od.nih.gov/oba/rac/ic/pdfs/temp_pdf.pdf)

   We acknowledge and agree with the reviewer that there is a delicate balance in introducing the idea of possible benefits into a phase 1 trial. We agree that the likelihood of a clinical benefit is unknown, but to state that no direct benefit is anticipated would not be consistent with the evidence shown in preclinical studies where MPSIIIB mice dosed with the study vector had prolonged survival. Furthermore, it would be inconsistent with other phase 1 gene transfer trials that have been afforded the same language of "potential for direct benefit" based solely on preclinical studies (see RAC Protocol # 1210-1188, for example). Finally, a statement of no direct benefit, if true, would then prohibit the study under 45 CFR 46.405 and 46.406 for the proposed patient population (see RAC review comments to Protocol #1301-1200, for example). Therefore we request to keep this language unchanged as provided.

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6. **Page 14: People … authorized to use, disclose and receive PHI**

   The list is overly inclusive. The IRB, OHRP, DSMB and the FDA rarely if ever need identifiable PHI.

   We concur that the need for any of the listed institutions to receive identifiable PHI is rare and typically not necessary. However, the language used is required by our institution's Institutional Review Board as part of our hospital's policy for when such a rare event occurs and we are required to release PHI to any governing parties. Such event includes any possible audits or possible reporting of serious life-threatening events. It is our IRB's policy to have the rare situations covered should such an event arise.

7. **Page 15: Use of samples and data for research.**

   Suggest adding a third choice offering the participants (or their representatives) the option of asking that they be informed of specific future research studies as they arise, so they can decide at that time whether or not they want to participate.

   We appreciate this suggestion. However, it will be impractical to re-consent patients for each potential future study for which their de-identified samples might be used, and have therefore declined to add a third choice.