U.S. FOOD & DRUG ADMINISTRATION

Preliminary Meeting Responses

Our Reference: PTS# PS008879/4 Meeting ID: 21494

DATE: July 22, 2025 **PAGES:** # 15

TO: Rebecca Ahrens-Nicklas, MD, PhD
The Children's Hospital of Philadelphia (CHOP)
3501 Civic Center Blvd
Philadelphia, PA. 19104
[redacted]

FROM: [redacted]

SUBJECT: Type B, pre-IND meeting to obtain specific feedback as to whether the proposed studies are adequate to support a study of LNP1.PAH.ABE1 in adults and adolescents with PKU

PRODUCT: Lipid nanoparticle (LNP) based editing therapeutic comprising lipid excipients identical between the two DPs, with a messenger RNA (mRNA) drug substance (DS) encoding an adenine base editor 8.8-m (ABE8.8) variant that is >99% identical between the two DPs, and a single guide RNA (gRNA) drug substance (DS) that is >80% identical between the two DPs. / Product Name: CHOP- LNP.PKU.P281L, CHOP- LNP.PKU.R408W

PROPOSED INDICATION: Treatment of adolescents and adults with phenylketonuria (PKU) who are homozygous or compound heterozygous for a variant in the phenylalanine hydroxylase (*PAH*) gene that can be efficiently corrected by an adenine base editor (ABE) with an ABE8.8-m TadA deaminase, such as c.842C>T, c.1222C>T, [redacted], [redacted], [redacted] and [redacted].

FDA Participants:
[redacted]

This material consists of our preliminary meeting responses to your questions and any additional comments in preparation for the discussion at the meeting scheduled for July 24, 2025. We are sharing this material to promote a collaborative and successful discussion at the meeting.

Although we continue to reserve July 24, 2025 at 9:00 AM, with you regarding this product, if you find that our attached responses and advice are sufficiently clear and complete to obviate the need for further discussion, please inform us in writing as soon as possible, and 1 calendar day from the date of receipt of FDA's Preliminary Responses, so that we may clear the meeting time. These responses would then become the official FDA responses to your questions.

If you determine that discussion is needed for only some of the original questions, you have the option of reducing the agenda and/or changing the format of the meeting from Face-to-Face (virtual) to teleconference. If you have questions regarding specific responses or advice included in this preliminary response, please inform the RPM so that the appropriate members of the Review Committee can provide clarification during the reserved meeting time. Please refer to the *Respond to Meeting Request-Granted* communication you received for details about your scheduled meeting.

Please be aware that your future submission should include all components for a complete submission and should be in compliance with all appropriate statutes and regulations. For input on additional issues that were not posed in your meeting package or addressed in our preliminary meeting responses, you may submit a new meeting or a WRO request, as we may not be prepared to discuss or reach agreement on new topics at the meeting.

Please include a reference to PTS# PS008879 or Meeting ID# 21494 in your future submissions related to this product.

Preliminary Meeting Responses

Pharmacology/Toxicology

Question 1:
Does the Agency agree that the completed proof-of-concept (POC) studies of a nonclinical precursor LNP test article, with an identical mRNA (ABE8.8) and gRNA (PAH- 001) to those being used in one version of the clinical LNP1.PAH.ABE1 DP, in a mouse disease model with one PAH variant (PAH^{P281L}/PAH^{P281L})—documenting treatment-related effects on blood amino acid levels, PKU-related neurobehavioral changes, and biochemical changes in the brain—provide sufficient data to support an IND application for the administration of any of the six versions of the LNP1.PAH.ABE1 DP to PKU patients?

FDA Response: Based on the nonclinical information provided in Sections 6, 9, and 10 (pages 16-37, 38, and 39-56, respectively), we agree that completed POC studies documenting neurobehavioral analyses (hindlimb clasping, rotarod, elevated zero maze, and open field test) and biochemical changes in the brain provide sufficient data to support your IND. These endpoints do not need to be included in your proposed definitive Good Laboratory Practice (GLP)-like POC study (pages 41-43). [redacted] We have the following comments regarding the planned study:

1. Please quantify plasma Phe and Tyr at multiple time points throughout your proposed definitive POC study to correlate blood Phe and Tyr levels to neurobehavioral outcomes across studies.

2. [redacted] Per the definitive POC study experimental design described on page 42, editing of *PAH* in liver only and multiple tissues (e.g. liver, spleen, adrenal gland, testes/sperm, and ovary/oocytes) will be assessed at the Week 9 and Week 19 sacrifice time points, respectively.

    a. Your definitive POC study should permit assessment of the LNP1.PAH.ABE1 minimum effective dose level (as demonstrated via percentage on-target *PAH* editing in liver, plasma Phe level, etc.) and inform the clinical trial design. [redacted]

b. We recommend that you evaluate *PAH* editing in all tissues positive for the lipids or gRNA/editor. Please provide justification for all tissues selected.

Question 2:
Does the Agency agree that the proposed definitive pharmacology/POC mouse study of the LNP1.PAH.ABE1 DP, with both ABE8.8/PAH-001 or SpRY-ABE8.8/PAH-002, in a compound heterozygous mouse disease model (PAH^{P281L}/PAH^{R408W}) will provide sufficient data to support an IND application for all six versions of the LNP1.PAH.ABE1 DP?

FDA Response:
We agree that your proposed definitive POC study to be conducted in compound heterozygous (PAH^{P281L}/PAH^{R408W}) mice with ABE8.8/PAH-001 and SpRY-ABE8.8/PAH-002 appears adequate to support product activity for all six variants of LNP1.PAH.ABE1.

Question 3:
Does the Agency agree that the proposed definitive toxicology rodent study of the LNP1.PAH.ABE1 DP, with both ABE8.8/PAH-001 or SpRY-ABE8.8/PAH-002, in wildtype rats will provide sufficient data to support an IND application for all six versions of the LNP1.PAH.ABE1 DP?

FDA Response: To support administration of six versions of LNP1.PAH.ABE1 and to enhance the judicious use of animals, we recommend that you administer one LNP1.PAH.ABE1 variant to a single species (either wild type rat or non-human primate [NHP]). We defer to you on the appropriate species for study conduct. In your IND, please provide your rationale for the species selected. This study should be comprehensive and include the following elements in your IND:

1. Ideally all safety studies should be carried out in compliance with GLP as per 21 CFR Part 58. If technical limitations do not allow for this, it is acceptable to perform the study in a non-GLP testing facility. However, the study should: a) be conducted according to a prospectively written protocol, b) be performed in as nonbiased a manner as possible, c) have appropriate record keeping and documentation of all data, and d) include Quality Assurance measures such that we can be confident that the resulting data are of sufficient quality and integrity to support the proposed clinical trial. In addition, as directed by 21 CFR Part 312.23(a)(8)(iii), the final study report should state why the study was not conducted in compliance with GLP and specify any areas that deviate from the prospectively written protocol and the potential impact of these deviations on study integrity.

2. Assessment of safety endpoints to include daily clinical observations, body weights, clinical pathology parameters (hematology, serum chemistry, and coagulation), immunogenicity (anti-PEG and anti-ABE antibodies), complete

macroscopic exams, organ weights (brain, heart, lungs, spleen, liver, adrenal glands, kidneys and gonads), and histopathology of a comprehensive set of tissues. All in-life parameters should be evaluated at baseline and at several time points post-LNP1.PAH.ABE1 injection for all surviving animals at the specified time points. Interim and terminal sacrifice time points should reflect peak of product activity/expression and the durability of LNP1.PAH.ABE1 action determined in POC studies. Please provide the rationale for all selected sacrifice timepoints.

3. Evaluate the biodistribution (BD) of LNP1.PAH.ABE1 (lipid excipients) and ABE expression in a comprehensive panel of tissues (including blood) at sacrifice. For all samples that are positive for lipids, ABE expression should also be measured. If a particular tissue is negative for lipids at a specific time point, then that respective tissue does not need to be analyzed at later time points. If a particular tissue is determined to be negative for lipids, then that respective tissue does not need to be analyzed for ABE expression. However, all tissues, whether analyzed or not, should be archived for possible future analysis.

4. We recommend that you incorporate central nervous system, respiratory, and cardiovascular safety pharmacology endpoints into your study, if lipids or ABE expression is detected in heart, lung, and/or brain.

5. If you plan to use NHPs, please provide your rationale for the 'pre-dose' regimen of famotidine, diphenhydramine, and dexamethasone relative to the methylprednisolone immunosuppression regimen included in the proposed clinical trial protocol.

6. For all unscheduled deaths, please perform comprehensive clinical pathology, gross pathology, and histopathology on a complete panel of tissues to determine the potential cause of death.

Question 4:
Does the Agency agree that the proposed definitive biodistribution/toxicology nonhuman primate (NHP) study of the LNP1.PAH.ABE1 DP, with ABE8.8/PAH-001 only, in wild-type NHPs will provide sufficient data to support an IND application for all six versions of the LNP1.PAH.ABE1 DP?

FDA Response: Please see our Comment Nos. 1 to 6 in response to your Question No. 3.

Question 5:
Does the Agency agree that the proposed studies of the LNP1.PAH.ABE1 DP in rodents and NHPs will provide sufficient data to support re-dosing of patients with the LNP1.PAH.ABE1 DP?

FDA Response: We agree that the proposed definitive POC and safety/BD studies should be adequate to support repeat intravenous (IV) administration of LNP1.PAH.ABE1 in the proposed clinical trial.

Question 6:
Does the Agency agree that the proposed off-target editing studies of all six versions of the LNP1.PAH.ABE1 DP will provide sufficient data to support an IND application for the administration of any of the six versions of the LNP1.PAH.ABE1 DP to PKU patients?

FDA Response:

Pharmacology/Toxicology

1. We agree that the proposed cell types (HuH-7, primary human hepatocytes, and cultured or primary human cells nominated [via BD data] from the proposed, definitive POC and safety studies) are appropriate for the assessment of off-target editing for the administration of any of the six versions of the LNP1.PAH.ABE1 DP.

Bioinformatics

Your proposed strategy for off-target editing study is generally acceptable. However, we require additional information and supporting data to complete our evaluation of your methods. Please include the following information in your future IND submission:

1. A comprehensive off-target analysis report encompassing both guide RNA (gRNA)-dependent and gRNA-independent editing events at the DNA and RNA levels.
    a. For off-target nomination and screening, the report should include a detailed description of each method, and a list of all off-target loci nominated from each method with detailed annotation information for each site. Specifically, please state whether an off-target site is intergenic, intronic, exonic, or impacts splicing. For an exonic site, please indicate the impact on the amino acid sequence. If a subset of nominated off-target sites was selected for confirmatory testing, please provide the justification for the selection. All information regarding off-target loci should be provided in Microsoft Excel workbooks.

    b. For each validated off-target site, please conduct a comprehensive literature search on the potential functional consequences of the editing and provide a discussion on how these edits may impact product safety. This may include information regarding any potential risks associated with alterations to the encoded protein and known mutations in the genes and associated

functional outcomes. Please also provide confirmation of pseudogenes using publicly available bioinformatic resources.

c. For the list of differentially expressed genes (DEGs) identified via RNA-seq, please incorporate pathway and gene ontology (GO) enrichment analysis into your transcriptomic data evaluation. This analysis would help reveal whether specific biological processes, cellular pathways, or regulatory networks are disproportionately affected, and identify whether any of the enriched pathways involve key liver function genes, tumor suppressors, proto-oncogenes, or developmental pathways, which could raise concerns for genotoxicity, tumorigenicity, or off-target physiological effects. Please clearly state the statistical criteria used and include enrichment tables in your submission.

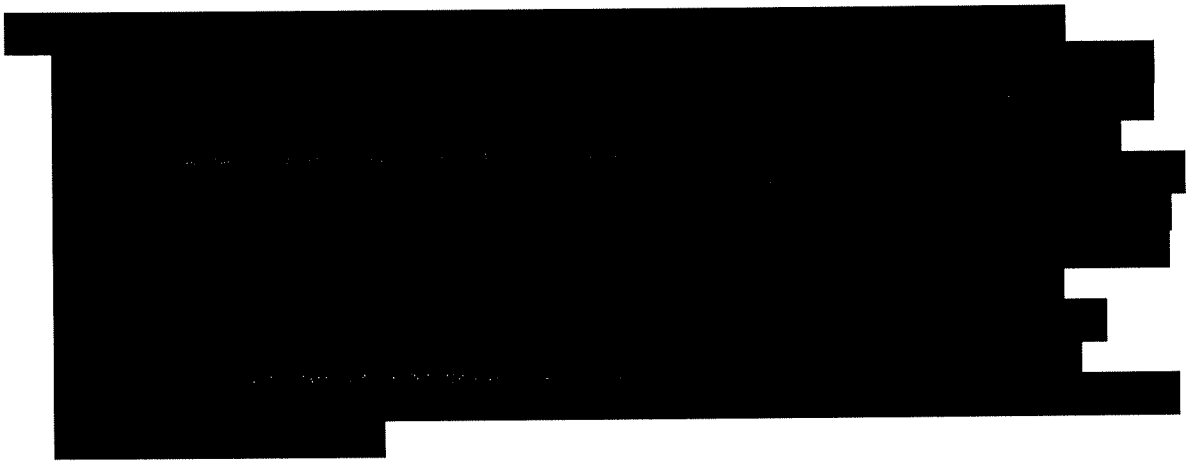
2. On page 54, you propose to assess LNP1.PAH.ABE1 off-target editing in HuH-7 cells, PHHs, and nominated primary human cells using a 'supersaturating' dose level (20x EC90). We acknowledge the rationale behind using a high dose of the gene-editing agent to maximize the likelihood of detecting rare or low-frequency off-target events. This approach is commonly employed during early-stage off-target risk profiling and can be useful in nominating candidate off-target sites. However, it is critical to contextualize findings by including testing at clinically relevant doses to assess whether off-target edits identified at high dose are still observed at expected therapeutic exposures. Please include sequencing-based analysis of the nominated off-target sites in PHHs edited with lower concentrations of LNP1.PAH.ABE1 and report the on- and off-target editing rates.

3. For all NGS-based methods used in your studies, please ensure the read depths are adequate for detecting off-target events and provide a comprehensive report on how the sequencing data was generated, how many samples or technical replicates were used, what metrics were used to assess the sequencing quality, and what bioinformatics analysis tools were used to process the data. We have provided a Microsoft Excel workbook (***NGS_qc+metadata_v0.3.xlsx***) as a convenience to you which contains generally accepted QC metrics for assessing NGS data quality. You should include any additional appropriate QC metrics to allow us to evaluate your sequencing quality more effectively.

4. A list of software tools used in your studies with detailed information such as the version of the tool, licensing information, publication information, repository information, OS/hardware system requirements, and command line interface (CLI) information. We have provided a Microsoft Excel workbook (***Software_tools_and_CLI_v0.3.xlsx***) as a convenience to you. You should provide software information used in each computational step of your data analysis in the worksheets of *Software_tools, Computational_steps* and *Annotation_resource*. This workbook does not contain an exhaustive list of important software attributes. You should include any additional information that would allow us to evaluate your bioinformatics methods effectively.

Question 7:
Does the Agency agree that the overall nonclinical development plan is sufficient to support an IND application for all six versions of the LNP1.PAH.ABE1 DP?

FDA Response: We cannot yet agree that the overall nonclinical development plan is adequate to support first-in-human, repeat administration of LNP1.PAH.ABE1 to [redacted] subjects with Phenylketonuria. In addition to the studies proposed, please address the following comments:



2. Provide a tabulated summary of the similarities and differences between the nonclinical product(s) administered in all completed nonclinical studies, including the products administered in the completed studies described on pages 30-37, and each variant of your intended clinical product (LNP1.PAH.ABE1). This summary should include physiochemical characteristics, including the lipid composition, total RNA content and ratio, RNA purity, encapsulation efficiency, zeta potential, particle size, and polydispersity index, guide ribonucleic acids (gRNAs), ABEs, manufacturing process, and major lot release criteria. For any differences identified, please discuss their potential impact on translatability of the nonclinical data to the proposed clinical trial.

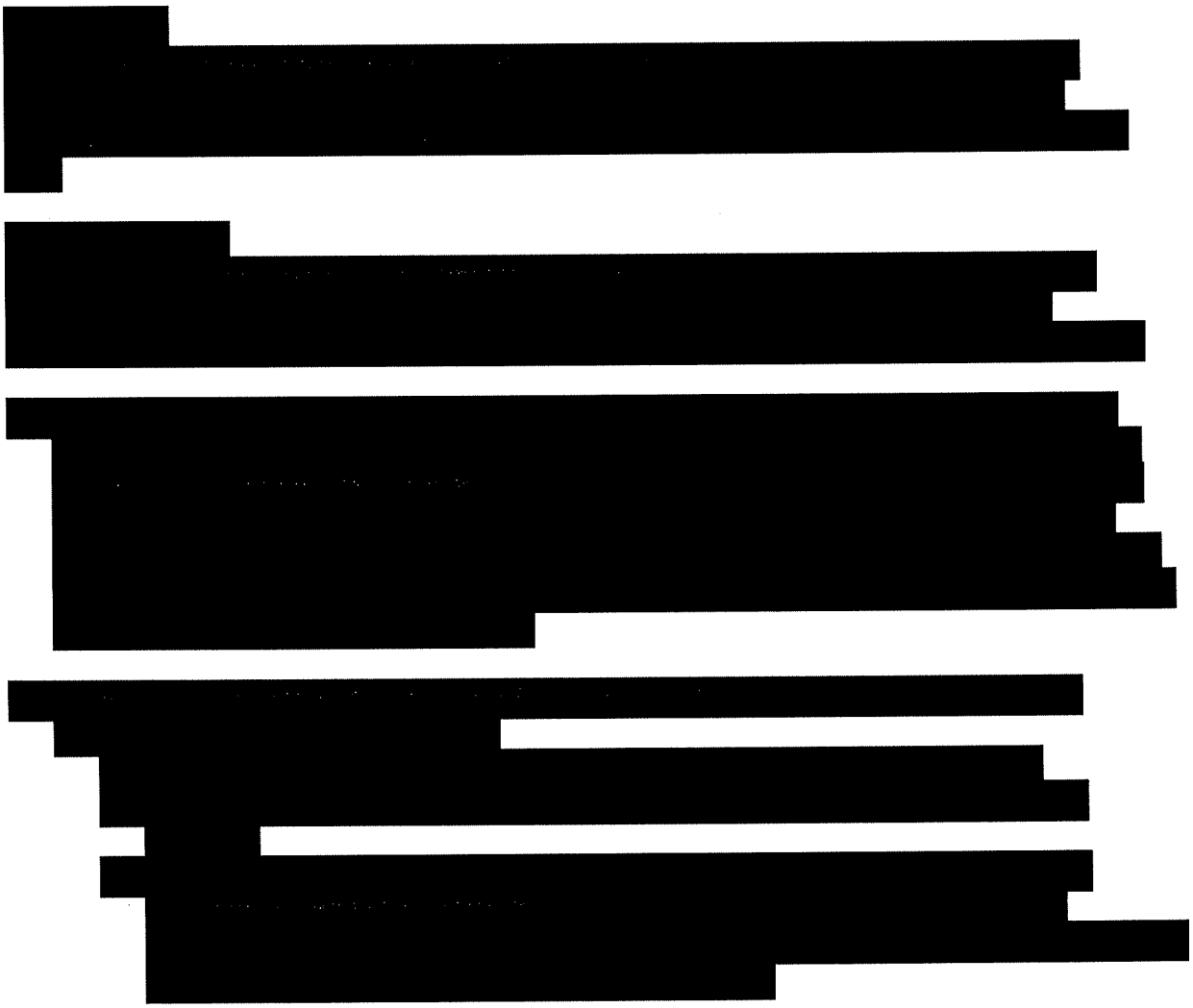
3. Provide your rationale for the clinical dose levels selected and repeat administration for each variant of LNP1.PAH.ABE1. This discussion should include data demonstrating the a) in vitro *PAH* editing efficiency of each variant of your intended clinical product and b) association between *PAH* editing efficiency and dose levels selected for each LNP1.PAH.ABE1 variant.

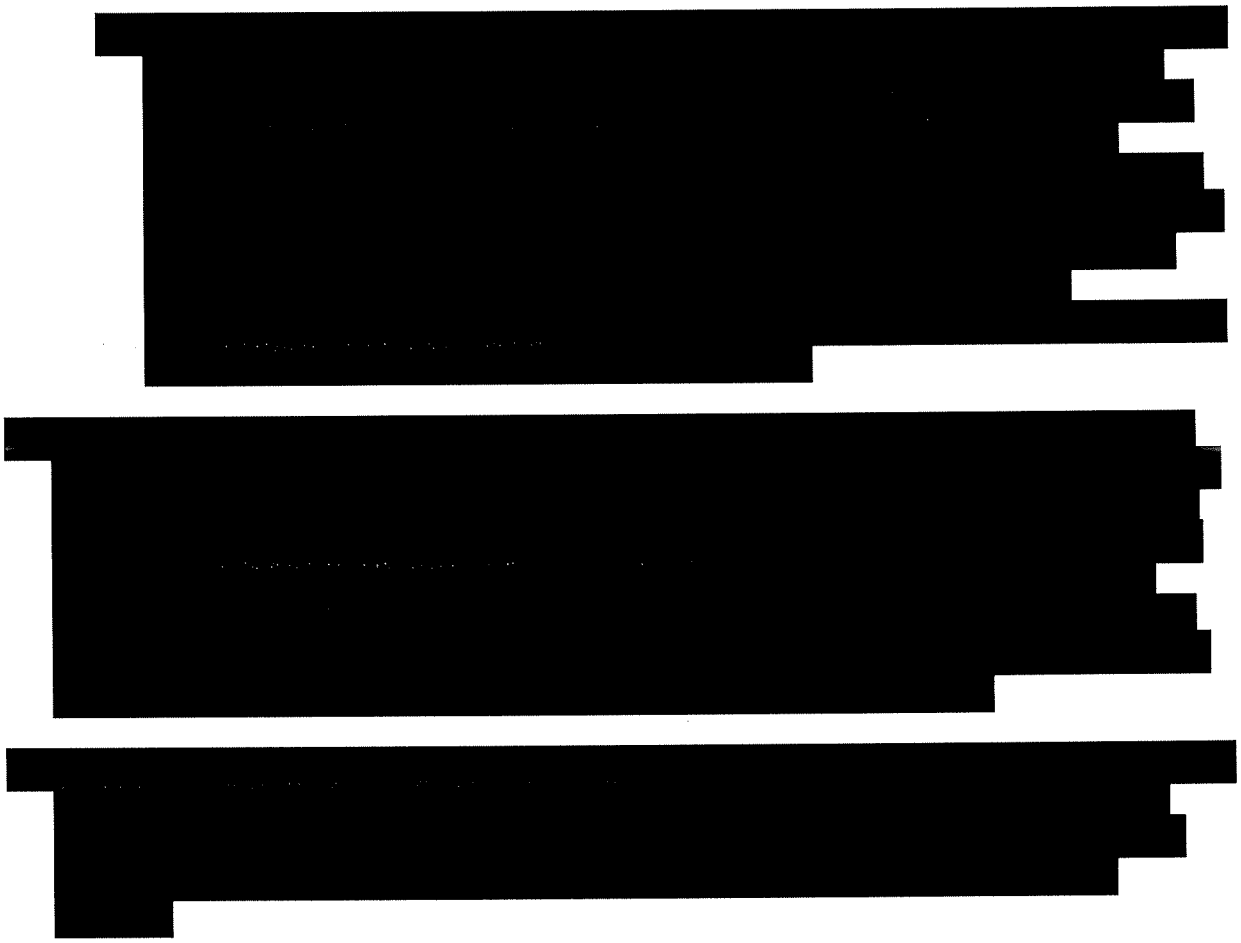
4. Provide your method of dose level extrapolation from animal to human and the rationale, with supporting data and/or publications, for this method.

5. Provide a detailed methodology for all neurobehavioral tests conducted and verification of the objective and stringent nature of these analyses (masked assessors, appropriate controls, etc.) of the testing procedure and resulting data interpretation.

6. Provide a discussion, with supporting data, on the potential for immunogenicity to the adenine base editor (ABE) protein due to repeat dosing of LNP1.PAH.ABE1 that may reduce the editing efficiency and activity of LNP1.PAH.ABE1.

7. Provide data from bench testing that confirm the compatibility of the nonclinical lots of LNP1.PAH.ABE1 (one variant) with the needle/syringe system used in each nonclinical study. Your evaluation should include the ability to consistently deliver accurate pre-specified dose levels of LNP1.PAH.ABE1. This can consist of assessment of LNP concentration after passage of each lot through the respective needle/syringe delivery system used in each study.





Question 9:
Does the Agency agree that the proposed potency assay for the LNP1.PAH.ABE1 DP is acceptable to support an IND application for all six versions of the LNP1.PAH.ABE1 DP?

FDA Response:
We agree that the proposed relative potency assay measuring editing efficiency in a lentivirus-transduced Huh-7 cell line is acceptable to support the proposed IND for all six variations of the LNP1.PAH.ABE1 DP. However, we do not agree with your proposal to use a Cas9 ELISA as a potency assay while completing your development of the relative potency assay. As described in the FDA guidance for industry, "Human Gene Therapy Products Incorporating Human Genome Editing", potency assays evaluating the ability of the GE components to perform the desired genetic sequence modification is recommended to support early phase clinical studies. While the Cas9 ELISA does measure some activity of the mRNA component of the DP it does not evaluate any activity of the gRNA. We recommend that you use the lentivirus-transduced Huh-7 cell line editing efficiency method to support initiation of the IND. It would be acceptable to use this assay with a minimum threshold of editing efficiency as the acceptance criterion while you continue to develop and qualify the assay as a quantitative relative potency assay.

Question 10:
Does the Agency agree that the general design, including the proposed safety and exploratory efficacy outcome measures, enrollment criteria, and long-term follow-up plan are appropriate for the Phase I/II umbrella trial protocol outlined in the protocol synopsis?

FDA Response:
Yes, we generally agree that the overall design including safety and efficacy outcome measures, enrollment criteria and long-term follow-up plan are appropriate for your proposed Phase I/II umbrella trial protocol. We have the following comments for you to address in your future IND submission:

[redacted]

Additional FDA Questions/Comments:

Chemistry, Manufacturing, and Controls

1. As product development continues, we recommend that you develop assays to measure the activity of the gRNA and mRNA and add these to the release specifications for each DS.

2. Information for the lipid components of the DP should be provided in eCTD section 3.2.P.4 Control of Excipients. Please note that the lipid components in the LNP are an integral part of the product due to their essential role in the delivery of the RNA payload to target cells. Any changes to these components could have an effect on the quality and performance, and therefore, safety and efficacy of the product. Therefore, the lipid components of LNPs are considered critical components that should be fully characterized, and relevant CMC information should be submitted in the IND. For more common lipid excipients, such as cholesterol, vendor and grade information and a certificate of analysis are sufficient for the IND. Novel lipids generally require additional CMC information to be provided in the IND to assure quality and safety (with a similar level of detail and a similar CTD organization as for a drug substance). Complete CMC information for each novel lipid may be provided in a separate section of the appendix, 3.2.A.3 Excipients. Please provide the following information for each novel lipid.
    a. The name and address of the lipid manufacturer.
    b. A certificate of analysis (CoA) for the lipid. If documentation for a lipid excipient is incomplete, testing for the incomplete attribute(s) of the lipid excipient should be performed.
    c. The full molecular structure of the lipid.
    d. A narrative description and flow diagram of the manufacturing process, including in process tests and controls.
    e. A description of all analytical methods used during lot release.
    f. A justification for acceptance criteria in the lot release specification.
    g. Stability study data and conclusions.
    h. The container closure system.
    i. A description of any reference standards used for testing.
    j. Please note that some or all of this information can be provided by cross reference to a master file (MF), if applicable. Please provide a signed letter of authorization from the MF holder detailing what information can be cross referenced.

Pharmacology/Toxicology

3. Regarding biodistribution assessment for gene therapy products, please refer to the document titled, *S12 Nonclinical Biodistribution Considerations for Gene Therapy Products: Guidance for Industry* (May 2023), available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s12-nonclinical-biodistribution-considerations-gene-therapy-products.

   For additional guidance regarding the nonclinical assessment of cell and gene therapy products, GLP testing requirements, and the contents of nonclinical study reports, please refer to: a) the document titled, *Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products (November 2013),* available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/preclinical-assessment-investigational-cellular-and-gene-therapy-products.
4. For a comprehensive summary regarding the nonclinical assessment of gene therapy products that incorporate genome editing, please refer to the document titled, *Human Gene Therapy Products Incorporating Human Genome Editing (January 2024),* available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-products-incorporating-human-genome-editing.

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