FDA U.S. FOOD & DRUG ADMINISTRATION

Preliminary Meeting Responses

Our Reference: PS009898 and Meeting ID 21202

DATE: April 25, 2025 **PAGES:** # 11

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POINT OF CONTACT:
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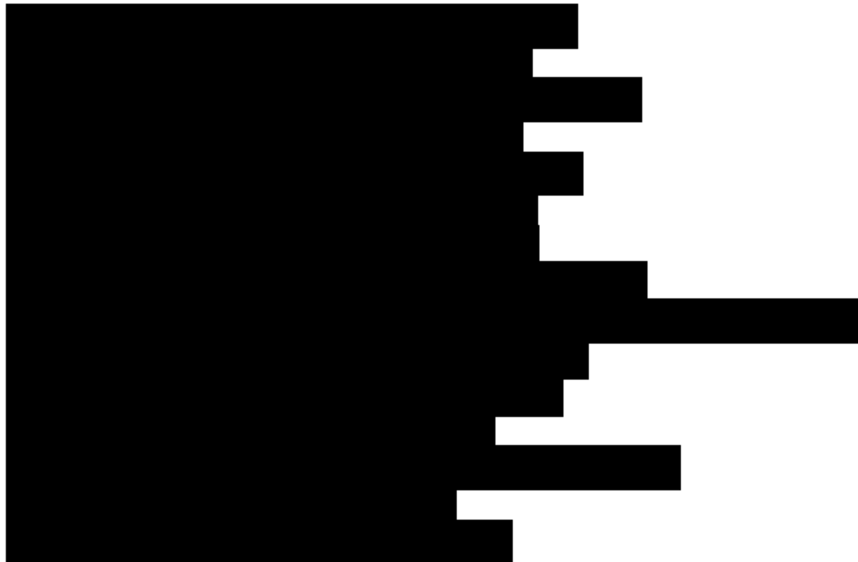
Kenisha Atkins -S Digitally signed by Kenisha Atkins -S Date: 2025.04.25 15:24:06 -04'00'

SUBJECT: INTERACT Meeting to seek early input from the FDA on the proposed chemistry, manufacturing and controls (CMC) and nonclinical development program for UW-LNP-ABE8E to support a first-in-human clinical trial (FIH) in patients with non-end stage LCA16 carrying a pathogenic W53X or T153I mutation. In addition, the Sponsor seeks input from the Agency on the proposed use of a platform technology approach to treating a single disease indication with unique drug products that differ only in the sequence of their sgRNA and a nominally altered Cas homolog.

PRODUCT: Lipid nanoparticle complex (LNP) containing messenger RNA (mRNA) encoding for Adenine Base Editor 8E (ABE8E) and single guide RNA (sgRNA) targeting the pathogenic W53X or T153I mutation in KCNJ13 / UW-LNP-ABE8 E

PROPOSED INDICATION: Treatment of non-end stage Leber Congenital Amaurosis 16 (LCA16), carrying a pathogenic W53X or T153I mutation

FDA Participants:



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 May 2, 2025. We are sharing this material to promote a collaborative and successful discussion at the meeting.

Although we continue to reserve May 2, 2025; 2:00PM – 3:00PM EST, 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 no later than 3 calendar days 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 PS009898 and Meeting ID 21202 in your future submissions related to this product.

Preliminary Meeting Responses

Question 1:
Does the Agency agree that the used in the manufacturing process is of sufficient quality for a First in Human (FIH) Trial?

FDA Response to Question 1:
We cannot yet agree that the is of sufficient quality to initiate the proposed clinical trial. Please be aware that novel lipids generally require additional CMC information be provided in the IND submission to assure quality and safety (similar to a drug substance). As such, we have the following comments, which you may wish to address in the preIND meeting packa
e to facilitate further discussion on this topic.

1. We recommend that you include tests for process related residual impurities such as solvents and elemental impurities.

2. Please include a more detailed description of the manufacturing process, including a description of what impurities may be present in the final lipid preparation.

3. Please be aware that it may not be necessary to test with the described bioassay during lot release provided there is suitable testing for purity and safety.

Question 2:
Does the Agency agree that the excipients that make up the LNP, other than , proposed for use in the manufacturing process are of sufficient quality for a FIH Trial

FDA Response to Question 2:
We agree that the lipid excipients (other than) are of sufficient quality to initiate the proposed clinical trial. We have the following additional advice.

1. Please be aware the should be compendial grade.

2. For the lipid excipients you should provide a Certificate of Analysis (CoA) or other documentation that describes the source and quality of the lipid excipient. The CoA or other documentation should be examined to ensure that it meets your established acceptance criteria for the specified attributes. If documentation for a lipid excipient is incomplete, testing for the incomplete attribute(s) of the lipid excipient should be performed. If the vendor of the material has a regulatory master file (MF) with the FDA, a signed letter authorizing you to cross-reference the information may be provided in the IND submission.

Question 3:
Does the Agency agree that the proposed characterization and release tests are appropriate to control the quality attributes of the excipients, DS and DP for FIH trial?

FDA Response to Question 3:
Please be aware that for clinical development programs with a very limited number of patients, such as those for ultra rare diseases, it is often necessary to leverage efficacy data from early phase clinical studies to support product licensure. Please be aware this may require you to expedite yo
r CMC development program.

We do not agree with your proposed testing plans for the gRNA drug substance (DS), mRNA DS, or UW-LNP-ABE8E drug product (DP). For information on testing of the lipid excipients, please refer to our responses to Questions 1 and 2. We have the following comments regarding testing which you may wish to address in your preIND meeting package.

1. Please provide complete specifications for your mRNA DS, gRNA DS, and UW-LNP-ABE8E DP. These specifications should include a table that details the attributes being tested, analytical methods used to assess each attribute, and acceptance criteria (AC) for lot release.

2. Based on the information provided in Table 12 of the meeting package, it is unclear which testing is performed as part of mRNA DS characterization and which tests will be used for lot release. We recommend your mRNA DS lot release specification include testing for the following attributes:
    a. appearance
    b. identity
    c. confirmation of mRNA sequence
    d. concentration
    e. mRNA purity
    f. mRNA capping efficiency
    g. mRNA polyA tail integrity
    h. bioburden
    i. endotoxin
    j. process and product related residual impurities.

3. We recommend that you include tests for the following attributes in your gRNA DS lot release specification.
    a. Solvent impurities, as appropriate
    b. Elemental impurities, as appropriate
    c. gRNA concentration

4. You propose to test gRNA identity using MS for lot release. While MS can be used to confirm gRNA molecular weight, it cannot confirm gRNA sequence identity. Sequence identity is considered a critical quality attribute for a gRNA as it has an

effect on both safety and efficacy of the final DP. We recommend you develop a NGS-based lot release test to confirm gRNA sequence identity. Please be aware we recommend an NGS-based assay also be used as an orthogonal assay for purity.

5. We recommend that you include tests for the following attributes in your UW-LNP-ABE8E DP lot release specification:
    a. mRNA purity
    b. gRNA purity
    c. ratio of mRNA to gRNA

6. We note you have included bioburden testing as part of lot release of the UW-LNP-ABE8E DP in Table 13. Please be aware the sample for this test should come from the bulk DP immediately prior to filter sterilization. You may consider this as an in-process test.

7. You propose to test UW-LNP-ABE8E DP identity using FT-IR for lot release. It is unclear what aspect of product identity you are confirming using FT-IR. Our expectation for identity testing during lot release of the UW-LNP-ABE8E DP is confirmation of gRNA identity, mRNA identity, and the identity of each lipid component. Moreover, considering that each batch of product will contain one of multiple potential gRNAs, your chosen method needs to be capable of sufficiently resolving the identities of each gRNA.

8. We note you plan to test UW-LNP-ABE8E DP strength by measuring the weight of the product. It is unclear from the meeting package how you plan to determine the dose for patient administration. We recommend a direct measure of the active pharmaceutical ingredients such as the concentration mRNA and gRNA for a determination of dose.

Question 4:
Does the Agency agree that the proposed off-target editing studies are adequate and that there are no anticipated further requirements for analysis of off-target editing to support a FIH clinical trial with UW-LNP-ABE8E?

FDA Response to Question 4:
Your proposed strategy for off-target editing study is acceptable, however, we require additional information and supporting data to complete our evaluation of your methods. Please include the following information in your future preIND or IND submission:

1. A comprehensive off-target analysis report encompassing both 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.

2. For all NGS-based methods used in your studies, please ensure the read depths are adequate in detecting off-target events and provide a comprehensive report on how the sequencing data was generated, 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 (*see* worksheets *Library_metadata*, *Run_DNA*, and *Run_RNA*). You should include any additional QC metrics to allow us to evaluate your sequencing quality more effectively.

3. 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 workbooks (***Software_tools_and_CLI_v0.3.xlsx***) as a convenience to you. You can 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 to allow us to evaluate your bioinformatics methods effectively.

Question 5:
For each mutation (W53X and T153I), does the Agency agree that assessment of the in vitro on-target editing and in vitro functional recovery of patient-derived iPSC-RPE cells after treatment with UW-LNP-ABE8E are sufficient proof-of-concept studies to support the proposed mechanism of action and a FIH clinical trial for UW-LNP-ABE8E?

FDA Response to Question 5:
Based on the nonclinical studies summarized in Sections 5.1.5-6 and 5.1.8-9 (pages 20-22 and 25-28, respectively), we agree that in vitro assessment of patient-derived iPSC-retinal pigment epithelium (RPE) cells exposed to UW-LNP-ABE8E specific for each mutation may be adequate. We have the following comments that you should address in your pre-IND meeting package:

1. You plan to leverage data for the W53X mutation resulting from nonclinical studies performed in vitro in iPSC-RPE cells and a planned dose-range finding study (DRF) in vivo in the LCA16 murine model (Sections 5.1.14-15; pages 34-35) to inform dose level selection for patients with mutations without an available murine model (e.g., T153I). Please provide:

a. Methods, with supporting data (e.g. percent editing, changes in cellular membrane potential, membrane channel localization), for dose level extrapolation to bridge the in vitro and in vivo dose levels.

b. Details of the adenine base editor(s) you plan to use for each mutation. The use of different adenine base editors may impact your ability to leverage existing nonclinical data. Please see Additional Comment #3 regarding this.

Question 6:
Does the Agency agree that the novel reporter cell line is a viable quality control measure and agree with its use as a release assay for UW-LNP-ABE8E?

FDA Response to Question 6:
We agree the described bioassay may be sufficient to support DP release for the proposed FIH clinical study. However, we disagree with the proposed bioassay output being binary and qualitative in nature. Considering that you may need to leverage efficacy data from your proposed clinical study to support product licensure, your potency assay(s) should be suitable for late-stage product development prior to initiating the proposed clinical study. As such, we recommend that you establish the described HEK-based bioassay as a quantitative measure of potency and use it for lot release and stability testing during all phases of clinical development.

Please be advised that you are required to establish a potency assay and qualify it prior to initiating clinical studies that are intended to provide the primary evidence of effectiveness to support a marketing application. For additional guidance on potency assay development, please refer to the 2023 draft FDA guidance for industry: “Potency Assurance for Cellular and Gene Therapy Products” (https://www.fda.gov/media/175132/download).

Question 7:
Does the Agency agree that the selection and proposed animal model species selection and design are acceptable for supporting a FIH clinical trial?

FDA Response to Question 7:
Based on the information provided in your INTERACT meeting package, we agree that the animal model species selected (LCA16 mouse model [*Kcnj13*$^{W53X/+\Delta R}$] and non-human primates [NHPs]) and the study design for your proposed nonclinical studies are acceptable to support the proposed FIH clinical trial. We have the following comments regarding the planned nonclinical studies that you should address in your pre-IND meeting package:

1. Regarding the dose-range finding and biodistribution (BD) study in the *Kcnj13*$^{W53X/+\Delta R}$ mouse model summarized in Sections 5.1.14-15 (pages 34-35):

a. In addition to the summary provided in Section 5.1.10 (pages 28-29), please provide a comprehensive discussion regarding the biological relevancy of the *Kcnj13*$^{W53X/+\Delta R}$ mouse model to the proposed patient population. Your discussion should include: i) progression of the abnormal phenotype observed in this model; ii) the lifespan of this model; iii) the similarities and differences between this model and humans with LCA16 (e.g., pathophysiology, biochemistry, and functional changes); and iv) the timing of vector administration in the mouse model relative to disease onset and progression compared to the target clinical population.

b. Please provide your rationale for the selected time points. Please note that sacrifice time points should be selected based on the kinetics of ABE8E editing and [redacted] persistence following administration of UW-LNP-ABE8E. At a minimum, your selected time points should coincide with the peak and plateaued editing of the ABE8E.

2. Regarding the pilot toxicology study in NHPs outlined in Section 5.1.16 (pages 35-36):

   a. You state that you will enroll 6 animals with a total of 12 eyes treated. However, you also state that 4 eyes will receive 200 µg of drug substance (DS), 4 eyes will receive 300 µg of DS, and 2 eyes will receive vehicle injection, for a total of 10 eyes. Thus, it is not clear how many NHPs will be enrolled in this study and how many eyes will be dosed. Please clarify the number of animals in this study and provide your rationale for the number of eyes injected.

   b. Please provide a comprehensive description of the device and procedure used to deliver UW-LNP-ABE8E. Information should include, at a minimum, number of injections; injection volume/site; injection rate and other factors (when applicable); and any differences as compared to the intended clinical delivery device and administration procedure.

Question 8:
Does the Agency agree that the proposed DP does not constitute a significant risk of germline editing, and that the proposed biodistribution studies are sufficient to eliminate the potential for germline transmission?

FDA Response to Question 8:
Your plan to use data obtained from BD studies in mice discussed in Sections 4.3.1 and 5.1.14 (pages 14-15 and 34-35, respectively) to inform the need for studies assessing the probability of germline transmission is reasonable. However, without these BD data, we cannot yet agree that your DP does not constitute a risk of germline editing. Please provide any available, relevant data in your pre-IND meeting package to facilitate further interaction on this issue.

Question 9:
Does the Agency support a clinical trial design recruiting two patient groups, in which the biodistribution for UW-LNP-ABE8E-W53X and in vitro efficacy data for UW-LNP-ABE8E-W53X or UW-LNP-ABE8E-T153I will be used to support the inclusion of patients harboring either the W53X or T153I mutation, and in which either UW-LNP-ABE8E-W53X or UW-LNP-ABE8E-T153I will be administered?

FDA Response to Question 9:
Yes, the information submitted in the meeting briefing package and the nonclinical data, including BD studies in mice and in vitro activity data, supports enrollment of patients with either *W53X* or *T153I* mutation. For additional feedback, please refer to FDA Response to Question 8.

Question 10:
Does the Agency agree that demonstrating benefit in the FIH clinical trial targeting either W53X or T153I mutations would potentially support the amendment of this proposed clinical protocol for UW-LNP-ABE8E to include patients with other pathogenic KCNJ13 A-base mutations in this ultra-rare, serious disease that results in irreversible loss of vision?

FDA Response to Question 10:
Yes, from a clinical perspective, your proposal is reasonable. We recommend that you seek advice from FDA, on the CMC and PT data that would need to be submitted to the IND in order to expand the clinical protocol to treat patients with additional pathogenic mutations in *KCNJ13*.

Additional FDA Questions/Comments:

General Considerations:

1. We recommend that you request a pre-IND meeting with CBER/OTP when ready, to obtain formal nonbinding comments regarding your product development plan from the three CBER/OTP review disciplines, consisting of product manufacturing (CMC), pharmacology/toxicology (P/T), and clinical. Please be advised that you should consider and address all recommendations provided in these INTERACT comments when you submit a pre-IND meeting package.

2. We refer you to *OTP Learn*, a series of online presentations provided by the Office of Therapeutic Products (OTP) which address important topics in the development of products regulated by OTP. You may find some of these presentations useful in your preparation of regulatory submissions and briefing materials for meetings with FDA. *OTP Learn* is available at: https://www.fda.gov/vaccines-blood-biologics/news-events-biologics/otp-learn.

Chemistry, Manufacturing, and Controls

3. Based on the information included in the meeting package, it is unclear whether you will be employing two different adenine base editor variants to correct the two target mutations. In your preIND meeting package, please clarify what, in addition to the gRNA, may be different between the products used to correct these two mutations.

Nonclinical

4. You state that you intend to use research-grade materials for your proposed pilot non-GLP safety study in NHPs outlined in Section 5.1.16 (pages 35-36). This is acceptable. However, the material used in the nonclinical and clinical studies should have similar critical product quality attributes, as feasible, so that the nonclinical data inform the design of the definitive safety study and clinical trial. In your pre-IND meeting package, please provide a table detailing the similarities and differences between each product used in the completed nonclinical studies and the intended clinical product UW-LNP-ABE8E. Please discuss how any differences may impact extrapolation to a potentially therapeutic clinical dose level for each of the target variants, especially given that editing efficiency may differ for each sgRNA.

5. In Figure 6D in Section 5.1.5(pages 20-21) you observed 31% correction efficiency with your control ribonucleoprotein sgRNA compared to 53% correction efficiency with your W53X sgRNA. In Section 5.1.8 (pages 25-26), you observed higher membrane potential in your lentiviral-mediated Kir7.1 augmented cells compared to your control cells in which you corrected the W53X variant. Therefore, please provide your rationale for the appropriateness of the control cells and control articles used in each nonclinical study.

6. All genotoxicity studies should be performed using the final LNP-formulated mRNA product UW-LNP-ABE8E. We recommend that you conduct the following studies to evaluate the potential genotoxicity of each UW-LNP-ABE8E: a) an in vitro micronucleus test and b) an in vitro mammalian cell hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene mutation test or a mouse lymphoma assay. If any positive signals for genotoxicity are detected, we recommend that you also conduct an in vivo micronucleus test. The following document may be referenced for additional guidance on this assay: *Guidance for Industry S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (https://www.fda.gov/media/71980/download).

7. Please provide the physiochemical characteristics, including the lipid composition, total RNA content and ratio, RNA purity, encapsulation efficiency, zeta potential, particle size, and polydispersity index for UW-LNP-ABE8E.

8. Please provide in vitro data evaluating the degradation profile of your novel LNP component in appropriate biosamples (e.g., tissues or cells) from mice, NHPs, and

humans to assist in translation of metabolism and clearance data from the selected animal models to humans.

9. Regarding BD studies:

   a. Please provide LC-MS characterization of your novel LNP component.

   b. For all samples that are positive for LNP component presence, ABE8E levels should be measured. If a particular tissue/biofluid is negative for LNP components at a specific time point, then that respective tissue/biofluid does not need to be analyzed at later time points. If a particular tissue is determined to be negative upon PCR analysis, then that respective tissue does not need to be analyzed for ABE8E expression at later time points. However, all tissues, whether analyzed or not, should be archived for possible future analysis.

10. If complete study reports are not available for your proof-of-concept (POC) studies, it is acceptable to submit a copy of all key publications that are cited to support the safety and scientific rationale for use of UW-LNP-ABE8E in subjects with LCA16. However, you should also provide a summary of each publication, including a discussion of the similarities and differences of the product(s) evaluated in the publication and UW-LNP-ABE8E.

11. For a comprehensive summary regarding the nonclinical assessment of gene therapy products for retinal disease indications, please refer to the document titled, *Human Gene Therapy for Retinal Disorders: Guidance for Industry* (January 2020), available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-retinal-disorders.

12. 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.

Clinical

13. We acknowledge your plan to discuss the design of the proposed FIH clinical trial under a future, preI-ND meeting. Please submit a detailed synopsis of your proposed clinical protocol in the pre-IND briefing package for further review and comments.

END