U.S. FOOD & DRUG ADMINISTRATION

Annotated Preliminary Meeting Responses

Our Reference: PTS #PS009261
CRMTS #15682

DATE: July 17, 2024 **PAGES:** 17

TO: Steven Gray, PhD
UT Southwestern Medical Center
[redacted]

POINT OF CONTACT: [redacted]

SUBJECT: INTERACT meeting to discuss specific CMC concerns regarding the manufacture of the dual AAV vector therapeutic, receive early feedback on the proposed set of IND-enabling pharmacology and toxicology studies to set the direction of pending mouse proof-of-concept studies, as well as to a clinical trial strategy, which the Sponsor recognizes has complex considerations due to the availability and nature of existing SMA therapeutics.

PRODUCT: Two recombinant serotype 9 adeno-associated virus vectors, encoding separate halves of the complete expression cassette
Product Name: DualAAV9/SMABE

INDICATION: Treatment of Spinal Muscular Atrophy (SMA)

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 12, 2024. We are sharing this material to promote a collaborative and successful discussion at the meeting.

Although we continue to reserve July 12, 2024 from 1:00 PM – 2:00 PM 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 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. 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 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 #PS009261 and/or CRMTS #15682 in your future submissions related to the subject product.

Preliminary Meeting Responses

Question 1:
DualAAV9/SMABE is composed of two separate AAV9 vectors that are intended to be administered together, at the same time, at a 1:1 ratio. They would never be expected to be administered alone. Our position is that the combined dual vectors would be considered a single drug product. However, we would prefer to manufacture and vial each of the two vectors separately, combining them just prior to administration to the patient. Does the agency agree with this position and strategy? Are there any specific considerations that we should be aware of when taking this position?

FDA Response to Question 1:

Your proposed approach where each vector would be manufactured, vialed, and quality control (QC) released separately, with the vectors being combined at a 1:1 ratio at the clinical site just prior to administration to the patient, is an acceptable manufacturing strategy. Please note that for this approach FDA would consider each vector to be a separate Drug Product (DP). Your therapy would therefore consist of two vector DPs (i.e., AAV9/SMABE-N and AAV9/SMABE-C), mixed 1:1 prior to administration, but this would not be considered a combination product. Please see the following specific considerations, comments, and advice:

1. When utilizing an approach where each vector is manufactured, vialed, and QC released separately you should address the following:
    a. The mixing procedures used to achieve a 1:1 ratio of the two vectors at the clinical site should be described in a Pharmacy Manual. This should include a detailed description of how the two vectors will be transported, stored, thawed, and mixed at the clinical site(s) for administration.
    b. The mixing procedures should be clear and unambiguous. In your IND, please provide detailed information on the devices or materials that will be used for mixing (e.g., syringe, needle, etc.). Please note that the devices used for mixing should be pre-defined. Please describe whether you intend to supply a mixing kit to the clinical site(s) with the DPs.
    c. Your IND should include data demonstrating that the mixing procedure results in the intended ratio of AAV9/SMABE-N and AAV9/SMABE-C.
    d. Please be advised that with an approach where each vector is separately vialed and QC released, future potency assay development may require generation of individual reference standards for each vector.
2. An alternative approach to what you proposed would be to generate a single DP consisting of the two vectors pre-mixed at a specific ratio (e.g., 1:1). The single DP consisting of the two vectors would undergo Quality Control (QC) release and cryopreservation. Please see the following considerations of this approach:
    a. To ensure the accuracy of the quantity and ratio of the two vectors in a single DP, please design your manufacturing process to achieve the intended ratio and measure the concentration of each vector in the final

DP as part of DP lot release testing. You should develop appropriate release testing acceptance criteria, taking into consideration the planned ratio of the two vectors in the final formulated DP and assay variability.

b. Because the single DP would contain the two vectors, you should develop a specific vector strength (concentration) assay for each vector. This could involve a multiplex assay in the case of ddPCR.

Question 2:
Are the release testing plan and specifications listed in section 1.5.1.5 appropriate for this product to be used in the initial Phase I/II clinical trial?

FDA Response to Question 2:

We cannot yet fully agree that the proposed release testing plan is appropriate for initiation of the proposed Phase 1/2 clinical study. Please see the following comments and advice:

1. We note that you are using methods from the European Pharmacopoeia (Ph. Eur.) for sterility, endotoxin and mycoplasma testing. To support a United States IND, compendial methods should be performed according to the United States Pharmacopeia (USP). Therefore, please test for sterility (i.e., USP <71>), endotoxin (i.e., USP <85>), and mycoplasma (i.e., USP <63>) according to USP methodology. In your IND submission, please provide the full name and designation of all compendial assays. If non-compendial methods are used, you should submit a detailed description of how the methods compare to the corresponding USP methods as well as data demonstrating that your method has equal or greater sensitivity than the methods described in the USP.
2. We understand that your manufacturing process is under development. However, you did not describe testing of the unprocessed bulk (UPB) harvest prior to lysis, treatment with nuclease, and clarification. Therefore, please test the UPB for mycoplasma (i.e., USP <63>) and *in vitro* adventitious viral agents (AVA) in three indicator cell lines following the method (i.e., a conventional 28-day AVA test) described in the FDA guidance for industry titled “Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications” published in February 2010 (i.e., https://www.fda.gov/regulatory-information/search-fda-guidance-documents/characterization-and-qualification-cell-substrates-and-other-biological-materials-used-production). We also recommend that you test the UPB for bioburden.
3. Your proposal to test the potency of each vector using a cell-based assay that quantifies transgene mRNA expression following transduction of Lec2 cells is insufficient for initiation of the proposed Phase 1/2 clinical study. Given the complexity of your product (a dual AAV split-intein system expressing a novel genome editing approach) and the availability of licensed therapies for your proposed indication, additional assurance of the activity of your product is needed.

a. In addition to your mRNA expression assay, please develop and implement an *in vitro* genome editing assay for lot release to confirm that each lot of your product is capable of single nucleotide conversion of *SMN2* exon 7 C6T. Given that you plan to vial each vector (i.e., AAV9/SMABE-N and AAV9/SMABE-C) separately, you will need to determine if this assay will use a to-be released lot of vector-N paired with a to-be released lot of vector-C or if a well-characterized reference materials will be used.

b. Regarding the use of a well-characterized reference material:

    i. If relevant, please describe how you plan to incorporate reference materials to test the potency of each of the vectors separately.

    ii. When possible, we recommend reporting potency for each lot as a percentage of the potency of the reference lot (assessed in parallel with the test lot).

    iii. The manufacturing, testing, and characterization of the reference lot should be comparable to that used for clinical lots. A robust qualification and stability plan for the reference material should be put in place. Additionally, you should develop a plan to bridge reference lots prior to exhaustion of the original reference material.

c. Please note that a qualified quantitative potency assay to assess the activity of your dualAAV9/SMABE product is required prior to the initiation of clinical studies intended to collect efficacy data in support of licensure (i.e., Phase 3 clinical study), and this assay should be validated prior to submission of a Biologics License Application (BLA).

d. For additional recommendations on the development of a potency plan, please refer to the FDA guidance for industry: “Potency Tests for Cellular and Gene Therapy Products” (January 2011) (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/potency-tests-cellular-and-gene-therapy-products). You may also refer to the draft FDA guidance for industry: “Potency Assurance for Cellular and Gene Therapy Products” (December 2023) (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/potency-assurance-cellular-and-gene-therapy-products).

4. Regarding lot release testing, we have the following comments:

a. We note that you defined the acceptance criteria (AC) for several lot release tests as “Report Value” or “Report Results”. In your IND, please define preliminary AC for potency, full:empty capsid ratio, and particle aggregates based on your data from engineering, toxicology, and/or clinical manufacturing. These AC may be set relatively wide to initiate Phase 1/2 clinical studies and should be progressively narrowed as product and process development continues, based on experience.

b. We recommend you include additional testing for the following process-related impurities: lysis reagent (i.e., detergent), transfection reagent (e.g.,

polyethylenimine, PEI), and residual affinity chromatography leached ligand.

c. We note that the DP will be formulated in 5% (w/v) D-sorbitol and 0.001% Pluronic F-68. Please include a test to quantify the amount of Pluronic F-68 (also known as Poloxamer 188) in the final DP (e.g., SEC-MALS).

Question 3:
Is the plan presented in section 1.5.1.9 appropriate, to bridge the nonclinical lots with the future clinical drug product based on analytical comparability and in vitro potency?

FDA Response to Question 3:

You propose to bridge the nonclinical lots with the clinical lots based on an analytical comparability study where the specifications of the clinical lots are expected to meet or exceed the specifications of the toxicology lots including the potency attribute. This approach is acceptable. In general, we recommend that Sponsor's provide a tabular listing of the product lots and their intended purpose (e.g., nonclinical, clinical, etc.) and whether the same critical starting materials, manufacturing process, and analytical procedures were used to manufacture the nonclinical and clinical lots. If different materials, process, and/or analytical procedures were used, please describe how data generated with the product lots used in the nonclinical toxicology studies will support product quality for the intended clinical study. Please consider a variety of quality attributes, including vector genome concentration, potency, and purity (e.g., endotoxin and process- and product-related impurities that may affect product safety).

Question 4:
We plan to manufacture the nonclinical non-GMP drug product batches (for pivotal pharmacology and toxicology studies) in a different facility than the drug product, which will be manufactured under GMP quality. Although the facility manufacturing the GMP drug product may use a slightly different overall process than that for the nonclinical drug product batches, our strategy would be to conduct analytical comparability studies to assure that the critical quality attributes of the GMP drug product meet or exceed the specifications of the nonclinical drug product batches. Does the Agency agree with this strategy? Would the Agency provide any specific guidance towards acceptance criteria for the manufacture of non-GMP and GMP qualities, which we could follow with the respective vendors?

FDA Response to Question 4:
Regarding manufacturing non-clinical product batches in a different facility than the product batches intended for clinical use please see our response to Sponsor Question 3. Regarding advice on setting clinical batch acceptance criteria please see our response to Sponsor Question 2. For advice manufacture of non-GMP lots please see the Pharmacology/Toxicology section of the Additional Comments at the end of this letter.

Question 5:
Our primary pharmacology strategy is to establish a minimally effective dose and a prospect for direct benefit to conduct dose-ranging efficacy studies in a humanized mouse model of SMA, with dualAAV9/SMABE alone or in combination with nusinersen, using survival as the primary efficacy outcome. Are the nonclinical pharmacology studies proposed in section 1.5.2.2 appropriate to evaluate the prospect for direct benefit of our gene editing approach in the target pediatric SMA patient population?

FDA Response to Question 5:
Based on the information provided in Section 1.5.2.2 (pages 21-26) of your INTERACT meeting package, we agree that your planned nonclinical pharmacology studies appear appropriate to evaluate the biological activity and prospect for direct benefit of your gene editing approach in pediatric SMA patient population. We have the following comments for clarification regarding your planned proof-of-concept (POC) studies in neonatal/ juvenile non-symptomatic and symptomatic Δ7 SMA mice that you should address in your pre-IND submission:

1. Please provide a justification, with supporting data, for the routes of administration (ROA) to be assessed in your planned pharmacology studies.

2. Please provide a detailed methodology of the nonclinical pharmacology studies including the product administration (e.g., location(s) of the administration, injection volume, delivery device, etc.) and the scientific justifications for each aspect of product administration. For the proposed co-administration of dualAAV9/SMABE and nusinersen in neonates (Figure 12A), if both will be given via ICV administration, please clarify a) whether dualAAV9/SMABE and nusinersen will be given sequentially or as a pre-mixture; b) if given sequentially, please justify the timing and preferred order of each injection.

3. Please provide a justification for the dosing regimen and dose levels administered for all test articles (e.g., dualAAV9/SMABE and nusinersen). In addition, please provide your methods for dose extrapolation to humans (e.g., vg/g body weight, vg/CSF volume, vg/brain weight, etc.) to allow for comparison to the proposed clinical dose levels.

4. Please provide a scientific justification for the study duration, assessments, and the selected evaluation and/or sacrifice time points.

Question 6:
The strategy for our pivotal toxicology studies is to conduct non-GLP and long-term safety assessments in mouse models, and further complement the safety data with a GLP non-human primate (NHP) study to specifically investigate the immune responses and biodistribution of our investigational drug product. Would the Agency consider the relevant nonclinical pharmacology and toxicology studies proposed in sections 1.5.2.3 (mouse studies) and 1.5.2.4 (NHP studies) appropriate to assess the safety of our gene editing approach to enable a human trial?

FDA Response to Question 6:
Based on the limited information provided in your briefing package and the early stage of your product development, we do not agree that your proposed animal toxicology and biodistribution studies as described in Sections 1.5.2.3-4 (pages 30-32) will be adequate to assess safety of dualAAV8/SMABE to support the initiation of your first-in-human (FIH) trial. We recommend that you refrain from initiating the planned pivotal safety studies until you have finalized the manufacturing process of your intended product and received additional feedback from us in a future pre-IND meeting. We recommend that you complete your proposed POC studies and provide a detailed summary of each study in your pre-IND meeting package. This information should help to inform the design of your pivotal safety studies. Please address the following general comments in your pre-IND submission:

Regarding your proposed 90-day GLP toxicology and biodistribution (BD) study in NHPs:

a. We recommend that you submit the definitive nonclinical safety study protocol(s) for feedback in your pre-IND package.

b. Please provide a justification for the dose levels administered as well as the methods used to extrapolate the dose levels used in your nonclinical studies to a starting clinical dose level that is safe and potentially biologically active. Please consider leveraging your nonclinical pharmacology and safety studies in mice to inform an appropriate study design of your definitive toxicology study in NHPs, including the determination of appropriate dose levels, study duration, and potential organ toxicities for observation, etc., as applicable

c. Your definitive studies should be of sufficient duration to evaluate potential acute, subacute, and long-term toxicities, bioactivity, and durability of observed effects. If product-related toxicities are observed and persist without stabilization or resolution at your planned 90-day timepoint, additional data from longer term studies may be warranted. Please note that sacrifice time points should also be selected based on the kinetics of transgene expression following administration of dualAAV9/SMABE and include assessments at peak and plateaued transgene expression levels.

d. Please provide a comprehensive discussion, with supporting data to address potential safety concerns regarding the persistence and immunogenicity of the adenine base editor (ABE).

e. On page 32, you state that vector genome BD will be assessed in key tissues (e.g., brain, spinal cord, dorsal root ganglia, bicep muscle, liver and heart). Please provide a justification for the limited tissues planned for vector BD. We recommend BD evaluation of a comprehensive list of tissues/organs to also include the site of administration, other highly perfused organs, gonads, and blood, as feasible. For any tissues where vector presence is detected, please

also evaluate transgene expression levels. This will help determine whether any histopathological changes are test article-related.

a. Regarding your proposed non-GLP 18-month safety assessments in mice to evaluate long-term safety related to off-target editing and histopathological changes in BD-positive tissues:

   a. Please provide your rationale for the study duration of 18 months.

   b. We recommend a stepwise approach to characterize the in vivo persistence and BD of ABE prior to conducting a long-term safety study. Please provide data for your product or published literature regarding in vivo persistence/expression kinetics for similar ABEs for justification. This information is important for selection of the appropriate sacrifice time points and study durations for your toxicology studies.

   c. Please refer to Question 7 for additional comments for off-target editing analysis.

Question 7:
Our off-target editing strategy is to evaluate the prospect of this approach primarily using in vitro studies in human cellular models, and to complement it with long-term mouse studies to understand the general kinetics of short-term versus long-term off-target editing events. This is described in more detail in section 1.5.2.3. Is our plan to evaluate off-target editing sufficient to evaluate this risk?

FDA Response to Question 7:
We do not agree with your proposal to implement CHANGE-seq using HEK293T genomic DNA. To assess off-target safety risks in the human genome please use human cells or genomic DNA in your studies and address the following in your IND submission:

a. Your proposed in vitro CHANGE-seq assay uses Cas9 that is known to create double strand breaks (DSBs) in the genome. This type of edit is different from the single-strand nicking event imparted by an ABE-editor. Due to the distinct DNA modification events, we are concerned that the off-target sites nominated by CHANGE-seq would not inform potential off-target editing risks in ABE-edited cells. Please use an appropriate assay that allows assessment of off-target editing in ABE-edited cells. Alternately, you could use currently available next-generation sequencing (NGS)-based method(s) to sequence the genome of edited and unedited cells to identify and report off-target edit sites. We recommend that you perform this analysis in 3 or more biological replicates.

b. We recommend that you use two or more orthogonal approaches and include replicates and appropriate experimental parameters for your off-target assessment. For additional information, please refer to the Guidance for Industry: Human Gene Therapy Products Incorporating Human Genome Editing which can

be found at https://www.fda.gov/media/156894/download. Please include in your IND a detailed report of the orthogonal approaches you used for the off-target nomination, criteria for selecting a subset of nominated off-target sites for confirmatory testing, and a detailed annotated list of off-targets you identified from orthogonal assays.

b. For your confirmatory analysis, please include a detailed description of your confirmatory testing method you implemented and provide a detailed report of your findings from this analysis. Please report the findings of this analysis as an annotated list of all off-target sites, associated edit rates you measured at each site, and on-target edit rates you determined for all samples processed for confirmatory testing. If you identify a confirmed off-target site, then please provide your assessment of its functional consequence in edited cells both short-term and long-term.

c. You did not provide sufficient information on the chimeric SpyMac Cas component of your drug product (DP). Specifically, it is not clear whether SpyMac has any DNA editing activity and the type of DNA edit it can impart. To address this, please perform additional analysis to characterize the editing activity of your chimeric Cas and provide data to support your conclusion.

d. To rule out the presence of small/large indels or bystander edits at the on-target site, we recommend that you use long-read sequencing-based analysis and report the findings of this analysis as a list of on-target indels you detected and the associated frequencies in each sample tested. Please report the frequencies of any bystander edits you may identify in ABE-edited cells and include an assessment of the functional and transcriptional impacts resulting from bystander editing.

e. Please perform transcriptomic analysis in cells that are representative of your intended target tissue of action in vivo. These cells should be from biological replicates of either healthy or patient cells. We recommend using the transcriptomic data to assess the level of RNA editing and differential gene expression in ABE edited cells. Please provide a risk assessment when reporting your analysis.

Meeting Discussion for Sponsor Question 7:

The sponsor referenced the Kim et al paper to justify the use of CRICLE-seq for off-target nomination. The sponsor also proposed to perform confirmatory testing on all sites identified from CIRCLE-seq assay. FDA agreed with the sponsor's proposed method. FDA recommended that the sponsor use genomic DNA from different sources to enable testing in biological replicates. The sponsor proposed using genomic DNA from other cell lines to which the FDA agreed and stated that they will review the data when the sponsor submits it to FDA. Regarding the use of appropriate cell type for confirmatory testing, FDA recommended that the sponsor use samples that are

representative of their intended target tissue. However, FDA also stated that if the sponsor is unable to find an appropriate cell type that is amenable to editing in vitro, then the sponsor can use alternative samples and provide justification to support their approach. Regarding the use of long-read sequencing-based methods for testing the presence of large indels, the sponsor clarified that their ABE harbors mutation(s) that make it amenable to only create a DNA nick. In light of this new information provided by the sponsor, FDA agreed that large indel analysis may not be necessary. FDA also recommended that the sponsor provide detailed information for all the mutations and modifications that their ABE harbors.

FDA agreed with sponsor's proposal to use short read sequencing for bystander mutation analysis and recommended that the sponsor provide a detailed report of all the bystander mutations they may have identified for their drug product (DP).

Regarding the gRNA-independent RNA editing analysis, FDA agreed with the sponsor's proposal to measure A to I conversions in edited cells. FDA also recommended that if the sponsor plans to provide references to published data to support minimal RNA editing effects of their editor, then they should provide relevant data to show that the editor in the publication is identical to the editor used in their DP. Otherwise, they may have to perform these studies and submit their findings to FDA. Finally, the sponsor agreed to perform in silico off-target nomination as another orthogonal off-target assay.

Question 8:
We recognize that the expressed base editor may be viewed as a foreign antigen and stimulate an anti-transgene immune response of this is not properly controlled. We proposed to incorporate a published immunomodulatory regimen consisting of corticosteroids, tacrolimus, and sirolimus appropriate for CRIM (-) individuals. Safety monitoring is focused on potential adverse immune responses. Would the FDA consider if the clinical immunosuppression regimen and safety monitoring strategy outlined in section 1.5.3 are suitable for this Phase I/II trial?

FDA Response to Question 8:
Please provide your rationale and supporting data for the proposed triple immune suppression regimen, consisting of prednisone/prednisolone, tacrolimus and sirolimus.

Your proposed safety monitoring strategy appears reasonable. We have the following additional comments:

a. We recommend primary safety endpoints as below:

Incidence of Adverse Events (AEs), Adverse Events of Special Interest (AESIs), and Serious Adverse Events (SAEs)
Physical examination findings including Vital signs.
Safety laboratory data
Electrocardiogram (ECG)
EMG (dorsal root ganglia toxicity)

b. For this first-in-human, dose-escalation study, we recommend defining dose limiting toxicity (DLT), as any Grade ≥3 AE or any Grade ≥2 not resolving within 14 days, assessed to be related to the study product and not due to any intercurrent illness. Toxicity grading can be determined using the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (NCI-CTCAE v5.0).

c. We recommend that you include the following as the primary safety endpoints:
   i. Incidence of Adverse Events (AEs), Adverse Events of Special Interest (AESIs), and Serious Adverse Events (SAEs)
   ii. Physical examination findings including Vital signs.
   iii. Safety laboratory data
   iv. Changes in Electrocardiogram (ECG) and EMG (dorsal root ganglia toxicity)

d. We recommend staggering enrollment with 28 days interval between enrollment of consecutive patients in each dose cohort. The Data Safety Monitoring Board should review the safety data from the low-dose cohort prior to initiating enrollment in the high-dose cohort.

e. You proposed “Safety follow-up will continue over a two-year period that incorporates the active phase of the protocol. Individuals will then transfer to an annual monitoring program where data will be collected from annual standard care visits for up to 5 additional years.” Due to the application of gene-editing technology, we recommend a total follow-up period of 15 years.

Question 9:
In brief, we plan to enroll subjects that are taking nusinersen according to normal treatment guidelines. Our rationale is that patients may receive additional benefit of dualAAV9/SMABE over nusinersen alone. During future evaluation and development of dualAAV9/SMABE, there may be the possibility to use dualAAV9/SMABE as a stand-alone treatment. Does the agency agree with the proposed patient population and enrollment strategy outlined in Section 1.5.3?

FDA Response to Question 9:
The proposed single-arm, dose-escalation study design in patients with SMA types 1, 2, and 3, ranging in age from 0 to 12 years old appears reasonable. We have the following comments regarding the proposed patient population:

As a secondary outcome, you proposed to assess initial efficacy of your product compared with the standard treatment using nusinersen. However, the proposed patient enrollment criteria include both nusinersen and risdiplam as standard treatments. We recommend that you consider enrolling only patients who are receiving nusinersen as the standard treatment or stratifying enrollment based on the baseline treatment.

SMA types I, II, and III differ in clinical presentation and disease progression. It is very challenging to draw conclusions regarding preliminary efficacy with the current study design.

Question 10:
Using the intrathecal route of administration of AAV9, germline cells are not expected to be transduced to any appreciable extent, making the risk of germline editing negligible. Our position is that a detailed germline transmission study would therefore not be necessary for our drug product at this stage of development (i.e., first-in-human studies). Does the agency agree with this position?

FDA Response to Question 10:
Based on the information provided in your briefing package, it is challenging at this point, to determine whether further assessment for potential germline transmission would be needed for the initiation of your FIH trial. We recommend a stepwise approach to address the risk of inadvertent germline transmission, including characterization of vector and/or transgene biodistribution in the reproductive tissues in your in vivo studies. To facilitate further interaction in the pre-IND meeting, please provide a comprehensive discussion, with supporting data and your strategy, on how you plan to address the risk of inadvertent germline transmission for your product.

Meeting Discussion for Sponsor Applicant Question 10:

The sponsor proposed to determine the expression of the genome editor and assess editing in germ cells collected from the humanized SMA mouse model. The FDA agreed that the proposed approaches are reasonable. FDA stated that sponsor should provide a discussion of these approaches in their pre-IND along with their overall stepwise plan to assess the risk of germline transmission.

Additional FDA 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

1. Regarding the assay for measurement of vector strength (e.g., vector genome titer by ITR ddPCR), we have the following comments:
    a. To ensure that an accurate dose is administered, this assay must be qualified and must be shown to have adequate performance prior to Phase 1/2 clinical studies.
    b. The qualification study should be performed using a product-specific test material.
    c. Please provide a detailed protocol for the qualification study and the SOP for the assay.
    d. Please provide the full qualification study report with data documenting accuracy, precision (repeatability and intermediate precision), specificity, range, and linearity. The coefficient of variation (CV) for intermediate precision should be ≤ 15%. A precise assay is necessary to ensure that subjects receive the intended doses, to support consistent dosing throughout the clinical study, and to monitor product stability.
    e. Please describe any deviations that occurred during the qualification study.
    f. To ensure consistent dosing between clinical and preclinical studies, we recommend using the same qualified assay for measuring the vector genome titer of the preclinical and clinical lots.
    g. The assay to determine vector strength should be validated prior to the initiation of any clinical study that is intended to provide the primary evidence of effectiveness for a marketing application.
2. Regarding the master cell bank (MCB) and working cell bank (WCB) of HEK293 cells, please provide the following information in your IND to support the use of the cell line in the manufacture of dualAAV9/SMABE:
    a. A narrative description of the derivation history, propagation, generation, and qualification testing of the cell bank(s).
    b. A complete list of the animal-derived raw materials used in the derivation of the cells that includes the source, qualification, and testing of the raw material.
    c. Please provide adequate details on the tests used to qualify the cell bank(s). Testing reports and a Certificates of Analysis (COA) for the MCB and WCB should be documented in the IND.

d. If the MCB will be derived at a manufacturing facility which is also the site for manufacturing other AAV products, we recommend testing the MCB for the presence of commonly used AAV serotypes.

e. Please describe how you will verify the identity of the cells (in the MCB and WCB) from other similar cells maintained in the same facility.

3. Regarding the plasmids used in the manufacture of dualAAV9/SMABE, please provide the following information in your IND:

   a. The name and address of the plasmid manufacturer.

   b. Annotated maps for all plasmids used in manufacturing, identifying all open reading frames, genetic components (i.e., promoters, introns, known coding sequences, polyadenylation signals, and untranslated regions). We recommend that you sequence each plasmid master cell bank to ensure the correct plasmid sequence and to identify any unexpected sequences.

   c. Certificates of analysis (COAs) for all plasmids used in product manufacturing. COAs should indicate the grade of plasmid.

   d. A narrative description and/or flow diagram of the plasmid manufacturing process.

   e. A description of the Quality Unit and procedures in place at the plasmid manufacturer to prevent cross-contamination from other plasmid DNA lots made prior to your plasmid DNA lots (i.e., segregation, tracking and changeover systems for manufacturing of the plasmid DNA lots).

4. Regarding the proposed stability protocol, we note that you are testing for sterility (USP <71>) at timepoints M12, M24, and M36. We recommend you replace the sterility test with a container closure integrity test (CCIT) with the vialed drug product. For more information, please refer to FDA guidance for industry titled "Container and Closure System Integrity Testing *In lieu* of Sterility Testing as a Component of the Stability Protocol for Sterile Products" from February 2008 (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/container-and-closure-system-integrity-testing-lieu-sterility-testing-component-stability-protocol). We also recommend you include assessment of aggregation.

5. Regarding the planned in-use stability and delivery device compatibility study, we have the following comment: This study should demonstrate that administering dualAAV9/SMABE using the delivery device will not negatively affect the product's quality and that you understand the quantity of product that will be delivered to the recipient (i.e., preliminary dose accuracy data). These data should be collected with the final formulated DP under the worst-case conditions expected in the clinical trial (such as thaw, dose preparation procedure, duration of storage after preparation, dose/volume range, flow rate/pressure, duration of delivery, temperature, simulated delivery into worst case target tissue anatomy, etc., as applicable). The device compatibility data should support the instructions for product handling and storage during formulation in the pharmacy and clinical

administration (e.g., Pharmacy Manual, Administration Manual, Surgical Manual, etc.), as applicable.

6. For additional advice regarding your product development, please refer to "Guidance for Industry: Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)," from January 2020 (https://www.fda.gov/media/113760/download).

Pharmacology/Toxicology

1. Regarding the vector lots and delivery devices used in your nonclinical studies, please address the following comments in your pre-IND:

   a. You mentioned that you may consider switching variants of your lead candidate base editor that have equivalent on-target editing efficiency but a more favorable (i.e., less) off-target editing profile (page 26). Please be advised that your definitive nonclinical studies should use dualAAV9/SMABE that is identical to the intended clinical product, as feasible. In your pre-IND/IND, please indicate the vector lots used in each nonclinical study and provide a tabulated summary of the similarities and differences between these lots. A comprehensive discussion on the potential impact of any differences on interpretation of the POC and safety data from animals to humans should be provided.

   b. Please provide detailed information regarding the assays and standards that were used to quantify each nonclinical lot. For any assay that differs with that used for the clinical lot, please retest any retained material from the nonclinical lots using the assay for the clinical lot, as feasible. The vector dose levels administered in the nonclinical studies should be recalculated based on this analysis.

   c. We recommend that you retain samples from your nonclinical vector lots for possible future analysis.

   d. Please provide a summary table comparing the delivery device, administration procedures, and anatomic site(s) of administration used in each nonclinical study to the planned clinical device delivery and procedures. This comparison should include any modifications that were made to accommodate anatomic differences. All deviations from the proposed clinical trial should be discussed and the potential impact on interpretation of the data should be provided. Additionally, please discuss how differences in the delivery device/procedure factor into the extrapolation of the starting clinical dose level from nonclinical studies.

2. For a comprehensive summary regarding the nonclinical assessment of cell and gene therapy products, please refer to 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.

END