SPONSOR RESPONSES TO FDA ADVICE FROM PRE-IND WRITTEN RESPONSES

Table of Contents

1. REGULATORY

1.1. Sponsor Question 1:

Does the agency agree that this non-profit charitable sponsor could be granted an exemption by OTAT from eCTD rules and submit their future IND as non eCTD (PDFs with CTD structure) for the first in human study for this ultra-rare disease, with electronic filing requirements after the first in human study?

1.1.1. FDA Written Response to Sponsor Question 1:

You may submit a waiver request for exemption from eCTD requirements. After you have requested and received the IND number from FDA, please send an email to esubprep@cber.fda.gov and formally request the waiver. Once your waiver request has been approved by the esubprep staff, you may submit the IND via DCC email at: cberdcc_emailsub@fda.hhs.gov.

A waiver was submitted but esubprep confirmed none was required for a research (non-commercial) IND.

2. PHARMACOLOGY/TOXICOLOGY QUESTION

2.1. Sponsor Question 2:

Does the Agency agree that the GLP toxicology study in Sprague Dawley rats, coupled with the ex vivo and in vivo data from various studies, supports the intrathecal dosing of Melpida in patients with SPG50?

2.1.1. FDA Written Response to Sponsor Question 2:

Based on the limited information provided in Sections 14.3 (pages 12-15/99), 15.3 (pages 36-50/99) and 17.5 (pages 65-99/99) of your briefing package, we cannot yet agree that the preclinical studies described will be sufficient to support the proposed clinical trial. Please address the following comments in your IND submission.

1. The proposed clinical trial will involve administration of Melpida to pediatric subjects. For clinical investigations associated with more than a minor increase over minimal risk involving children, these risks must be justified by a prospect of direct clinical benefit (PDB) to the children (21 CFR 50 § 50.52). Preclinical data used to support PDB at an optimal range of clinical dose levels should be derived from studies conducted in a biologically relevant animal model that demonstrates improvements in a comprehensive battery of clinically meaningful biochemical, pathophysiological and functional parameters in addition to durability of effect. As a result, we have the following comments regarding your ongoing study #2020- 06 (page 43/99; Section 15.3.4),

a. There is insufficient information in your pre-IND briefing package regarding the Ap4m1-/- mouse model to determine its suitability for use in establishing proof-of-concept (POC) and PDB for Melpida. Please provide a comprehensive discussion, with accompanying data, regarding the biological relevancy of the Ap4m1-/- mouse model to the proposed patient population, including: i) life-span of the model, ii) onset and progression of the abnormal phenotype (i.e., biochemical, morphological, functional), iii) the similarities and differences in this model and the human disease (e.g., pathophysiology, biochemistry, functional changes, etc.), and iv) the timing of Melpida administration relative to the disease progression in the proposed patient population.

The Ap4m1 knockout mouse was generated specifically for the study of MELPIDA; there are no data or publications available describing this model. The details requested are described further in **Module 2.4**.

b. In addition to the proposed behavioral outcomes, we recommend that you conduct assessments of a more comprehensive set of disease parameters in this animal model, including survival and relevant biochemical and pathophysiological changes, over time. A comprehensive rationale should be provided for any parameters that are not evaluated.

More comprehensive disease parameters were included as follows: survival, hindlimb clasping, and motor function (rotarod), as well as assessing vector biodistribution and transgene expression. Using these measures, a benefit was observed from treatment of MELPIDA in the mouse model, which is detailed in **Module 2.4.** Other behavioral outcomes are being explored, and will be evaluated for rescue within the limits imposed by the animal disease model.

c. Please provide justification for evaluating only two dose levels of Melpida in this study.

Based on published studies that a maximum feasible dose (MFD) of AAV9 by this route of administration will still result in sub-saturating transduction efficiency across the brain, it is anticipated that anything less than the MFD will result in reduced efficacy. With that rationale, a dose approximating the MFD (5E11 vg) was tested, along with a 4-fold lower dose (1.25E11 vg) in mice 7-10 days old in study 2020-06. A more detailed dosing study comprising 3 dose levels (1.25E11, 2.5E11, and 5E11 vg) was conducted in mice dosed at postnatal day 90.

2. Recent published data indicates the potential for AAV-mediated toxicity in the dorsal root ganglion (DRG) and peripheral nerves in non-human primates following intrathecal administration (e.g., Hordeaux et al, 2020). At this time, it is unclear whether rodents are sufficiently sensitive to adequately characterize these toxicities. As a result, given the nature of your clinical product, route of administration, and target population, we recommend that you comprehensively evaluate the potential

for vector biodistribution and toxicity in the DRG, spinal cord, and peripheral nerves of NHPs in addition to your ongoing GLP rat study (Study #CRL555008). We have the following additional details regarding the design of this study:

d. The NHP study should use the intended clinical delivery device, administration procedure and dose levels that bracket the range of proposed clinical dose levels.

An additional NHP study was performed (study CRL-5550014), which evaluated the toxicity to DRG, spinal cord, and peripheral nerves, which specifically addresses this concern (See additional correspondence in Section 5.3).

The NHP study would be compromised if it used the proposed human delivery device, due to differences in size. However, the syringe, tubing, and needle used for all animal studies used comparable materials to the human device. Binding to the NHP injection materials was assessed (**Device Report, Module 3.2.P.2**). The dose levels used were 8.4E13 (1.1 fold proposed human dose) and 1.68E14 vg and the final study report is submitted in **Module 4.2.3.1**.

e. Please incorporate a comprehensive battery of safety assessments including both neuropathological and functional assessments (i.e., neurological examinations, electrophysiological assessments, etc.) at multiple timepoints over a study duration that enables characterization of the onset, progression and potential recovery from any toxicities.

These were included. The protocol is available in **module 4.2.3.1.**

f. Please provide justification if this study is not completed for your initial IND submission and include a comprehensive discussion of the benefit/risk profile for administration of Melpida in your proposed study subjects in the absence of this data.

The final study report is provided in **module 4.2.3.1.**

3. With regards to Study #05 (page 44; Section 15.3.1), you indicate that a number of 7-week-old WT C57Bl/6J mice receiving Melpida developed elevated liver enzyme levels and hepatocellular adenomas at 12 months. To address the risk of AAV vector integration which has been reported to cause tumor formation in mice (e.g., Li et al, 2020), please provide the following:

g. Comprehensive data regarding the incidence of tumors in vehicle control and low and high dose level groups and the time points at which they were observed.

This information is provided in the final study report for study 05 (**Module 4**), Section 4 of the Investigator's Brochure (**Module 1**), and the nonclinical overview (**Module 2.4**). Briefly, 2 males and 1 female mouse had hepatocellular adenomas by microscopic examination of major tissues/organs; up to 51% of male WT mice

naturally develop these adenomas as they age (see reference DHHS, 2019, provided in **Module 4.3**).

h. Data from analyses conducted to assess the causative nature of tumors from archived tissue such as integration analysis and histopathological assessments of tumor and surrounding normal tissues.

We assessed transgene expression in histological sections by RNAscope, reasoning that if AAV genome integration caused the tumors we would see higher transgene expression localized to the tumorigenic portion of the liver. All of the livers of the mice showing tumors showed normal or slightly below normal transgene mRNA by RNAscope, with no visual evidence of clonal homogeneity from an AAV integration event in the tumor regions.

i. A comprehensive discussion, supported by data, regarding the risk of AAV integration and oncogenesis for your clinical product to include any risk factors inherent to your target population that may impact potential tumorgenicity.

This information is included in **module 2.4**

j. Based on data collected from Study #05, we recommend that you comprehensively evaluate the potential for AAV-mediated tumor formation post-administration in Study #2020-06 (page 43/99; Section 15.3.4) and consider extending the study duration for a group of animals, as feasible per the life-span of the Ap4m1-/- mouse model, to enable further characterization of this risk. This data should be accompanied by a discussion regarding any inherent pathologies in this mouse model that may impact tumor formation and/or study readouts.

Please see the response to query 3b. Additionally, based on this feedback and the lack of literature describing this mouse phenotype, the study has been extended to at least 18 or 21 months (the study is currently at 12 months with no impact on survival). Eight-month interim data are described in **module 2.4**

4. We have the following general comments regarding your preclinical development program.

k. Regarding the preclinical vector lots used in your pivotal studies:

i. Please ensure the same assay is used to determine the concentrations of your preclinical and clinical vector lots and provide detailed information on the assays and standards that were used.

The same ddPCR assay was used for the lots manufactured by Viralgen Vector Core (VVC) ([redacted] [research grade] and [redacted] [cGMP]) and the University of North Carolina Vector Core (UNC VC). The UNC VC lots were used for the non-GLP pharmacology/efficacy studies and tittered at 9.9E13 vg/mL. The [redacted] lot was used for the 90-day GLP toxicology

study in Sprague Dawley Rats (CRL-5550008) and the GLP NHP study (CRL-5550014) and tittered at 5.43E13 vg/mL. Full details of which lots were used for each nonclinical study is available in **module 3.2.P.5.4. and Module 2.4**

ii. Please retain adequate material from each preclinical lot so that it can be retested if the assays for future clinical vector lots change. The vector dose levels administered in these preclinical studies should be recalculated based on this analysis.

Adequate retains of each lot has been stored for this purpose, as availability allows.

iii. Please provide a tabulated summary of the similarities and differences between preclinical and clinical vector lots, including vector identity and composition (e.g., capsid, regulatory elements, transgene, etc.), vector titer, proportion of empty to full capsids, presence of aggregates, formulation, production site, and overall manufacturing process. Please note that the dilution buffer, container, and delivery device should reflect what will be used clinically, as feasible.

The same vector was used in the production of the batch for the pivotal toxicology studies ([redacted]) and that proposed for the initial human clinical study ([redacted]). The manufacturing of these batches is the same other than the scale (50L vs 250L) and was conducted at the same manufacturing site, Viralgen . Detailed information regarding the manufacture is presented in module 3.2.S.2.2, module 3.2.S.3.3 and Drug Master File (DMF) [redacted]. The materials used in the production of these batches are the same and is detailed in module 3.2.S.2.3 and Drug Master File (DMF) [redacted]. The batch analysis results including vector titer, proportion of empty to full capsids, presence of aggregates is presented in **module 3.2.S.4.4 and module 3.2.P.5.4**. A tabular summary of the batches is also presented in **module 3.2.P.5.4**.

l. Please provide data supporting the reproducibility and accuracy of vector delivery using the respective delivery device and administration procedure for each pivotal preclinical study. If vector loss is observed, please provide the actual administered vector dose level in the study report and data tables.

The device for vector delivery into humans is approved (510(k)#911202). Vector delivery in the nonclinical studies used different (approved) syringes and needles with a smaller gauge to the clinical device. Vector compatibility studies across all clinical and nonclinical delivery devices showed minimal to no vector loss in conditions mimicking the dosing events. Specifics are included in **Module 2.4. and the Clinical Protocol and Pharmacy Manual.**

m. Please ensure that the technical personnel tasked with dosing the animals are appropriately trained. All instances of suspected mis-dosing should be documented in the raw data and included in the final study report.

All personnel are trained per institutional or laboratory Standard Operating Procedures (SOPs), and all mis-dosings are provided in the final study reports for each study.

n. For behavioral assessments, please provide a detailed methodology for each test to verify the objective and stringent nature (i.e., masked assessors, appropriate controls, etc.) of the testing procedure and resulting data interpretation

The behavioral assessment methodology is provided in the study protocol and/or study report for each study as appropriate. In all studies, assessors were masked to treatment and genotype, and there were vehicle control groups for comparisons.

o. Please ensure that you retain comprehensive set of tissues from your definitive studies and archive all unused tissues for possible future analysis, as feasible.

A comprehensive set of tissues were retained from the definitive Rat toxicology study (CRL-5550008) and the NHP study (CRL-5550014); unused tissues were frozen and archived. Remaining tissues from nonGLP studies (#2020-06 and #05) were similarly archived.

p. For all unscheduled deaths, please perform comprehensive clinical pathology, gross pathology and histopathology on a complete list of tissues, and other analyses, as appropriate, in order to determine the cause of death.

Comprehensive evaluation was planned to determine the cause of death for all unscheduled deaths in any study conducted with MELPIDA, and carried out whenever possible.

q. Please ensure that all attempts are made to minimize potential study bias, including: i) inclusion of appropriate control groups; ii) randomized assignment of animals to study groups; iii) appropriate staggered dosing of animals across groups; and iv) masked assessment of selected in-life and post-mortem parameters by qualified personnel.

All attempts were made to minimize potential study bias. The details are provided in each study report in **Module 4.**

r. Please provide your rationale for the preclinical dose levels evaluated and timing of study assessments and sacrifice timepoints.

The rationale for the dose levels, study assessments and sacrifice timepoints for each study is provided in the study reports for each study in **Module 4.**

s. Please provide a comprehensive justification for the proposed clinical dose levels, dose volumes and route of administration. Please note that these elements should be supported by data from your preclinical studies.

A comprehensive justification for the proposed clinical dose level, volume and route of administration is provided in **Module 2.4**, Investigator's Brochure (**module 1.14.4.1**) and the clinical protocol (**m5.3.5.2**).

t. Please provide your method of dose level extrapolation from each animal species used to humans. Additionally, please include your rationale, with supporting data, for this method. For example, if CSF volume is used in your dose extrapolation method, please provide a tabulated summary of CSF volumes for all neonatal, juvenile and adult animals used in your preclinical POC and safety studies in addition to the corresponding values in human pediatric subjects. Please provide the dose levels of Melpida using the appropriate units (e.g., vg/mL of CSF) to allow for comparison across species in your IND.

This information is provided in **Module 2.4** and the Investigator's Brochure (**module 1.14.4.1**).

2.1.2. Additional FDA Pharmacology/Toxicology Comments

1. Statements regarding the adequacy of any preclinical study to support a particular clinical trial or fulfill a specific regulatory requirement are made based solely on the information provided in your pre-IND meeting package and are considered preliminary. A final determination regarding the adequacy of the studies cannot be made without CBER review of complete materials that should be submitted in the IND.

Complete materials have been submitted for all nonclinical reports in **Module 4**.

5. In your IND submission, please provide complete study reports for all preclinical studies used to support the safety and rationale of your proposed clinical trial. These reports should include, but should not be limited to: a) a prospectively written protocol and all protocol amendments or a detailed methodology; b) a detailed description of the study design (e.g., description of the test system used, animal species/animal models, control and test articles administered, dose levels, detailed procedures for test article administration (including delivery device description), and collection of all study protocol parameters, etc.); c) results for all parameters evaluated for each animal on study; and d) your analysis and interpretation of the study data.

Complete study reports are provided for all nonclinical studies in **Module 4.**

6. For each toxicology study performed, please provide documentation showing that the study was conducted in compliance with Good Laboratory Practice (GLP) as per 21 CFR Part 58. If the study was not GLP-compliant, as directed by 21 CFR Part

314.50(d)(2)(v), you should provide a brief statement of the reason for the non-compliance in your IND submission. In addition, please specify in the study report any areas that deviate from the prospectively written protocol and the potential impact of these deviations on study integrity. Each study should: a) be conducted according to a prospectively written protocol, b) performed in as nonbiased a manner as possible, and c) have appropriate record keeping and documentation of all data.

Documentation on compliance with GLP is provided in each study report in **Module 4**. The definitive rat toxicology study (CRL-5550008) and NHP toxicology study (CRL-5550014) were performed to GLP standards.

7. We strongly recommend oversight of the conduct of all non-GLP toxicology studies and each resulting final study report by a Quality Assurance (QA) unit/person that is independent of the personnel responsible for the conduct of this study, as per 21 CFR Part 58.35. This QA oversight is important to assure study conduct according to sound procedures and to ensure the quality and integrity of the resulting data.

There was no QA oversight of the conduct of the non-GLP toxicology in C57BL/6J mice (Study #5), but scientific rigor such as randomization and masked assessments was incorporated to the full extent possible. There was QA oversight for the pivotal GLP rat study (CRL-5550008) and GLP NHP study (CRL-5550014).

8. In Module 4 of your IND, please provide a copy of all key publications cited that support the safety and rationale for administration of your investigational product in the proposed clinical trial. In Module 2 of your IND, please include a comprehensive summary for each publication. The summary should provide the reason for including the publication (i.e., how it directly supports safety/activity of your product) and a discussion regarding the comparability of the product(s) used in the publication to the final clinical product.

As there are no publications on the mouse model used to assess efficacy and the remaining information is compiled from various sources, this has not been provided.

9. Please ensure that you have adequately addressed all CBER pre-IND comments and include these responses in the IND submission.

All comments have been provided in this document and where possible also addressed and cross-referenced in the IND. This document resides in **module 1.12.1.**

10. Please provide an Investigator Brochure (IB) in the IND submission. For additional recommendations on the preparation and content of your IB, please refer to Section 7 of the document titled, E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) - Guidance for Industry (March 2018), available at:

https://www.fda.gov/regulatory-information/search-fda-guidance- documents/e6r2-good-clinical-practice-integrated-addendum-ich-e6r1.

The Investigator's Brochure is included in **Module 1.14.4.1** of the IND.

11. For a comprehensive summary regarding the preclinical assessment of cell and gene therapy products, we refer you 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; and b) the OTAT Learn Webinar Series, available at: http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm.
12. Regarding biodistribution (BD) assessment for gene therapy products, we recommend reading the 2018 International Pharmaceutical Regulators Programme (IPRP) reflection paper titled, Expectations for Biodistribution (BD) Assessments for Gene Therapy (GT) Products, available at: https://admin.iprp.global/sites/default/files/2018-09/IPRP_GTWG_ReflectionPaper_BD_Final_2018_0713.pdf.
13. The preclinical program for any investigational product should be individualized with respect to scope, complexity, and overall design, to maximize the contribution and predictive value of the resulting data for clinical safety and therapeutic activity. As recommended in Section III.B.8 of the Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products, we encourage you to explore opportunities for reducing, refining, and replacing animal use in your preclinical program. For example, it may be appropriate to use in vitro or in silico testing to complement or replace animal studies. We encourage you to submit proposals and justify any potential alternative approaches.

3. CLINICAL QUESTIONS

3.1. Sponsor Question 3a:

Does the Agency agree with the design elements and enrollment criteria of the Phase I/II trial, the dose strategy and route of administration as summarized in Section 14.4.2?

3.1.1. FDA Written Response to Sponsor Question 3a:

We have the following comments intended to better protect the safety of subjects and the design of the proposed open-label, single-arm study:

u. For a rare condition such as spastic paraplegia type 50 (SPG50) caused by the AP4M1 gene mutation with heterogenous manifestations, the more efficient clinical development path is to conduct a randomized clinical trial with a concurrent control

group and blinding as early as possible, even in the first-in- human trial. A concurrent control group with appropriate blinding:

i. facilitates interpretation of safety data and provides a comparator for assessments of safety, activity and efficacy. This will also help you to better plan for a more robust late phase trial, including more appropriate sample size estimation.

ii. may speed development of your product, by potentially enabling results from earlier phase studies to provide supportive evidence of effectiveness in support of a future marketing application.

iii. maximizes the use of valuable patient resources.

> The sponsor agrees with the desire to conduct adequate and well-controlled studies as early as possible in development. This Phase 1 study is designed to be open-labeled as it involves the administration of MELPIDA to young children via the intrathecal route where sham controls would be ethically challenging.

An adequately designed and well-controlled early phase study has the potential, depending on the study results, to provide evidence of effectiveness to support a marketing application. For additional information, please refer to our recently published draft Guidance: Human Gene Therapy for Neurodegenerative Diseases (https://www.fda.gov/media/144886/download). We therefore recommend that you modify your protocol to incorporate the following elements: randomization of study subjects, inclusion of a concurrent sham-procedure control group (e.g., performing a sham lumbar puncture without penetration of the dura and using placebo instead immunosuppression drugs, to help maintain adequate blinding of treatment group assignment), and blinding of subjects and evaluators.

> The sponsor proposes conducting this clinical study with 2 subjects, totaling 3 subjects with the subject who has already been dosed in Canada. The sponsor recognizes the robustness of a well-controlled study and will address this once the current protocol is underway.

v. You plan to measure baseline neutralizing AAV9 antibody titers at your study screening. However, it is not clear from the meeting package whether you will use this result as a patient selection criterion. Please specify whether patients with pre-existing AAV9 antibodies, including neutralizing antibodies, will be eligible and provide the rationale for either including or excluding such patients.

> Patients will be screened for AAV9 neutralizing antibody (NAb) titers in serum but not excluded due to the intrathecal route of administration being used, which is minimally impacted by systemic NAb titers (Gray SJ, Nagabhushan Kalburgi S, McCown TJ, Jude Samulski R. Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates [published correction appears in Gene Ther. 2013 Apr;20(4):465]. Gene Ther. 2013;20(4):450-459. Doi:10.1038/gt.2012.101).

w. You plan to enroll subjects with "Clinical history or examination features consistent with SPG50 and that include neurologic dysfunction." Please clarify the term "neurologic dysfunction." Please clarify whether you plan to enroll subjects with lower limb spasticity at baseline.

"Neurologic dysfunction" is a degenerative neurologic disease with loss of motor function, seizures and severe cognitive deficit.

x. You plan to evaluate only one dose level. To increase the likelihood of identifying a safe and efficacious dose, we recommend more substantial dose exploration.

The study proposes the dosing of up to 2 subjects, and is based on the availability of drug product. The single dose level equates to the maximum feasible dose that can be administered to these patients, thereby offering them the highest potential for benefit, even though this is likely to be less than the maximal efficacious dose needed. In preclinical studies in the Ap4m1 KO mouse model, a 2-fold lower equivalent dose than the proposed human dose didn't provide clear evidence for benefit.

y. You state that "Stopping criteria are based on development of unacceptable toxicity defined as the occurrence two or more Grade 3 (Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0) or higher unanticipated treatment-related toxicities." To limit the number of subjects being exposed to unknown but potentially significant risks, please revise the stopping criteria to occurrence of any Grade 3 or higher unanticipated toxicities independent of attribution.

Stopping criteria have been revised to the occurrence of any Grade 3 or higher unanticipated toxicities independent of attribution. The protocol is provided in **Module 5.3.5.2.**

z. You plan to administer several immunosuppressive drugs, including sirolimus, corticosteroid and tacrolimus before and/or after product administration. To maintain a favorable benefit-risk profile, please provide your justification with sufficient data to support the proposed dose and dosing regimen and treatment duration for each immunosuppressive drug.

This information is provided in the Investigator's Brochure in Section 6 which is located in **Module 1.14.4.1.**

aa. You plan to perform multiple lumbar punctures (LPs) to obtain cerebrospinal fluid (CSF) during the 2-year follow-up period. To minimize the risks to the subjects, please decrease the number of LPs. Please justify with data that each of the LPs post product administration is essential and unavoidable.

The number of LPs in the clinical study has been reduced to 4.

3.2. Sponsor Question 3b:

That since the ratio of central nervous system mass to whole body mass changes with age, the dose will be scaled by age correlated to brain mass and not body weight based. Does OTAT agree with both the rationale for dosing based on age (≥ 4 years old), or by approximate brain size for subjects < 4 years old (Table 6)?

3.2.1. FDA Written Response to Sponsor Question 3b:

The overall dosing strategy seems reasonable. In the IND submission, please provide sufficient data to support your proposed brain volume-based dose in children younger than 4 years of age and a fixed dose for subjects 4 years of age and older.

Data to support the proposed brain volume dose is provided in **module 2.4** and the Investigator's brochure in **module 1.14.4.1.**

3.3. Sponsor Question 3c:

That SPG50 is an infant onset progressive disease, associated with severe morbidities and caused by biallelic mutations in AP4M1. The primary outcome for the proposed clinical study is safety, however, exploratory efficacy outcomes will be collected. Does the Agency agree with the proposed safety and exploratory efficacy outcome measures, based on the known cause and natural history of the disease (Section14.4.2)?

3.3.1. FDA Written Response to Sponsor Question 3c:

We have no objection to the proposed safety and exploratory efficacy outcome measures. In addition, we recommend that you also assess Clinical Global Impression of Overall Change by Physician (CGI).

CGI has been added to the clinical protocol (**module 5.3.5.2**).

4. ADDITIONAL FDA QUESTIONS/COMMENTS:

4.1. CMC

1. Please note that as the IND sponsor you are responsible for providing all the CMC information necessary to assess product safety for the planned Phase 1/2 trial (either as part of the original submission or via a cross-referenced file). Please refer to the Guidance for Industry: Chemistry, Manufacturing, and Controls (CMC) Information for Human Gene Therapy Investigational New Drug (IND) Applications, January 2020, for our comprehensive recommendations. https://www.fda.gov/regulatory-information/search-fda-guidance- documents/chemistry-manufacturing-and-control-cmc-information-human-gene- therapy-investigational-new-drug

2. Please be aware that if you intend to reference CMC information in your IND that was previously submitted to the Agency under another IND or BB-Master File (MF), then you should clearly specify (preferably in a tabular format) the information to be referenced, including the nature of the information (e.g., reagents, testing, manufacturing, etc,), file name, reference number, eCTD module, and page number where the information can be found. This information should also be clearly stated in the letter of authorization (LoA) provided by the cross-referenced IND sponsor or MF holder.

3. Regarding the ddPCR assay that will be used to measure vector genome titer (vector strength) of your product, please note the following:

bb. The assay used to determine vector/product strength (vector genomes/ml) must be qualified prior to Phase I clinical studies. Failure to submit adequate information supporting assay suitability will result in your IND being placed on clinical hold.

The ddPCR assay protocol has undergone extensive qualification and the associated qualification report is provided in **Module 3.2.S.4.3.**

cc. Please be aware that the qualification data should be collected for the product under study, AAV9-AP4M1, and should include appropriate product-specific controls.

dd. In the IND, please provide a detailed protocol for the qualification study or the SOP used to qualify your assay, including information about the reference standards, controls, and assay optimization.

The ddPCR assay protocol has undergone extensive qualification and the associated qualification protocol is provided in **Module 3.2.S.4.3**.

ee. Please provide the study report with data documenting assay qualification, including accuracy, precision (repeatability and intra-assay precision), specificity, range, and linearity. We recommend that the precision of the assay be <15% coefficient of variation (CV).

The ddPCR assay protocol has undergone extensive qualification and the associated qualification protocol and report are provided in **Module 3.2.S.4.3.**

ff. Please describe any deviations that occurred during the qualification study.

There were no/were significant deviations during the qualification. All deviations encountered are detailed starting on page 45 of 49 of the final qualification report in **Module 3.2.S.4.3.**

gg. Please plan to validate the assay prior to the conduct of clinical studies that will assess product efficacy for licensure.

We thank the FDA for their advice. Validation of the ddPCR assay will occur following the conduct of this initial clinical study.

14. Please provide your plan to ensure that the cumulative endotoxin exposure of the pediatric subjects planned in the trial will not exceed the 0.2 EU/kg/hr (USP<85>). In the description of your plan please take into account the following when calculating the potential maximum endotoxin exposure:

hh. The endotoxin levels in the clinical batch of the product

The CoA for the clinical batch indicates endotoxin levels of <0.05 EU/mL. Assuming the product has 0.05 EU/mL, and a minimum body weight of 6 kg, a volume of 10 mL provides a total EU dose of 0.08 EU/kg in the 10 min intrathecal infusion (1 mL/min), which is well below the 0.2 EU/kg/hr limit.

ii. The acceptance criterion/limits set for endotoxin levels in the testing plan for DP lot release

The acceptance criterion for endotoxins in the drug product is ≤0.2 EU/mL.

jj. The endotoxin from the delivery devices planned for product administration

The products used for administration of MELPIDA are endotoxin free.

kk. The maximum delivery time allowed, and the maximum product volume allowed

The maximum delivery time is 10 mins with a maximum volume of 10 mL.

ll. The minimum and maximum weight expected of subjects enrolled in the highest dose cohort and the weight range expected of subjects enrolled in all cohorts

The minimum expected weight is 6 Kg, which is ~25% less than the average weight of a 1-year-old female (males are heavier). The maximum weight is not relevant for this calculation as the total dose will be 1E15 vg regardless of weight.

mm. Whether a contrast agent will be used at the time of product delivery or soon after within the hour, and the endotoxin levels expected from the contrast agent

There will be no contrast agents used.

nn. Whether a diluent will be used for product formulation in the pharmacy, and the endotoxin levels expected from the diluent

[redacted] The acceptance criterion for endotoxins is <0.2 EU/mL. Use of a diluent is not expected for this initial lot of drug product, since it was formulated to the desired concentration of 1E14 vg/mL.

oo. The acceptance criterion/limits set for endotoxin levels in the testing plan for diluent lot release

The acceptance criterion for endotoxins is <0.2 EU/mL in the diluent, which will be below the endotoxin limit of an intrathecal drug as long as the injection volume does not exceed 1 mL per kg body weight.

15. You propose to administer the drug product intrathecally using a Pajunk Atraumatic Sprotte Needle, 60" Marquette Medical IV extension tubing, Braun Discofix 4-way stopcock, 20 mL BD syringe, and an infusion pump. To ensure the devices are being used safely in the context of your proposed clinical study, please provide the information below in your future IND for all delivery devices that will be used to administer the drug product in your proposed clinical study.

pp. Please indicate whether the devices will be supplied by the sponsor or clinical site.

Clinical site.

qq. For each device that is FDA-cleared or -approved, please provide the following:

i. The submission number (e.g., 510(k) or PMA number).

These details are provided in **Module 2.4**, the Investigator's Brochure (**module 1**) and the clinical protocol and pharmacy manual (**module 5**). The needle for intrathecal administration is a US FDA approved device being used per labeling.

ii. A comparison of the cleared or approved indications for use and how the devices will be used in the clinical study, as well as a risk assessment for the proposed use in the clinical study.

The device will be used for the administration of MELPIDA rather than anesthesia. Device compatibility was conducted to confirm MELPIDA was not impacted by the needle selected for administration. The device compatibility report is provided in **Module 3.2.P.2.**

iii. Define the essential performance criteria of the device constituents required for the safe use of the device in the context of the IND. Please determine if the defined

essential performance criteria are within the cleared or approved indications and specifications and provide performance testing to verify the essential performance criteria if the devices are being used outside of the cleared indication or environment of use.

The administration device is being used within the cleared indication.

iv. If you wish to leverage data from the 510(k) or PMA submission, please provide a letter of authorization for cross reference to that submission.

Not applicable; all devices are approved and used for their intended purpose

rr. For each device that is not previously cleared or approved, please provide the following:

i. A detailed description of the delivery device, including, but not limited to: a description of each component and any accessories that will be used with the device; the manufacturer and trade name; the principle of operation; pictures, diagrams, or engineering drawings; materials of construction; and identification of directly and indirectly (e.g., via fluid path) patient-contacting components.

Not applicable as the device for administration is being used as indicated.

ii. Information to establish safety of the delivery device for the proposed clinical use, including but not limited to biocompatibility, sterility, endotoxin, packaging, shelf life, electrical safety (if applicable), electromagnetic compatibility (if applicable), software (if applicable), essential performance requirements (EPRs), and performance testing demonstrating that the device will accurately deliver the drug to the target site within acceptable limits, and identification of how the device may cause harm or may fail to accurately deliver drug during clinical study. Please note that examples of infusion pump EPRs include but are not be limited to delivery accuracy and/or consistency, bolus dose accuracy (if applicable), and delivery status feedback (e.g., visual, audio, or tactile feedback for delivery start, delivery progress, unintended stoppage, and delivery complete).

Not applicable.

iii. We recommend that you provide the information in c.ii above in the form of a tabulated risk analysis with references (hyperlinked) to corresponding test reports or other supporting information or test reports provided in your submission or cross referenced elsewhere, as applicable.

Not applicable.

iv. If you intend to cross reference a device master file regarding any of this information, please provide a letter of authorization for the master file with the specific location of the information being referenced within the master file.

Not applicable.

v. Please also refer to the following guidance documents for additional information:

- Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process" (https://www.fda.gov/regulatory-information/search-fda- guidance-documents/use-international-standard-iso-10993- 1-biological-evaluation-medical-devices-part-1-evaluation- and).
- Infusion Pumps Total Product Lifecycle (https://www.fda.gov/regulatory-information/search-fda- guidance-documents/infusion-pumps-total-product-life-cycle).
- Recommended Content and Format of Non-Clinical Bench Performance Testing Information in Premarket Submissions (https://www.fda.gov/regulatory-information/search-fda- guidance-documents/recommended-content-and-format-non-clinical-bench-performance-testing-information- premarket).

ss. We recommend that the clinical study protocol include requirements to capture any delivery device failures/malfunctions.

The clinical study manual will provide guidance on how to capture and report delivery device failures/malfunctions.

16. The drug product, Melpida, is intended to treat Spastic Paraplegia Type 50, which is a rare disease. As discussed in the draft FDA guidance document “Human Gene Therapy for Rare Diseases,” from January 2020 (https://www.fda.gov/regulatory-information/search-fda-guidance- documents/human-gene-therapy-rare-diseases), an adequately designed and well-controlled early phase study has the potential, depending on the study results, to provide evidence of effectiveness to support a marketing application. As the devices used to administer the drug product are an important factor in demonstrating the safety and effectiveness of your investigational treatment, we strongly recommend that your clinical studies utilize the delivery devices you would intend to be used with the drug product upon licensing. Furthermore, we recommend that you consider your licensing and labeling strategy as it relates to the devices used to administer the drug product early in your product development to ensure that appropriate devices would be available (i.e. FDA- cleared or approved) to deliver the licensed product. Additional information may be needed depending on your proposed delivery devices and marketing strategy, and you may need to work with a device manufacturer(s) to ensure the feasibility of this approach. We recommend communication with OTAT early in product development regarding your delivery device strategy.

Noted. The sponsor will communicate with OTAT on this issue once the IND is active.

17. Please plan to conduct device compatibility studies to support vector stability in the delivery devices. Please note that failure to submit sufficient information supporting vector stability in the delivery device will result in your IND being placed on clinical hold. In the IND, you should describe the dose preparation (in the pharmacy) and provide the report of the delivery device compatibility study performed to simulate the procedure for dose preparation and product administration at the clinical site.

The sponsor has provided the device compatibility protocol and report in **module 3.2.P.2** and a pharmacy manual in **module 5.3.5.2** for dose preparation.

Please note the following recommendations for the conduct of the study:

tt. Please ensure that the product lot used in the compatibility study is manufactured and formulated in a manner comparable to the clinical lot(s). The supporting manufacturing, qualification and testing information for the product lot used in the compatibility study should be submitted in the IND.

The product lot used for the compatibility study is [redacted] which is the drug lot used for all the pivotal toxicology studies and was manufactured using the same process as the clinical lot ([redacted]). The only difference was the batch size.

uu. Please assess the amount (vector genomes) and activity (infectious units or potency) of the product following exposure to the clinical delivery device.

The device compatibility study report is provided in **module 3.2.P.2**.

vv. Please be aware that the study should include tests conducted over the planned dose-range and should take into account the expected time between thaw of the product and infusion.

The device compatibility study was conducted at temperatures between 2 and 8°C for a hold time of 4 hours to account for the time between thawing and administration to the patient.

ww. Please perform device compatibility testing for the product under conditions that mimic the clinical scenario (i.e., hold time, formulation/concentration, temperature, presence of contrast agent, etc.); the study design should consider the worst-case scenario (e.g., low product concentration, maximum hold time).

This has been accounted for. Please see Device compatibility report in **module 3.2.P.2**.

xx. The device compatibility data should support the post-thaw product handling instructions provided in the Instruction to Pharmacy/Pharmacy Manual document that is supplied with the product to all the clinical sites.

The device compatibility study supports the handling in the Pharmacy Manual.

18. You should describe how the drug product will be shipped from the site of manufacturing to clinical sites in the US. You should conduct appropriate shipping study (using representative material) in order to evaluate the impact of shipping on product quality (product purity, sterility and potency). Accordingly, please plan to develop mitigations plans for temperature excursion. Please note that the shipping study should be qualified to support late phase studies and validated by licensure.

Shipping instructions are provided in the pharmacy manual in **Module 5.3.5.2.**

19. If a diluent will be manufactured to support the preparation of the final dose in the clinic, please describe the manufacturing and release testing plan for the diluent (i.e., specifications for diluent release). This information should be documented under Section 3.2.P-Diluent, separate from the information for the DP (3.2P- Vector). Also, please ensure that the diluent meets the requirements for subvisible particulates per USP <787>. The IND should also include information on how you will monitor the stability of the diluent during storage and shipping.

The manufacturing and testing of the diluent are described in a separate set of 3.2.P sections “3.2.P-diluent”. The diluent meets the requirements for subvisible particulates per USP <787>. The diluent sections detail stability monitoring of the diluent.

5. SUBSEQUENT INTERACTIONS/CLARIFICATIONS OCCURRING AFTER PREIND RESPONSES AND PRIOR TO IND SUBMISSION

5.1. Sponsor Email May 17, 2021

The sponsor requested clarification on the PreIND WROs as follows:

- Question 2, response 3d concerning extending Study#2020-06 for the life span of the Ap4m1-/- mouse, currently planned for 12 months:

  We are planning to submit with 8 months of in-life efficacy data in the KO mouse model, along with 12 months terminal data in WT mice with the IND submission planned for 4Q this year. We support extending Study #2020-06 for an additional 6 months and want to confirm those data could be submitted once the IND is active,

and that clinical dosing would not be suspended until these 18 month data were submitted.

- Question 2, response 2 concerning the recommendation to evaluate NHPs with Melpida for DRG toxicity.

  We are actively discussing a study to evaluate DRG with Melpida and will submit a protocol by early June to the review team for feedback prior to commencing additional work. Please confirm this would be acceptable.

Agency feedback to these questions is included under Section 5.3.

5.2. Sponsor Email May 20, 2021:

The sponsor raised a concern regarding amount of drug product available to do the study the review group recommended in a large animal model – regardless of species (dog, pig, NHP). As this is a charitable organization, there is only one batch available.

Therefore, we also want to ask the CMC reviewer if stability could be performed every 6 m out to 3 years (36m), which would free up about 9 vials for performing additional nonclinical work. We anticipate all 3 subjects would be dosed within the first 12 months of the clinical study starting, and AAVs are known to be highly stable.

Response received by email on 24 May, 2021:

- **Considering the limited availability of the single clinical batch, your proposal to assess product stability every 6 m out to 3 years (36m) is reasonable.**
- **Please note that In lieu of sterility testing beyond T=0 (in your stability plan), you may consider container closure integrity testing. Please refer to FDA's 2008 guidance "Container and Closure System Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products."https://www.fda.gov/regulatory-information/search-fda-guidance-documents/container-and-closure-system-integrity-testing-lieu-sterility-testing-component-stability-protocol. This will approach will further save samples from the clinical batch for the planned study.**
- **Please plan on adequate retains from the clinical batch for future analyses (e.g., comparability studies to support future manufacturing changes).**

5.3. Formal amendment to PIND PTS#PS006503 July 2, 2021

The sponsor submitted a cover letter and a proposal for the design of the NHP study that the agency suggested in the PIND WROs:

The purpose of this submission is to request feedback/written confirmation **by 9 July** that the NHP study outlined below will be acceptable to support the first-in-human clinical study for patients with SPG50, following the agency's PIND response #2 to Sponsor's

Question 2 concerning the recommendation to evaluate non-human primates (NHPs) with Melpida for dorsal root ganglia (DRG) toxicity (Agency's response below).

Feedback received 14 May 2021 pertaining to PTS PS006503; meeting ID 13219:

2. *Recent published data indicates the potential for AAV-mediated toxicity in the dorsal root ganglion (DRG) and peripheral nerves in non-human primates following intrathecal administration (e.g., Hordeaux et al, 2020). At this time, it is unclear whether rodents are sufficiently sensitive to adequately characterize these toxicities. As a result, given the nature of your clinical product, route of administration, and target population, we recommend that you comprehensively evaluate the potential for vector biodistribution and toxicity in the DRG, spinal cord, and peripheral nerves of NHPs in addition to your ongoing GLP rat study (Study #CRL555008). We have the following additional details regarding the design of this study:*
    a. *The NHP study should use the intended clinical delivery device, administration procedure and dose levels that bracket the range of proposed clinical dose levels.*
    b. *Please incorporate a comprehensive battery of safety assessments including both neuropathological and functional assessments (i.e., neurological examinations, electrophysiological assessments, etc.) at multiple timepoints over a study duration that enables characterization of the onset, progression and potential recovery from any toxicities.*
    c. *Please provide justification if this study is not completed for your initial IND submission and include a comprehensive discussion of the benefit/risk profile for administration of Melpida in your proposed study subjects in the absence of this data.*

<u>The proposed NHP study addresses the agency's feedback with the following design:</u>

Title: An Acute Intrathecal Injection Toxicity Study Of A Test Item In The Cynomolgus Monkey (CRL-341547).

This is a non-GLP study where Melpida will be administered as a single one-time intrathecal (IT) dose to 6 male NHPs ~4 years of age, with a 60-day observation period post treatment. An immunosuppression regimen will be provided consisting of methylprednisolone succinate (10 mg/kg iv, 30 min infusion) immediately preceding surgery, methylprednisolone acetate (1 mg/kg, IM) with the first dose the morning preceding surgery and then daily for 60 days post dose, and rapamycin (0.01 mg/kg IM) twice daily starting 2 weeks prior to surgery and continuing for 60 days.

Group (N, sex)	Total Dose (vg)	Dose Volume (mL)	Dose Conc (vg/mL)	Human Dose Equivalent	Evaluations
1 (N=2 males)	Vehicle	1.55	0	N/A	• Mortality/morbidity twice daily, daily food consumption, weekly body weight,

2 (N=2 males)	8.42E13	1.55	5.43E13	= Human Dose (1E15 vg)	• Neurological examinations pretreatment, 4 to 6h post dosing, Day 2, 7, 28 and ~60, nerve conduction velocity (NCV) on Day 28 and ~60, • Hematology, clinical chemistry and urinalysis pretreatment, Day 2, Weeks 1 and 4 and 60, • Cytokines pre-dose Day 1, then 30 min, 4 and 24h post dose, • Histopathology: brain, spinal cord, DRG, liver, spleen, lung, kidney and heart, • Tissue bioanalysis: brain, spinal cord, CRG, liver spleen, kidney, skeletal muscles, sciatic nerve, testis, thymus and heart,
3 (N=2 males)	1.68E14	3.1	5.43E13	= 2 x human dose (2E15 vg)	

NB: a 7th NHP will be available as a replacement.

While the ongoing GLP rat toxicology study is viewed as the pivotal toxicology study for Melpida, this NHP study was designed to supplement the rodent study to identify any severe adverse effects that would preclude initiation of a human trial due to an unacceptable risk:benefit.

The proposed study will utilize a single time point of 60 days for post-mortem assessment, which is within the 1 to 6 month window reported by Hordeaux et al (2020) when DRG toxicity findings were consistently observed. We propose to dose 2 NHPs with a dose equivalent to the target 1E15 vg human dose of Melpida. Two additional NHPs will be dosed with a 2x higher dose equivalent to a 2E15 vg human dose. The higher dose represents a 2x overdose that will be achieved by a higher-than-typical injection volume in the NHPs, and this is a maximum feasible dose that can be tested in NHPs due to injection volume limitations and an inability to concentrate Melpida further. Two NHPs will be injected with vehicle, as control comparators.

The number of animals reflects the limited vector available; there will be no remaining vector to conduct further studies or additional NHPs, in order to have enough available to dose at least a single patient in the clinic. The proposed study includes 3 subjects but this will be dependent on the product yield (amount and final concentration) at the end of the current manufacturing run.

This NHP study is starting mid-July at Charles River and we are requesting feedback prior to this mid-July date. We are able to start this early because (1) these NHPs were a charitable gift from another sponsor who decided they didn't need them (which was a blessing for this program, as the cost involved was beyond our charitable funds), and (2) Charles River has this slot available; no other slots are available until early 2022. IND submission is expected September 2021 with clinical dosing shortly thereafter. We expect to file the IND before the final toxicology report from the NHP study is available. The IND will be filed with an interim report on in-life data to assure that no serious adverse clinical findings are observed, and the IND will be amended afterwards with the final report including histological findings when available.

Agency Feedback to this request was received July 8, 2021 as follows:

Pharmacology/Toxicology

1. Please note that the adequacy of the proposed NHP study and Study #2020-06 to address the safety and activity of your product for the clinical trial proposed is contingent upon review of the complete data submitted in the IND and a corresponding assessment of the overall benefit/risk profile for Melpida. If significant issues are identified during the review of your IND, additional preclinical data may be needed. In your IND, please ensure that you provide a point-by-point response to our comments provided under CRMTS# 13219 and this amendment.

Point-by-point responses to the agency's comments are included in this document which is in **module 1.12.1.**

2. For your NHP study evaluating DRG and peripheral nerve toxicities, we note that a limited number of animals are available for use and you propose to evaluate two dose levels, one equivalent to the proposed clinical dose level (1E15 vg or 7E12 vg/mL of CSF per Table 5 of your Pre-IND briefing package) and one 2X higher dose level, over the course of 60 days. You propose to submit interim data at the time of the IND that will include only in-life assessments evaluating any significant adverse clinical findings.

We refer you to comments 2 and 4 under Question 2 of CRMTS#13219 and the following additional comments regarding your proposed NHP study:

a. We recommend that you use dose levels that bracket the proposed clinical dose level and are equivalent to your pivotal ongoing proof-of-concept (POC) study #2020-06 (1.25E11 vg/animal or 3.6E12 vg/mL of CSF and 5E11 vg/animal or 1.42E13 vg/mL of CSF per Table 5 of your Pre-IND briefing package).

The doses chosen were 1.1-fold above the proposed clinical dose and 2-fold over the clinical dose (8.4E13 and 1.68E14 vg respectively).

b. You indicate that only one timepoint, 60 days, will be used for post-mortem analysis. If feasible, we recommend that you consider extending the study to 90 days post-administration. Furthermore, please note that using a single time point will not allow for an assessment of the onset, progression and potential resolution of any toxicities.

The study was extended to 90 days.

c. We encourage you to submit your IND with comprehensive gross and histopathological assessments of a comprehensive list of tissues, including the DRG and peripheral nerves. However, if complete terminal assessments (e.g., gross pathology, histopath, etc.) will not be ready at the time of IND submission, please be sure that you provide comprehensive in-life data in your IND including, at a minimum, body weight, clinical observations and comprehensive functional assessments (i.e neurological and electrophysiological assessments, etc) conducted at multiple timepoints in your interim report. For these assessments, please provide a detailed methodology for each test to verify the objective and stringent nature (i.e. masked assessors, appropriate controls, etc.) of the testing procedure and resulting data interpretation. Please note that these assessments will be especially important considering you only have a single necropsy timepoint.

Gross and histopathological assessments of tissues at both doses at 94 days are included in the CRL-5550014 report.

d. Please be sure to archive a comprehensive list of tissues, including DRG and spinal cord tissue, from animals in this study for any future analysis.

All tissues were archived.

e. As stated in our previous comments, please ensure that all attempts are made to minimize potential study bias, including: i) randomized assignment of animals to study groups; ii) appropriate staggered dosing of animals across groups; and iii) masked assessment of in-life and selected post-mortem parameters by qualified personnel.

All attempts were made to minimize the potential for study bias and are included in the study report for CRL-5550014 in **Module 4.**

f. We note that this will be a non-GLP study. We strongly recommend oversight of the conduct of all non-GLP toxicology studies and each resulting final study report by a Quality Assurance (QA) unit/person that is independent of the personnel responsible for the conduct of this study, as per 21 CFR Part 58.35. This QA oversight is important to assure study conduct according to sound procedures and to ensure the quality and integrity of the resulting data.

The sponsor updated this study to be conducted in accordance with GLP guidelines, with associated QA oversight.

3. With regards to you request for clarification on Question 2 comment 3d concerning extending Study #2020-06, we tentatively agree with your proposal to submit 8 month interim data from KO mice and 12 months terminal data in WT mice for Study #2020-06 in your IND. We also refer you to comments 3a-c under Question 2 of CRMTS# 13219.

The 12-month report for this study is included in **Module 4**

5.4. Formal amendment to PIND PTS#PS006503 19 September, 2021

Dear CMC Reviewer

Following on from our PIND meeting (PTS#PS006503), we have additional CMC questions that impact our AAV9/AP4M1 product (MELPIDA) which is the subject of an upcoming IND, and request a response from the agency in order to satisfy the CDMO, who requires these responses prior to releasing product.

MELPIDA is intended for pediatric patients with extremely rare neurological conditions. The final fill-finish process involves the application of a semi-manual process where a sterilizing filter is present immediately upstream to the fill. The fill is conducted in an ISO 5 Biological Safety Cabinet with a single operator conducting the fill in a short duration (due to the limitations of material). The ISO 5 BSC is located within an ISO 7 clean room.

The following queries originates from the relatively small number of vials from our process (approximately 50 total vials at a 1 mL fill volume) and the significance of product to treat patients.

A summary of the overall manufacturing process for AAV9/AP4M1 (MELPIDA) is provided as an attachment following these questions for reviewer convenience:

QUESTION 1:

We would like to propose a sampling scheme departing from EP 2.6.1 / USP<71>, in consideration of USP and FDA documents. Specifically, these documents include:

- "Amendments to Sterility Test Requirements for Biological Products" published by FDA in 2012, and
- USP <1071> (though we remain cognizant to the technologies addressed – rapid versus traditional microbiology)

Out of an awareness of these publications and the controls in our process, we propose the following:

1. [redacted] This scheme enables us to capture the integrity of the filling environment for the duration of the process.

2. If an unplanned intervention is encountered during the fill, we will include the vial immediately after such as a part of the sterility testing.

3. To provide an additional layer of assurance, we will conduct full USP <71> testing for the formulation buffer, which is filled in the same manner to serve as a drug product diluent, to ensure components retain sterility.

This strategy allows us to consider the potential environment of fill throughout the duration, and enable us to identify the potential impact of unexpected interventions. We believe this strategy encompasses the intent of Amendments to Sterility Test Requirements for Biological Products to assure both product integrity and feasibility to manufacture clinical doses.

Does the FDA agree to the proposed sterility testing plan?

QUESTION 2:

We would like to propose a sampling scheme departing from EP 2.9.19 and USP<788>.

Specifically, we would like to propose to use a total of 5 vials of product for the particulate matter determination per the following:

1. We will collect 5 vials throughout the filling process. This scheme enables us to capture the potential subvisible particulates throughout the duration of the aseptic filling process.

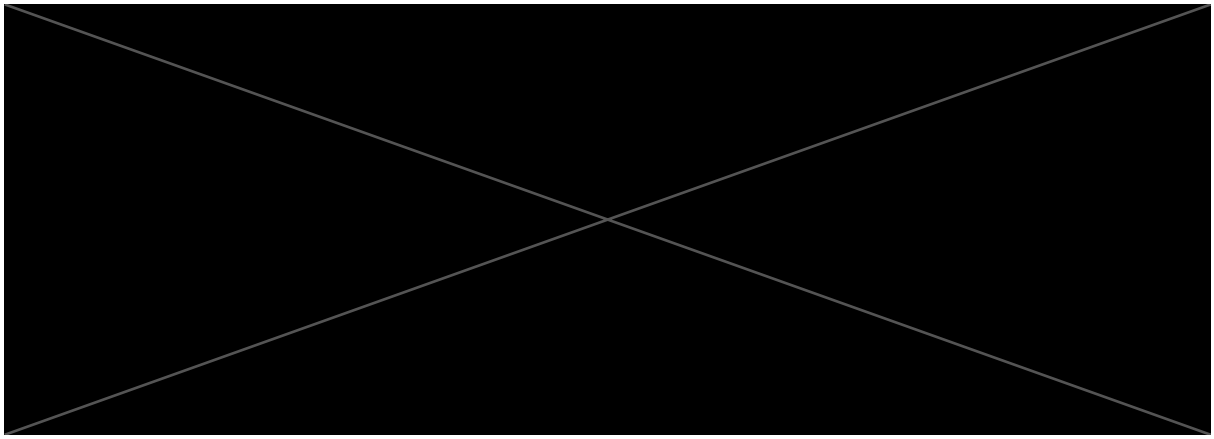
2. These 5 units will be combined and tested to EP 2.9.19 and USP<788>.

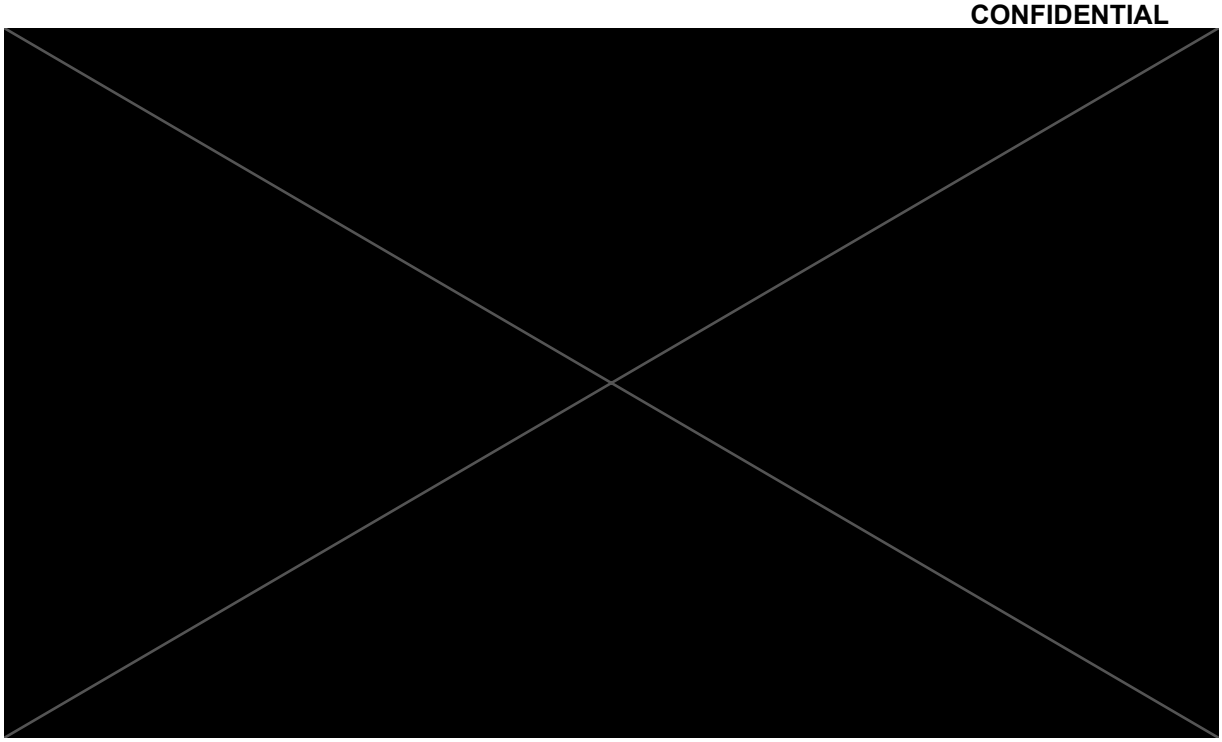
Does the FDA agree to the proposed subvisible particulate testing plan?

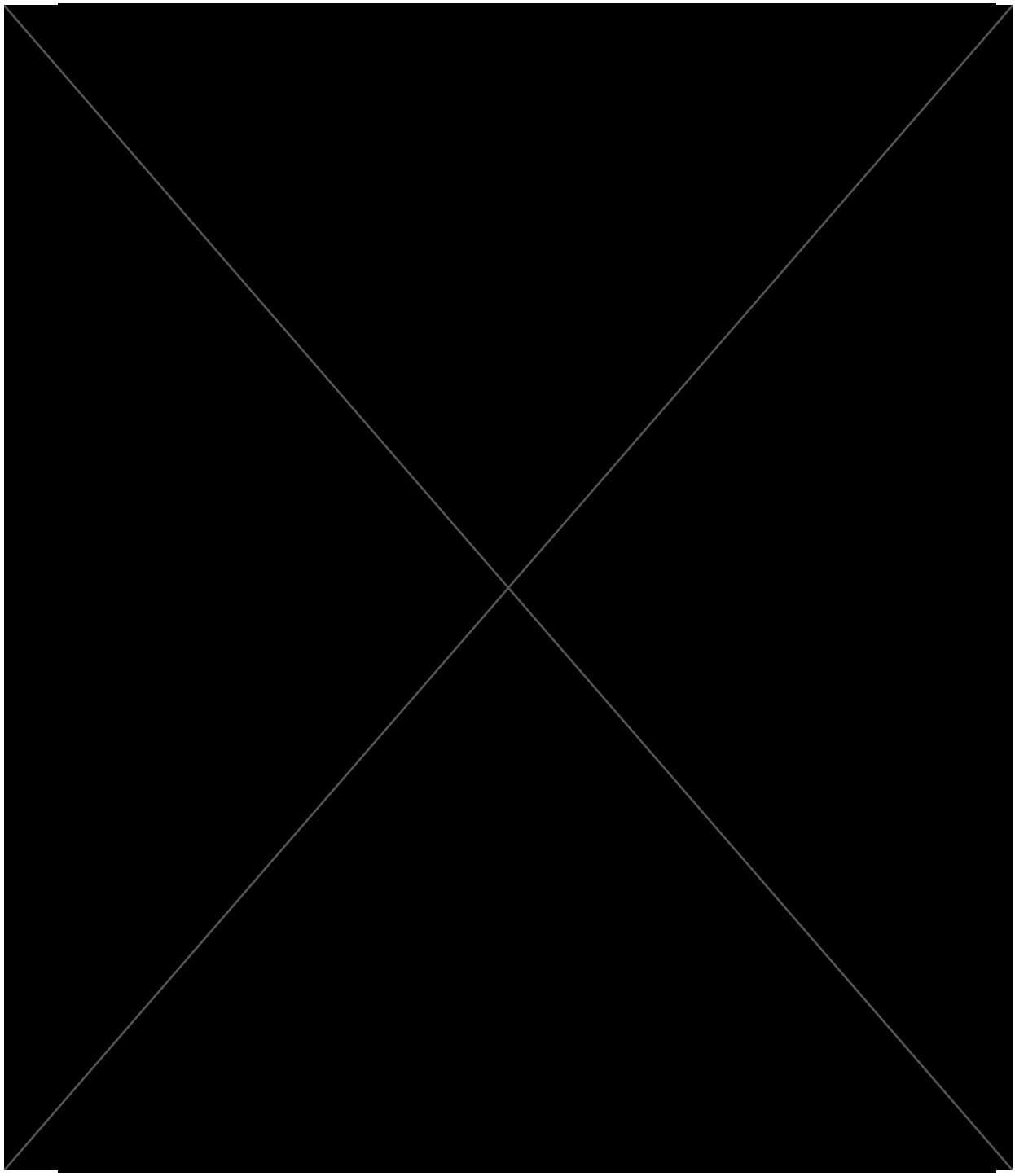
QUESTION 3:

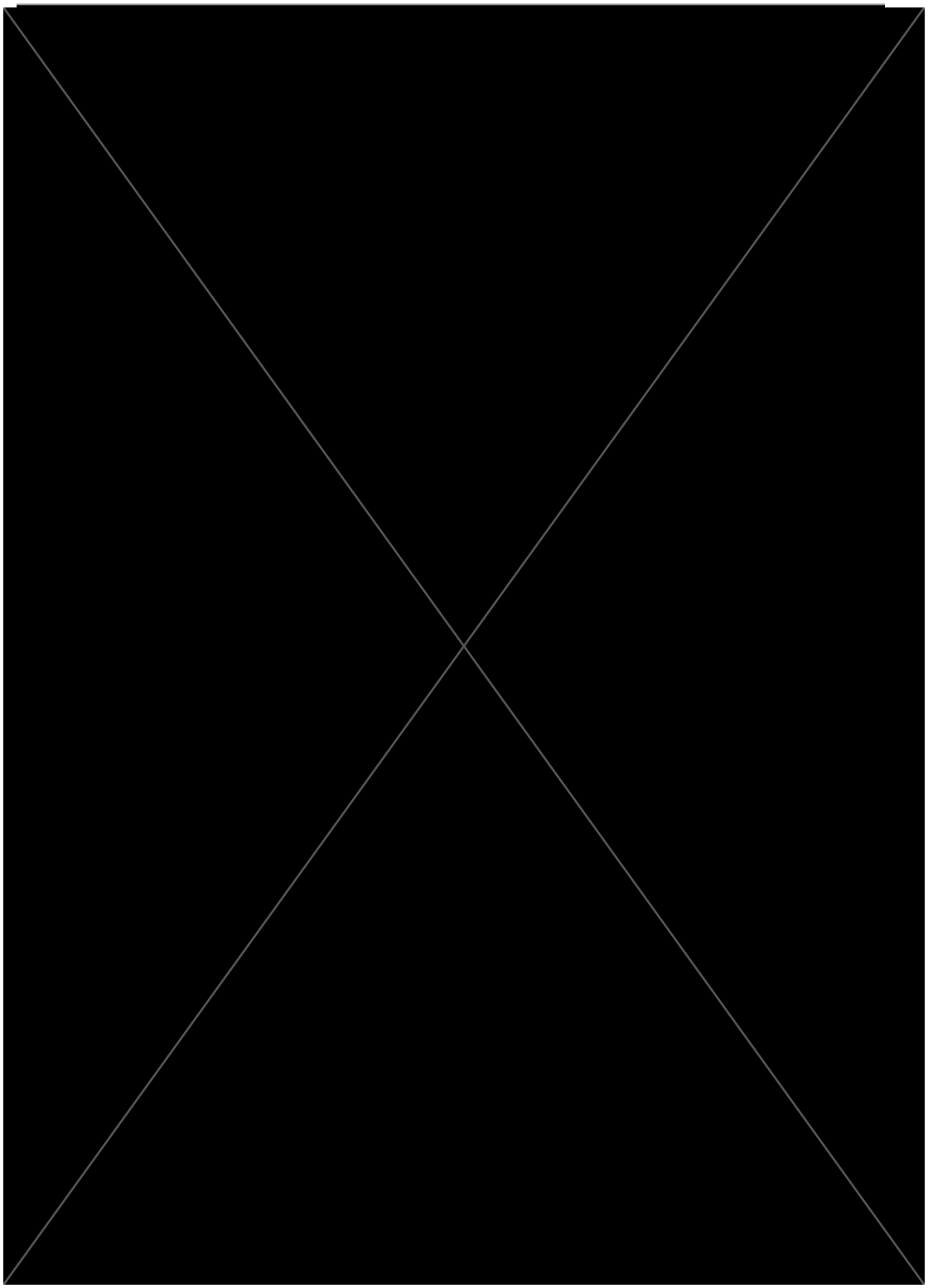
We would like to propose a stability plan for the clinical trial batch (Batch No. [redacted]) inclusive of the 0, 6, 12, and 24 month timepoints. This is due to the design of the clinical trial which is a single injection to a very small cohort of patients (1-3 total patients); therefore, the proposed stability plan covers the intended duration of use of the clinical trial batch (Batch No. [redacted]).

Additionally, this approach is supported by the stability program for the pivotal nonclinical batch (Batch No. [redacted]) which included assessment at the 0, 3, 6, 9, 12, 18, 24, 36, 48, and 60 month timepoints. It is noteworthy that the manufacturing process for Batch No. [redacted] are identical other than the scale. Batch No. [redacted] has remained within stability parameters through 6 months.









Question 1:

Your proposal of sampling vials for sterility testing from the beginning, middle and end of the vailing process might be acceptable when manufacturing a batch able to treat only a small number of subjects in early phase studies. Please indicate the number of subjects

you anticipate will be treated with each product batch in your IND. The sampling plan should also be documented in the IND when describing the methodology for release testing of Drug Product. However, please be aware that for licensure, you need to follow the guidelines provided under USP<71> for the sampling size.

There will be sufficient product to treat 2 patients from lot [redacted]. The sampling plan is documented in the IND in **Section 3.2.P.5.4 and Section 3.2.P.5.6**.

Question 2:

Due to sample constraints, we recommend that you test the final drug product according to the USP <787> general chapter which allows for smaller test product volumes and smaller test aliquots to determine particulate matter content in lieu of USP<788>. Also, if you intend to use a diluent for the final formulation of the product in the pharmacy, then please ensure that the testing of the diluent is performed according to compendial USP<788> and related limits should apply as the diluent would not be a therapeutic protein (USP<787>) or have volume limits for testing (as you have noted for the DP).

Subvisible particles tested according to USP<788> and EP 2.9.19 with 5 vials of Drug Product (5 mL) with a 1/5 dilution The sampling plan for the drug product is documented in the IND in **Section 3.2.P.5.4 and Section 3.2.P.5.6.** The diluent was tested to USP<788> with its related acceptance criteria as presented in **Section 3.2.P.5.1-diluent and Section 3.2.P.5.4-diluent**.

Question 3:

We do not have adequate information regarding your prior stability conducted study using Batch No. [redacted] to determine if your stability plan for the clinical batch (Batch No. [redacted]) is appropriate. Please provide all supporting stability data during the IND submission. We will evaluate the adequacy of the stability testing plan and data collected during the IND review. We have the following recommendations regarding your stability testing plan for the clinical GMP material:

All available drug product stability information is provided in the IND, **Section 3.2.P.8.1 and Section 3.2.P.8.3.**

a. Please set acceptance limits in your stability plans. Please note that under 21 CFR 312.23(a)(7)(ii), you must conduct stability testing in all phases of the IND, to demonstrate that the product is within acceptable chemical and physical limits for the planned duration of the proposed clinical investigation. Considering that you may have limited stability data at the time of IND submission, the acceptance criterion for each measure in the stability plan should consider the release testing data/manufacturing history, criticality of the product attribute and assay variability.

The stability specification including tests and acceptance criteria is presented in **Section 3.2.P.8.1.**

b. We recommend additional testing time points in your plan; i.e., 3, 9 and 18 months, in addition to the planned testing at 6 months and annual testing. Please note that the initial DS and DP shelf-life must be supported by real-time stability data generated using representative lots (i.e., similar manufacturing scale, release testing and storage conditions, etc.).

The stability protocol for Batch No. [redacted] is presented in **Section 3.2.P.8.1** and includes timepoints at 0, 3, 6, 9, 12, 18, 24, 36, and 48 months. Real time stability data through 9 months is provided for Batch No. [redacted] in **Section 3.2.P.8.3.** Due to limited material, the stability protocol for Batch No. [redacted] includes timepoints at 0, 6, 12, and 24 months (**Section 3.2.P.8.1**). Batch No. [redacted] conditions.

c. In addition to the real-time stability under normal storage conditions, please also assess the stability of the DP under accelerated storage and forced degradation conditions to allow for the identification of stability-indicating assay early in the clinical development.

We thank the Agency for their advice. In subsequent stability testing the drug product will be assessed under accelerated storage and forced degradation conditions.

d. Please monitor the long-term stability of all product lots (i.e., engineering, IND-enabling, clinical GMP lots and reference lots).

The long-term stability of the two primary batches of drug product produced to date are being monitored, Batch No. [redacted] (pivotal nonclinical studies) and Batch No. [redacted] (proposed initial clinical study). Information on the stability of these bathes is provided in **Section 3.2.P.8.1 and Section 3.2.P.8.3.**