About the PaVe-GT AAV9-hPCCA Type-C Documents

The following documents are communications between the National Institutes of Health (NIH) National Center for Advancing Translational Sciences (NCATS) and the U.S. Food and Drug Administration (FDA) Center for Biologics, Evaluation and Research (CBER) regarding a Type-C meeting. The Type-C meeting focused on follow up discussions after the pre-IND meeting for the development of AAV9-hPCCA (NCATS-BL0746), a gene therapy for propionic acidemia (PA) resulting from a deficiency of Propionyl-CoA Carboxylase, alpha subunit (PCCA) as part of the Platform Vector Gene Therapy (PaVe-GT) program.

PaVe-GT is a pilot project testing the hypothesis that the efficiency of gene therapy trial startup can be significantly improved by using similar processes across gene therapies for four different rare diseases. An important goal of PaVe-GT is to share project results and lessons learned with the public in such a way that the information is useful to any party interested in developing a gene therapy efficiently. Specifically, we will make processes, study results, regulatory documentation and knowledge gained from the PaVe-GT program publicly available. To ensure access to the latest information, please visit the PaVe-GT website, subscribe to project updates, and explore the full set of available resources at pave-gt.ncats.nih.gov

Some portions of this document—primarily sections that are highly specific to PCCA-related PA and therefore not relevant to other AAV gene therapy efforts—have been formatted, edited or redacted to improve the clarity of materials, and/or support other project objectives. Modified sections are typically identified with italics, brackets, and highlighted *[as shown here].* The text within the brackets describes the original content. It is important to note that these programs are continually evolving, and some information has changed since the Type-C meeting. Some of the data presented in the package below have now been published elsewhere.

Disclaimer: NCATS, NHGRI, and NIH provide no warranties, representations or guarantees that PaVe-GT resources will work for any given project or disease condition. The information is specific to each program, and the following documents are meant to serve only as examples. The mention of trade names, commercial products and organizations does not imply endorsement by the U.S. government. Further, NIH disclaims any liability and provides no indemnification. For a full list of terms and conditions for use of PaVe-GT resources, visit pave-gt.ncats.nih.gov/

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Documents

October 2nd, 2024

U.S. Food and Drug Administration
Center for Biologics Evaluation and Research
Document Control Center
10903 New Hampshire Avenue
WO71, G112
Silver Spring, MD 20993-0002
Copy: *[Name of Regulatory Project Manager]*

Re: *[FDA-assigned Pre-IND number]*
Sponsor: National Center for Advancing Translational Sciences (NCATS)
Drug name: AAV9-hPCCA
Indication: Treatment of *PCCA*-related propionic acidemia (PA)
Re: Request for Type C meeting

Reference is made to the Pre-IND Meeting to support questions related to the development program for AAV9-hPCCA for the treatment of *PCCA*-related PA held on July 10, 2023, and associated meeting minutes sent on August 9, 2023. A clarification to the pre-IND meeting minutes was sent by the Sponsor on 13 October, 2023. An INTERACT Meeting was also held with the Agency on July 14, 2021 *[FDA-assigned INTERACT number]*.

In accordance with this notification, NCATS hereby requests a Type C meeting to obtain FDA feedback on specific chemistry, manufacturing, and control (CMC) and clinical questions for the AAV9-hPCCA investigational product. Specific questions are included in the Type C Meeting Request. A comprehensive Meeting Briefing Package will be provided not later than 47 days before the scheduled meeting.

Should you require any additional information, please contact me at *[phone number of primary contact],* or via email at *[email address of primary contact]*. If I am unavailable, please reach out to *[name of secondary contact]* at *[phone number of secondary contact]*, or via email at *[email address of secondary contact]*.

Sincerely,

[Signature, name and contact information of primary contact]

DISCLAIMER: This information may no longer be applicable due to subsequent improvements.

Type C Meeting Request

Center for Biologics Research and Evaluation, Office of Therapeutic Products

Request Date:	October 2nd, 2024
Pre-IND Number:	*[FDA-assigned pre-IND number]*
Drug Product:	AAV9-hPCCA
Formulation:	Sterile, aqueous buffered solution composed of the AAV9-hPCCA drug substance formulated in *[buffer composition]*.
Sponsor:	National Center for Advancing Translational Sciences 9800 Medical Center Drive Rockville, MD 20850 Ph: 301-594-8966

Confidentiality Statement

DISCLAIMER: This information may no longer be applicable due to subsequent improvements.

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1 APPLICATION NUMBER

[FDA-assigned pre-IND number]

2 PRODUCT NAME

AAV9-hPCCA; additional name, identifier: NCATS-BL0746.

3 CHEMICAL NAME, ESTABLISHED NAME, AND/OR STRUCTURE

AAV9-hPCCA is an Adeno-Associated Virus 9 vector expressing a functional human codon optimized cDNA encoding the Propionyl-CoA Carboxylase, alpha subunit (*PCCA*), under control of the *[specific]* promoter.

The AAV9-hPCCA vector transgene schematic and description are shown in **Figure 1**. The full plasmid map and sequence for the pAAV9-hPCCA plasmid, along with information on the helper plasmid and Rep-Cap plasmid, will be included in the Type C meeting briefing package.

Figure 1. *[Schematic diagram of vector cassette]*

4 PROPOSED REGULATORY PATHWAY

The proposed regulatory pathway is for biologic product under 351(a) of the Public Health Service Act (42 U.S.C. 262), with an application under section 505(b)(1) of the Food, Drug, and Cosmetic Act. An Orphan Drug Designation was obtained on September 27, 2021, and a Rare Pediatric Disease Designation was obtained on September 15, 2022.

5 PROPOSED INDICATION(S)

Treatment of *PCCA*-related propionic acidemia (PA).

6 TYPE OF MEETING BEING REQUESTED

Type C, virtual face-to-face meeting.

7 PEDIATRIC STUDY PLANS

As PA typically presents in the neonatal period, the clinical development program includes a pediatric population in the first-in-human study. As such, the AAV9-hPCCA development program will comply with the Pediatric Research Equity Act, and an initial pediatric study plan will be provided in accordance with applicable regulation.

8 HUMAN FACTORS ENGINEERING

Not applicable.

9 COMBINATION PRODUCT INFORMATION

Not applicable.

10 PURPOSE AND OBJECTIVES OF THE MEETING

The Sponsor seeks to evaluate the safety and preliminary efficacy of the AAV9-hPCCA gene therapy investigational product in a patient population with *PCCA*-related PA, with the intention to begin with adolescent or pediatric patients and then opening enrolment to eligible patients older than 3 years. The drug product formulation will consist of the AAV9-hPCCA drug substance in a buffered solution, to be administered as a one-time intravenous infusion. Drug substance and drug product will be manufactured in a GMP-compliant manner via triple transfection of a HEK293-S cell line. A stability program will be performed to support all storage conditions and durations for the drug product.

To date, the Sponsor has completed pharmacology studies *in vitro* (CRISPR-induced deletion mutagenesis in parental HepG2 cell line) and *in vivo* (mouse model with a CRISPR-induced $Pcca^{p.Q133LfsX41}$ mutation in Exon 5 of the *Pcca* gene; denoted $Pcca^{-/-}$), including biodistribution endpoints. The Sponsor has also initiated a pivotal efficacy/safety study ($Pcca^{-/-}$ mice), and intends to shortly commence a combined GLP biodistribution and dose escalation (by cohort) toxicology study (C57BL/6 mice).

Following the conclusion of the IND-enabling nonclinical development program, the Sponsor intends to perform a first-in-human clinical trial in a patient population with *PCCA*-related PA, with the intention to begin with adolescent or pediatric patients and then open enrolment to eligible patients older than 3 years. Given the rarity of the disease population, this dose-escalating study will include both safety and preliminary efficacy endpoints, in line with Agency commentary at the previously held INTERACT meeting (July 14, 2021) and pre-IND meeting (*[FDA-assigned pre-IND number]*). The Sponsor ultimately intends to submit a BLA marketing application pursuant to 351 (a) of the Public Health Service Act (42 U.S.C. 262) using the 505(b)(1) marketing pathway.

The proposed Type C meeting follows the afore-mentioned pre-IND meeting (*[FDA-assigned pre-IND number]*) held with the FDA on 10 July 2023. Since that meeting, the Sponsor has accumulated additional Natural History study data (under NIH protocol *[protocol number]*; NCT02890342) related to clinical biomarkers previously discussed (*[disease related biomarkers]*). The Sponsor has also continued manufacturing activities as lifecycle development approaches GMP compliance. Given these new developments since the pre-IND meeting, the Sponsor seeks to obtain targeted FDA feedback on specific chemistry, manufacturing, and control (CMC), and clinical questions for the AAV9-hPCCA investigational product. Specific questions are included herein.

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11 PROPOSED AGENDA

The following agenda is proposed (it is assumed respondents will introduce themselves upon speaking):

Brief Overview	5 minutes
Questions for Agency	50 minutes
Wrap up/Summary of Agreements	5 minutes

12 PROPOSED QUESTIONS

The briefing package will contain all background material relevant for the Agency's review in the context of the Sponsor's proposed questions.

12.1 Chemistry, Manufacturing, and Controls

Background for Question 1

FDA pre-IND (*[FDA-assigned pre-IND number]*) Preliminary Responses, July 7, 2023, Item #6 under responses to Question 1 (CMC): "We note that the amount of residual Host Cell DNA (HCD) in the 50L scale preclinical lot (TL-21-001-41) is $2.5x10^6$ pg/mL, which is significantly higher than the World Health Organization (WHO) recommended limit of ≤10 ng per dose. Because the 50L scale and 200L scale lots are manufactured using a similar process, it is expected that the residual HCD in the 200L scale GMP clinical product will also be significantly higher than the WHO recommended limit. In your IND submission, please justify your proposed limit(s) with manufacturing data that applies to your process or to the manufacturing platform. Accordingly, you should provide a comprehensive risk assessment that takes into consideration the highest dose that will be administered, levels and size of residual DNA in the product lots, the patient population, target tissue, and route of administration. You should also discuss process optimization plans to reduce the level of residual host cell DNA in the commercial product."

The Sponsor is presenting a risk assessment and approach in the briefing package.

Question 1:

Does the FDA agree with our risk assessment and approach to support the limits of residual host-cell DNA (HCD)?

Background for Question 2:

FDA pre-IND (*[FDA-assigned pre-IND number]*) Preliminary Responses, July 7, 2023, Items #2-4 under responses to Question 1 (CMC):

#2: We note that you include a Western Blot assay for hPCCA expression as the potency assay and have set an acceptance criterion (AC) of "PCCA Expression in HepG2-Knockout Cells". It appears that this assay is a qualitative assessment of transgene expression. This

approach may be acceptable for an early phase clinical trial designed to evaluate product safety and tolerability; however, if you intend to leverage the proposed Phase 1/2 clinical study to provide the primary evidence of effectiveness to support a marketing application, you should develop a quantitative, accurate, and precise potency assay early in clinical development. If available, you should submit the assay qualification protocol and report in your IND submission.

#3: We recommend that you develop a quantitative measure of the biological function(s) of your product related to the mechanism of action and use it for lot release and stability testing.

#4: We note that you include a Next Generation Sequencing (NGS) assay as a transgene identity test for DS lot release testing. An NGS assay that can ensure the DS sequence is correct, used in conjunction with a quantitative measure of transgene expression (mRNA or protein), may be adequate to ensure product potency. Please provide a detailed NGS protocol and a description of how you plan to assess the sensitivity, accuracy, and precision of the NGS assay to detect mutant vector sequences in the DS.

Question 2:

Does the FDA agree with our plans for testing and qualifying a potency assay matrix for AAV9-hPCCA?

12.2 Clinical

Background for Question 3:

PCCA-related PA is an ultra-rare disease which presents early in life with life threatening, multiorgan clinical manifestations. To date there does not exist an FDA-approved medicinal product for systemic treatment for PCCA, and thus there does not exist a surrogate biomarker that functions as the basis of drug approval. Significant work has been performed at the National Institutes of Health to determine correlations between surrogate biomarkers and disease severity of PCCA-related PA (and the associated acidemias PCCB-related PA, and MMUT-methylmalonic acidemia), as well as their response to liver-targeted enzymatic correction studying patients after an elective liver transplant. Data from these natural history protocols will be utilized to support eligibility criteria and pharmacodynamic measures in the proposed first-in-human gene therapy clinical trial. These biomarkers were discussed with Agency reviewers during the pre-IND meeting, regarding retrospective data collection in both nonclinical and clinical studies, and potential prospective clinical utilization.

Of biomarkers gauged to date, *[disease related biomarkers]* have shown statistically significant correlation to varying measures of clinical meaningfulness. The Sponsor seeks to leverage these data to help:

- Determinations of severity of PCCA-related PA to assist with gene therapy candidate patient selection
- Determinations of preliminary efficacy (pharmacodynamic response) of AAV9-hPCCA
- Determinations of sustainability of enzymatic correction, in the event of immunological reactions to the AAV vector or transgene in CRIM (cross-reactive

immunologic material/PCCA protein)-negative subjects, where a cytotoxic T-cell immune response can result in loss of transduced cells and PCCA activity.

- Independent Data Monitoring Committee assessment for clinical trial investigational product dose escalation

Updated Natural History study data and associated rationale will be provided in the meeting briefing package.

Question 3:

Does the Agency agree with the proposed approach for utilization of the specified surrogate biomarkers for evaluation of preliminary clinical efficacy (pharmacodynamic response) and associated measures?

13 LIST OF ATTENDEES

[list of attendees and their titles]

14 REQUESTED AGENCY ATTENDEES

Non-specifically, the Sponsor requests Agency attendees familiar with propionic acidemia (and/or associated organic acidemias), gene therapy lifecycle development, and clinical trials in a pediatric population. Where possible, the Sponsor requests attendance of parties present at the Pre-IND meeting held on July 10, 2023 due to their predicate understanding of the AAV9-hPCCA development program.

15 SUGGESTED DATE AND TIME OF THE MEETING

Date	Times Available
Dec 16, 2024	All day
Dec 17, 2024	All day
Dec 18, 2024	All day
Dec 19, 2024	All day
Dec 20, 2024	All day

16 MEETING FORMAT

A virtual-only, face-to-face teleconference/video conference is requested.

17 MEETING PACKAGE

The meeting package will be sent at least one month prior to the scheduled meeting date.

18 REFERENCES

Applicable references will be provided in the pre-IND briefing package with associated context described.

October 30th, 2024

U.S. Food and Drug Administration
Center for Biologics Evaluation and Research
Document Control Center
10903 New Hampshire Avenue
WO71, G112, Silver Spring, MD 20993-0002
Copy: *[name of regulatory project manager]*

Re: *[FDA-assigned Pre-IND number]*
Sponsor: National Center for Advancing Translational Sciences (NCATS)
Drug name: AAV9-hPCCA
Indication: Treatment of *PCCA*-related propionic acidemia (PA)
Re: Type C meeting Briefing Package

Reference is made to a Meeting Confirmation document received on October 23rd, 2024, confirming a Type C Written Response Only. In accordance with this notification, NCATS hereby provides a Meeting Briefing Package to support questions related to its development program for AAV9-hPCCA for the treatment of propionyl-CoA carboxylase subunit A (*PCCA)*-related propionic acidemia (PA). This Type C meeting follows a pre-IND meeting held with the Agency on July 10, 2023, and an INTERACT Meeting on July 14, 2021 *[FDA-assigned INTERACT number]*.
All Type C Briefing Package materials are contained in Module 1.6.2 (Meeting background materials) and specifically consist of the following information broken down by subject matter discipline:

- Type C Meeting Briefing Package
- Chemistry, Manufacturing and Controls Information
    - 2019-004-1 Study Report
    - NGS Executive Summary
    - mRNA Potency Assay Method
- Clinical Information
    - Clinical synopsis clean
    - Clinical synopsis tracked change

With this submission, we are also notifying you of a change to the alternate Sponsor's authorized representative, to *[name of representative]*. The primary Sponsor's authorized representative *[name of authorized representative]* remains the same.
[name and detailed contact information of sponsor's alternate representative]
Should you require any additional information, please contact me at *[phone number],* or via email at *[email address]*. If I am unavailable, please reach out to *[name, phone number and email address of sponsor's alternate representative]*.

Sincerely,

[Signature, name and contact information of primary contact]

***DISCLAIMER**: This information is specific to AAV9-hPCCA and it does not directly apply to other investigational products. The information may no longer be applicable due to subsequent changes.*

Type C Meeting Package

Center for Biologics Research and Evaluation, Office of Therapeutic Products

Date of Meeting:	Written response
Pre-IND PS Number:	*[FDA-assigned pre-IND number]*
Drug Product:	AAV9-hPCCA
Formulation:	Sterile, aqueous buffered solution composed of the AAV9-hPCCA drug substance formulated in *[buffer composition]*
Drug Substance:	AAV9-hPCCA
Indication:	Treatment of *PCCA*-related propionic acidemia (PA)
Sponsor:	National Center for Advancing Translational Sciences (NCATS) 9800 Medical Center Drive Rockville, MD 20850 301-594-8966

Confidentiality Statement

***DISCLAIMER**: This information is specific to AAV9-hPCCA and it does not directly apply to other investigational products. The information may no longer be applicable due to subsequent changes.*

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LIST OF TABLES

LIST OF FIGURES

***DISCLAIMER**: This information is specific to AAV9-hPCCA and it does not directly apply to other investigational products. The information may no longer be applicable due to subsequent changes.*

LIST OF ABBREVIATIONS

Abbreviation	Full Length Name
AAV	Adeno-associated virus
AC	Acceptance Criterion
BLA	Biologics License Application
bp	Base pairs
CMC	Chemistry, Manufacturing, and Controls
CRIM	Cross-reactive immunologic material
CV	Coefficient of variation
DS	Drug Substance
FDA	U.S. Food and Drug Administration
FIH	First-in-human
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GOI	Gene of Interest
HCD	Host Cell DNA
IP	Investigational Product
ITRs	Inverted Terminal Repeats
KO	Knockout
LT	Liver Transplantation
MMUT	Methylmalonic acidemia
NCATS	National Center for Advancing Translational Sciences
NGS	Next Generation Sequencing
NHGRI	National Human Genome Research Institute
NH	Natural History
NIH	National Institutes of Health
PA	Propionic Acidemia
PBS	Phosphate buffered solution
PCC	Propionyl-CoA Carboxylase
PCCA	Propionyl-CoA Carboxylase, alpha
PCCB	Propionyl-CoA Carboxylase, beta
PoC	Proof-of-Concept
RCT	Randomized, Controlled Trial
RoA	Route of Administration
US	United States
VRBPAC	Vaccines and Related Biological Products Advisory Committee
WHO	World Health Organization
WT	Wild type

***DISCLAIMER**: This information is specific to AAV9-hPCCA and it does not directly apply to other investigational products. The information may no longer be applicable due to subsequent changes.*

1 APPLICATION NUMBER

[FDA-assigned pre-IND number]

2 PRODUCT NAME

AAV9-hPCCA; additional name, identifier: NCATS-BL0746

3 CHEMICAL NAME, ESTABLISHED NAME, AND/OR STRUCTURE

AAV9-hPCCA is a pseudoserotyped Adeno-Associated Virus 9 (AAV9) virus containing a single stranded vector with human codon optimized Propionyl-CoA Carboxylase, alpha subunit (*PCCA*).

The AAV9-hPCCA vector transgene schematic and description are shown in Figure 1.

Figure 1: AAV9-hPCCA vector transgene

[Schematic describing components of the AAV9-hPCCA cassette]

4 PROPOSED REGULATORY PATHWAY

The proposed regulatory pathway is for biologic product under 351(a) of the Public Health Service Act (42 U.S.C. 262), with an application under section 505(b)(1) of the Food, Drug, and Cosmetic Act. An Orphan Drug Designation was obtained on September 27, 2021, and a Rare Pediatric Disease Designation was obtained on September 15, 2022.

5 PROPOSED INDICATION(S)

Treatment of *PCCA*-related propionic acidemia (PA).

6 DOSAGE FORM, ROUTE OF ADMINISTRATION, AND DOSING REGIMEN

AAV9-hPCCA drug product will be administered as an intravenous infusion in over approximately 30-60 minutes using a syringe pump. Pending review of initial data, dosing will proceed in two cohorts. Cohort 1 will receive a dose of *[starting dose]* of AAV9-hPCCA, and Cohort 2 will receive a dose of *[higher dose]*.

7 PEDIATRIC STUDY PLANS

As PA typically presents in the neonatal period, the clinical development program includes a pediatric population in the first-in-human study.

8 HUMAN FACTORS ENGINEERING

Not applicable

9 COMBINATION PRODUCT INFORMATION

Not applicable

10 LIST OF ATTENDEES

Not applicable as Written Response only

11 DEVELOPMENT HISTORY AND PRODUCT DEVELOPMENT STATUS

11.1 Brief History of Development

11.1.1 AAV9-hPCCA Gene Therapy Program

PA is a rare autosomal recessive disorder of organic acid metabolism in humans. It is caused by a deficiency of propionyl-CoA carboxylase (PCC), a ubiquitously expressed, heteropolymeric mitochondrial enzyme involved primarily in the catabolism of propiogenic amino acids, particularly isoleucine, valine, methionine, and threonine, as well as odd-chain fatty acids (Shchelochkov et al., 1993). The enzyme is composed of α- and β-subunits encoded by their respective genes, PCCA and PCCB.

Most frequently, PA presents in the neonatal period with hyperammonemia, vomiting, poor feeding and hypotonia, and progresses into a life-threatening metabolic crisis. Patients who survive suffer from recurrent metabolic instability and can develop multisystem complications, including cardiomyopathy. The long-term prognosis for survival in severely affected patients is poor, where PA patients with an early and severe clinical course experience increased mortality and disease associated morbidity (Shchelochkov et al., 1993, Surtees et al., 1992). The recalcitrant nature of the disorder to conventional medical management, including the dietary restriction of amino acid precursors, L-carnitine supplementation, and administration of metronidazole to reduce the generation of propionic acid by intestinal bacteria, has led to the implementation of elective liver transplantation (LT) as an experimental surgical treatment for PA. While not curative of all aspects of the disorder, successful LT in the setting of PA provides restoration of metabolic stability and protection from early death and therefore represents a clinical benchmark for gene replacement therapy that may increase hepatic PCC expression and activity. There is currently no US Food and Drug Administration (FDA)-approved drug or biologic for the treatment of PA, though it is noted the FDA has approved Carbaglu® (carglumic acid) as **adjunctive** therapy to standard of care for the treatment of acute hyperammonemia due to PA, among other indications.

The Sponsor is developing an AAV9 gene therapy candidate, AAV9-hPCCA, for the treatment of *PCCA*-related PA (proposed patient population does not include those with *PCCB*-related PA). Previously, an INTERACT meeting was held with the Agency on July 14, 2021, in support of this development program. Additionally, an Orphan Drug Designation was obtained on September 27, 2021, and a Rare Pediatric Disease Designation was obtained on September 15, 2022. A Type B pre-IND meeting was held on July 10, 2023.

11.2 Substantive Changes in Development Plans

Since the pre-IND meeting, revisions have been made to the proposed first-in-human (FIH) clinical trial specific to the proposed utility of the surrogate endpoints. A revised protocol synopsis including the changes discussed in this Type C meeting is included as an addendum to Section 15.2. All other Agency comments from the INTERACT and pre-IND will be addressed at the time of IND submission.

11.3 Current Status of Product Development

To date, the Sponsor has completed pharmacology studies *in vitro* (CRISPR-induced deletion mutagenesis in parental HepG2 cell line) and *in vivo* (mouse model with a CRISPR-induced $Pcca^{p.Q133LfsX41}$ mutation in Exon 5 of the *Pcca* gene; denoted $Pcca^{-/-}$), including biodistribution endpoints. The Sponsor is performing a pivotal efficacy study ($Pcca^{-/-}$ and wild type (WT) counterpart mouse pups) and biodistribution measures, as well as a combined Good Laboratory Practice (GLP) biodistribution and dose escalation (by cohort) toxicology study in C57BL/6 mice.

Following the conclusion of the IND-enabling preclinical development program, the Sponsor intends to perform an FIH clinical trial starting with adolescent and pediatric patients with PA, then opening enrollment to eligible patients older than three years of age. Given the rarity of the disease population, this dose-escalating study will include both safety and preliminary efficacy endpoints. The Sponsor ultimately intends to submit a Biologics License Application (BLA) marketing application pursuant to 351 (a) of the Public Health Service Act (42 U.S.C. 262) using the 505(b)(1) marketing pathway.

12 PURPOSE AND OBJECTIVES OF MEETING

The purpose of this Type C meeting is to obtain FDA feedback on the surrogate biomarkers in the clinical development program and for the AAV9-hPCCA chemistry, manufacturing, and control (CMC) specific questions are included herein.

13 AGENDA

Not Applicable as Written Response Only

14 LIST OF QUESTIONS FOR DISCUSSION

14.1 Chemistry, Manufacturing, and Controls

Background for Question 1:

FDA pre-IND *[FDA-assigned pre-IND number]* Preliminary Responses, July 7, 2023, Item #6 under responses to Question 1 (CMC): “We note that the amount of residual Host Cell DNA (HCD) in the 50L scale preclinical lot (TL-21-001-41) is $2.5x10^6$ pg/mL, which is significantly higher than the World Health Organization (WHO) recommended limit of ≤10 ng per dose. Because the 50L scale and 200L scale lots are manufactured using a similar process, it is expected that the residual HCD in the 200L scale Good Manufacturing Practices (GMP) clinical product will also be significantly higher than the WHO recommended limit. In your IND submission, please justify your proposed limit(s) with manufacturing data that applies to your

process or to the manufacturing platform. Accordingly, you should provide a comprehensive risk assessment that takes into consideration the highest dose that will be administered, levels and size of residual DNA in the product lots, the patient population, target tissue, and route of administration. You should also discuss process optimization plans to reduce the level of residual host cell DNA in the commercial product."

The Sponsor is presenting additional batch data, a risk assessment and approach in section 15.1.

Question 1:

Does the FDA agree with our risk assessment and approach to support the limits of residual host-cell DNA?

Background for Question 2:

FDA pre-IND *[FDA-assigned pre-IND number]* Preliminary Responses, July 7, 2023, Items #2-4 under responses to Question 1 (CMC):

#2: We note that you include a Western Blot assay for hPCCA expression as the potency assay and have set an acceptance criterion (AC) of "PCCA Expression in HepG2-Knockout Cells". It appears that this assay is a qualitative assessment of transgene expression. This approach may be acceptable for an early phase clinical trial designed to evaluate product safety and tolerability; however, if you intend to leverage the proposed Phase 1/2 clinical study to provide the primary evidence of effectiveness to support a marketing application, you should develop a quantitative, accurate, and precise potency assay early in clinical development. If available, you should submit the assay qualification protocol and report in your IND submission.

#3: We recommend that you develop a quantitative measure of the biological function(s) of your product related to the mechanism of action and use it for lot release and stability testing.

#4: We note that you include a Next Generation Sequencing (NGS) assay as a transgene identity test for drug substance (DS) lot release testing. An NGS assay that can ensure the DS sequence is correct, used in conjunction with a quantitative measure of transgene expression (mRNA or protein), may be adequate to ensure product potency. Please provide a detailed NGS protocol and a description of how you plan to assess the sensitivity, accuracy, and precision of the NGS assay to detect mutant vector sequences in the DS.

The Sponsor is presenting the proposed matrix and method protocols in section 15.1.

Question 2:

Does the FDA agree with our plans for testing and qualifying a potency assay matrix for AAV9-hPCCA?

14.2 Clinical

Background for Question 3:

PCCA-related PA is an ultra-rare disease which presents early in life with life threatening, multiorgan clinical manifestations. To date there does not exist an FDA-approved medicinal product for systemic treatment for PCCA, and thus there does not exist a surrogate biomarker that functioned as the basis of drug approval. Significant work has been performed at the National Institutes of Health to determine correlations between surrogate biomarkers/endpoints and disease severity of PCCA-related PA (and the associated organic acid disorders PCCB-related PA, and MMUT-methylmalonic acidemia), as well as their response to liver-targeted enzymatic correction studying patients after an elective liver transplant. Data from these natural history protocols will be utilized to support eligibility criteria and pharmacodynamic measures in the proposed first-in-human gene therapy clinical trial. These biomarkers were discussed with Agency reviewers during the pre-IND meeting, regarding retrospective data collection in both nonclinical and clinical studies, and potential prospective clinical utilization.

Of surrogate endpoints gauged to date, *[disease-related biomarkers]* have shown statistically significant correlation to varying measures of clinical meaningfulness. The Sponsor seeks to leverage these data to help:

- Determinations of severity of PCCA-related PA to assist with gene therapy candidate patient selection
- Determinations of preliminary efficacy (pharmacodynamic response) of AAV9-hPCCA
- Determinations of sustainability of enzymatic correction, in the event of immunological reactions to the AAV vector or transgene in CRIM (cross-reactive immunologic material/PCCA protein)-negative subjects, where a cytotoxic T-cell immune response can result in loss of transduced cells and PCCA activity.

The Sponsor is providing updated biomarker data and associated rationale in 15.2.

Question 3:

Does the Agency agree with the proposed approach for utilization of the specified surrogate biomarkers for evaluation of preliminary clinical efficacy (pharmacodynamic response) and associated measures?

15 DATA SUMMARIES

15.1 Chemistry, Manufacturing, and Controls

15.1.1 CMC Introduction and Lifecycle Development History

The AAV9-hPCCA gene therapy Investigational Product (IP) expresses a functional human codon optimized cDNA encoding the PCCA gene, under control of the *[specific]* promoter. The AAV9 capsid was selected to further enable hepatic and cardiac transduction. The therapeutic transgene cassette was designed with a *[specific]* promoter to enable wide expression. The inverted terminal repeats (ITRs) of the GMP AAV9-hPCCA have been optimized, strictly conserving all other elements of the research grade and feasibility lots of AAV9-hPCCA used in the PoC studies.

Cells from a HEK293-S working cell bank are expanded, grown to achieve the targeted cell density and batched up. Triple transfection with pAAV9-hPCCA, pRC9-Kan, and pHelper- Kan plasmids *[ratio]* is then performed, followed by cell expansion and subsequent harvest. Cells are lysed and impurities are removed across multiple steps, including filtration and affinity chromatography. Capsids are enriched via iodixanol gradient ultracentrifugation, and fractions are pooled (depending on yields), filtered, and buffered.

The formulated drug substance is filtered and then aliquoted into 2 mL or 5 mL Crystal Zenith® vials to produce the final drug product. The final drug product vials are labeled and stored at -80°C. The drug product formulation is a sterile, aqueous buffered solution composed of the AAV9-hPCCA drug substance formulated in *[buffer]*. Stability testing is proposed for frozen drug product (up to five years) and the clinical infusion formulation.

15.1.2 Host Cell DNA

Reference is made to the pre-IND *[FDA-assigned pre-IND number]* Preliminary Responses, July 7, 2023, Item #6 under responses to Question 1 (CMC). FDA noted that the amount of residual Host Cell DNA (HCD) in the 50L scale preclinical lot (TL-21-001-41) is $2.5x10^6$ pg/mL, which is significantly higher than the World Health Organization (WHO) recommended limit of ≤10 ng per dose. It was requested to justify the proposed limit(s) with manufacturing data, discuss process optimization and provide a comprehensive risk assessment in the IND.

The purpose of this section is to present the recent manufacturing process improvements and layout of the proposed quality risk assessment supporting HCD levels exceeding the WHO recommended limit of ≤10 ng per dose.

15.1.2.1. *Risk Assessment*

The use of human cell lines for an AAV product may result in residual human genomic DNA packaged within the AAV vector product, perhaps representing a risk of genotoxicity. The two types of genotoxic risk that have been identified from human-derived cell lines are oncogenicity and infectivity.

The concern that residual DNA impurities might express oncogenes led to existing guidelines that residual DNA amount and size be controlled; residual cell-substrate DNA should be ≤10 ng per dose, with a median DNA size of 200 bp or lower (WHO, 1998, FDA Vaccines and related biological product advisory committee meeting 1998). In some cases, however, the WHO guideline for residual host cell DNA quantity cannot be met for a vector dose exceeding 2×10^{11} vg. For AAV products it is therefore recommended to measure the amount, report it and for the Sponsor to determine levels that are shown to be safe (FDA CBER OTAT Town Hall, 2022). Furthermore, for a standard AAV product, the residual host cell DNA impurity is predicted to be present as single-stranded DNA fragments of ~4700 nt, which exceed the WHO guideline for size. A mitigating feature of residual host cell DNA fragments packaged by AAV vectors is its predicted single-stranded nature, rendering it unstable and likely to be degraded quickly following unpackaging in the nuclei of transduced cells (Wright J.F., 2014). Assuming the encapsidated residual host cell DNA represents AAV genome-sized fragments distributed randomly throughout the ~ $3x10^9$ nucleotide genome of human production cells, the predicted frequency of the packaging of any specific sequence within the host cell genome is ~1 copy per 10^8 vector genome

particles. While AAV encapsidated HCD falls under the existing regulatory guidelines regarding amount of residual cell substrate per dose and average size, these limits may require re-consideration for AAV gene therapy products based on the unique characteristics of this vector product-related impurity.

15.1.2.1.1. Safety margins

For genotoxic impurities with no identified threshold mechanisms, such as oncogenic and infectivity risks, the FDA Vaccines and Related Biological Products Advisory Committee (VRBPAC) considers a frequency of one in 10 million (10^{-7}) to be an acceptable level of risk (FDA Briefing document, 2012). If appropriate, this calculation will be performed for AAV9-hPCCA once data is available and presented in the IND.

15.1.2.2. *Clinical*

The patient population is Pediatric and adult patients, ≥3 years of age, *[and NIH Clinical Center specific criteria]* and with clinically, biochemically, and/or molecularly confirmed pathogenic variants in *PCCA* that cause PA. Target tissue is the heart and liver tissue. The highest dose currently proposed to be administered is *[higher dose]* via an IV infusion for the treatment of PCCA-related PA.

15.1.2.3. *Manufacturing process Optimization*

pAAV9-hPCCA is a *[XX]* base pairs (bp) AAV serotype 9 plasmid containing the gene of interest (GOI) with 5' and 3' *[AAV serotype]* ITR fragments. Three separate plasmids are used to generate AAV9-hPCCA. pAAV9-hPCCA is the GOI plasmid. The pHelper-Kan plasmid provides the adenoviral helper genes, E2A, E4, and VA for AAV packaging. The pRC-9-Kan plasmid provides the AAV rep and cap genes for AAV viral vector packaging.

HCD is considered a process-related impurity in AAV vectors with the safety concern of potential genotoxicity (Wright, 2014). Process-related impurities are derived from the manufacturing process of the raw materials and components, including cell substrate, cell culture medium, helper components, such as viruses and plasmid DNA, and purification-related process components, but are not structurally related to the product. Regulatory guidance suggests mitigating the risks by decreasing both the amount and the size of residual DNA.

15.1.2.3.1. Manufacturing process background

The AAV9-hPCCA drug substance has HCD by qPCR currently listed in the drug substance specification with the limit "To be determined".

In the downstream manufacturing process *[buffer concentration]* lysis buffer *[buffer composition]* and 10 U/mL Benzonase are added to the bioreactor, which is held at 37°C at 175 RPM for two hours. To inhibit the Benzonase activity, 5M NaCl is added to a final concentration of 0.5M. A series of depth filters are then used to remove cell debris and recover the AAV9 post cell lysis and digestion. The clarification filtration train is composed of *[filter types/pore sizes]*. The clarified material is concentrated and diafiltered into *[buffer composition]* buffer using tangential flow filtration (*[molecular weight cut-off]*) to remove the Tween-20 added during lysis

and to reduce the loading volume prior to affinity chromatography. Following diafiltration, the retentate is *[filter pore size]* filtered to generate the affinity chromatography load. *[product name]* AAV9 affinity chromatography is then used to capture the AAV9 vector and remove most impurities, such as host cell DNA and host cell protein.

15.1.2.3.2. Manufacturing process improvements

The first risk identified in the manufacturing process was the amount of benzonase used. Thus, for the manufacturing of the 200L engineering lot, the amount of benzonase was increased from 10 U/mL (used in the manufacturing of the 50L lot) to 30 U/mL. The residual total host cell DNA in the 200L engineering lot (drug substance) was $3.08x10^5$ pg/mL, an 8.1-fold reduction. The newly introduced 30 U/mL amount of benzonase will be used in all lots going forward.

The amounts of HCD in the AAV9-hPCCA product is shown in Table 1. Size of residual DNA will be tested and presented in the IND.

Table 1: Levels of residual DNA per lot

Lot	**Levels of residual DNA**
50L(TL-21-001-41)	$2.5x10^6$ pg/mL
200L (0110-ENG)	$3.08 x10^5$pg/mL

15.1.2.3.3. Characterization

Characterization of HCD has been performed on AAV9-hPCCA which is presented in *[SOP number]*, including the integrity of the GOI.

15.1.2.3.4. Conclusion

The successful manufacturing process optimization showing an 8.1-fold reduction in residual HCD in the 200L batch compared to the 50 L batch and the proposed IND risk assessment provides the justification for re-consideration of HCD levels in AAV-hPCCA drug product exceeding the WHO recommended limit of ≤10 ng per dose.

HCD will continue to be tested in all future lots to the specification “Report result” until a specification limit can be set based on batch data. The Sponsor will continue to characterize genes 18S (with and without DNAse) and E1A fragments.

15.1.3 Potency Assay

Reference is made to pre-IND *[FDA-assigned pre-IND number]* Preliminary Responses, July 7, 2023, Items #2-4 under responses to Question 1 (CMC):

#2: We note that you include a Western Blot assay for hPCCA expression as the potency assay

and have set an acceptance criterion (AC) of "PCCA Expression in HepG2-Knockout Cells". It appears that this assay is a qualitative assessment of transgene expression. This approach may be acceptable for an early phase clinical trial designed to evaluate product safety and tolerability; however, if you intend to leverage the proposed Phase 1/2 clinical study to provide the primary evidence of effectiveness to support a marketing application, you should develop a quantitative, accurate, and precise potency assay early in clinical development. If available, you should submit the assay qualification protocol and report in your IND submission.

#3: We recommend that you develop a quantitative measure of the biological function(s) of your product related to the mechanism of action and use it for lot release and stability testing.

#4: We note that you include a Next Generation Sequencing (NGS) assay as a transgene identity test for DS lot release testing. An NGS assay that can ensure the DS sequence is correct, used in conjunction with a quantitative measure of transgene expression (mRNA or protein), may be adequate to ensure product potency. Please provide a detailed NGS protocol and a description of how you plan to assess the sensitivity, accuracy, and precision of the NGS assay to detect mutant vector sequences in the DS.

In accordance with the FDA comment, the Sponsor is proposing the following matrix of potency assays:

1. GOI mRNA evaluation/quantification by ddPCR

[Method name] will be used as the proposed assay to determine the potency of recombinant AAV vector in cell culture preparation. The assessment is made through transduction of cells with the vector followed by extraction of RNA, reverse transcription and quantification using droplet digital PCR (ddPCR) resulting in the determination of amount of mRNA present. The *[method name]* will be qualified prior to release of the clinical material and presented in the IND.

2. NGS of the drug substance

The reliability, sensitivity and long turnaround time of traditional virus testing bioassays has long been a concern in the pharmaceutical industry and therefore the development of agnostic NGS virus testing approaches has shown to be a suitable alternative reconciling the need for a broad detection range, deep analytical sensitivity and rapid turnaround times (Beurdeley-Fehlbaum P., et al. 2023).

Genetic characterization, specifically nucleotide sequence testing of AAV-hPCCA will be done by NGS comparing the sample data set against a defined reference sequence. NGS Executive Summary summarizes the proposed method including specificity, repeatability, intermediate precision, limit of detection and robustness.

[SOP number] shows the NGS assay applicability to assess the integrity of encapsidated AAV2 genomes (percentage and overall length).

AAV9- hPCCA batch PD0108-502-24001 was tested by the proposed NGS method to confirm the nucleotide sequence and detect and characterize sequence variants relative to the defined reference sequence. *[Brief extraction and sequencing method summary]*. Results showed that the sample's

sequence matches 100.00% with the reference sequence with a coverage at *[XX]* depth minimum of 100.00%.

Potency testing with the proposed analytical methods for all manufactured lots will commence after completion of method development and full validation of both methods is planned post IND, prior to start of Phase 2.

15.2 Clinical

15.2.1. Introduction

Reference is made to the pre-IND meeting *[FDA-assigned pre-IND reference number]* held July 10, 2023. At the time of this meeting, the Sponsor presented a clinical synopsis for a proposed Phase 1/2, first-in-human clinical trial. Given the ultra-rarity of *PCCA*-related PA, this trial proposed two primary objectives across both safety and efficacy:

Safety: To assess safety and tolerability of intravenous administration of AAV9-hPCCA in research participants with *PCCA* type PA; and
Efficacy: To assess changes from baseline in response biomarkers, a.k.a., pharmacodynamic (PD) response, to AAV9-hPCCA

As noted in Question 3 and the associated Sponsor's position, there does not exist an FDA-approved medicinal product for systemic treatment for PCCA, and thus there does not exist an analogous surrogate endpoint that has functioned as the basis of drug approval for systemic treatment. However, researchers at the NHGRI have been running an ongoing Natural History study, "The Natural History, Physiology, Microbiome and Biochemistry Studies of Propionic Acidemia" (*[protocol number]*; NCT02890342), which seeks to better understand PA patient outcomes and their associations with clinical, pharmacological, laboratory and dietary factors. Provided within the pre-IND briefing package were aggregated results from *[XX]* patients with either PCCA- (N=*[XX]*) or PCCB-related PA (N=*[XX]*), with further delineation for disease severity and correlation with clinical parameters and biomarkers. This data summarized the rationale as to why the *[two disease related biomarkers]* could be predictive of clinical meaningfulness.

Given the data in hand, at the time of the pre-IND meeting the Sponsor proposed as a primary efficacy endpoint the absolute and percent change from baseline in *[two disease related biomarkers]* at *[XX]* weeks (interim endpoint for DSMB assessment) and at the end of the *[XX]* study period (primary endpoint). While the Agency noted the importance of maximizing the knowledge that could be gained from the study, inclusive of obtainment of robust efficacy data, at the time they did not agree with the use of a surrogate endpoint as a primary efficacy endpoint.

While the Sponsor appreciates the Agency's thoughtful advice, due to both the rarity and heterogeneity of PCCA-related PA there may not exist an acceptable primary efficacy endpoint at the outset of an open-label FIH gene therapy clinical trial. Given this, the Sponsor proposes a single primary objective for safety, with the secondary goal of evaluating pharmacodynamic endpoints for potential utility as future efficacy endpoints. These surrogate endpoints include the previously proposed *[two disease related biomarkers]*, as well as the additional [*another related*

biomarker] as an additional biomarker of interest. The *[XX]* biomarker was not thoroughly described at the time of the pre-IND meeting, but data and associated rationale are provided herein. While the Agency's recommendation in the pre-IND meeting was to categorize these as exploratory endpoints, the Sponsor believes the data in hand supports a linkage to PA severity such that correlations to treatment effect may potentially be made.

Given the length of the long-term follow up in the clinical trial along with a potentially prolonged rate of study accrual due to the number of participants available and interested, this collection of data should organically allow for juxtaposition with measures of clinical meaningfulness (i.e., other secondary and exploratory measures of efficacy) without sacrificing study progression. Continued data collection in the Natural History study will also be available as a direct comparator, as was also discussed at the pre-IND meeting. Analysis of the available totality of the evidence will guide the continued direction of the study.

This is believed to be in line with related Agency commentary received at the pre-IND meeting noting that if a randomized clinical trial was proposed, the Sponsor "*might consider offering the product to subjects in the control arm at the completion of the study period, provided the preliminary safety and efficacy data are favorable*." Analogously, upon analysis of adequate safety and efficacy data and pending Agency review the Sponsor may amend the protocol for additional enrollment, thereby potentially operating under a more fluid single pivotal study paradigm. While it is acknowledged this may need to be predicated upon robust signals of efficacy, a similar approach in a rare disease population was recently shown to be feasible in the exa-cel development program (CASGEVY; Vertex Pharmaceuticals, Inc.; Study 121, NCT03655678).

For additional context, responses to Agency commentary related to the proposed surrogate endpoints provided at the pre-IND meeting are shown in Table 2 below. A revised clinical synopsis (clean and tracked changes) is also provided. All additional Agency commentary will be addressed at the time of IND submission. Additional data supporting the utility of the proposed surrogate endpoints are contained in Section 15.2.2 of this document.

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Table 2. Responses to select FDA commentary received at the pre-IND Meeting

Question Number	Discipline	Question	FDA Response Number	FDA Response	NCATS Commentary
4	Nonclinical	Depending on whether the 4E13 vg/kg/dose (mid-dose in the GLP toxicity study) or the highest tested dose, 3E14 vg/kg/dose, is found to be the No Observed Adverse Effect Level, does the FDA agree that these doses support the proposed starting clinical dose *[starting dose]* and the proposed maximum clinical dose *[higher dose]*, respectively?	4d	Regarding your proposed definitive POC study described in Sections 15.2.1.2.6 and 15.2.3.3 (page 37 and 39, respectively) and outlined in *[protocol number]* in the neonatal Pcca-/- mouse model: Please assess the plasma levels of *[two disease related biomarkers]* , and plasma levels of *[another disease related biomarker]* in your metabolite bioanalysis.	While acknowledging these data are related to the nonclinical proof of concept study, these measures may provide additional evidence for the utility of *[two disease related biomarkers]* as surrogate efficacy endpoints.
7	Clinical	Does the Agency agree with the proposed first-in-human study design, including participant inclusion/exclusion criteria, dosing rationale, study population rationale, staggering of IP administration, stopping rules, safety oversight, and safety and efficacy endpoints?	1b	"...If you are able to develop appropriate product and release testing to support a pivotal study, we recommend that you consider designing this study as a larger randomized control trial or at least include a concurrent control group."	The proposed FIH clinical trial remains designed as a dose escalation study. However, the Sponsor will monitor emerging safety and pharmacodynamic data to inform evolving study design. Given the ultra-rarity of the disease, anticipated enrollment is low (a steady rate of accrual cannot be projected), and data from each individual participant will possess outsized importance. Given this pending ongoing data analysis, the Sponsor may consider study redesign via protocol amendment and additional Agency interaction.

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Question Number	Discipline	Question	FDA Response Number	FDA Response	NCATS Commentary
7 (continued)	Clinical	Does the Agency agree with the proposed first-in-human study design, including participant inclusion/exclusion criteria, dosing rationale, study population rationale, staggering of IP administration, stopping rules, safety oversight, and safety and efficacy endpoints?	3b	You plan to enroll subjects who have "biochemical evidence of PA (e.g., elevated *[disease related biomarkers]* in body fluids)." However, you did not provide the thresholds for the biomarkers that will be used to determine eligibility. To ensure a favorable benefit/risk and consistency in enrollment of subjects, please provide thresholds for *[two disease related biomarkers]* for inclusion into the study in your protocol.	This inclusion criterion will be refined in the final version of the clinical protocol submitted in the IND. The proposed study intends to utilize run-in/screening repeat measures, including two *[disease related biomarker]* measures plus third if the first two diverge (e.g., more than 50%, but a number needs to be finalized for PA patients, as a good measure of variability needs to be obtained). Additionally, the related exclusion criterion #2 was refined to include numerical parameters for *[two disease related biomarkers]* : *"High [disease related biomarker], as demonstrated by [biomarker assay]>[XX] [% dose oxidized * 0-60 min AUC] or >[XX]% cumulative [disease related biomarker] within [XX] minutes and [disease related biomarker]< [XX] µmol/L and [disease related biomarker] < [XX] µmol/L, as seen in PA patients with clinically mild disease or after LT."*
			4b	The Data Safety Monitoring Board will review the study data after the first three subjects are treated in Cohort 1. If there are signs of efficacy and an "acceptable safety profile," then three additional subjects will be treated in Cohort 1. However, if there are no signs of efficacy and no serious adverse events, then you will escalate the dose and one to three subjects will be treated in Cohort 2. Efficacy will be based on "clinical assessment and biomarker data (i.e., changes in *[four disease related biomarkers]* at Day *[XX]* vs baseline)."	This text has been revised to note it is anticipated six participants will be enrolled into cohort 1, at which point the DSMB will review aggregate safety and pharmacodynamic data.

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Question Number	Discipline	Question	FDA Response Number	FDA Response	NCATS Commentary
7 (continued)	Clinical	Does the Agency agree with the proposed first-in-human study design, including participant inclusion/exclusion criteria, dosing rationale, study population rationale, staggering of IP administration, stopping rules, safety oversight, and safety and efficacy endpoints?	4b-i	To ensure subjects safety, please clarify what you mean by "acceptable safety profile" and provide objective criteria that will be used to guide dose escalation or expansion. Please define the criteria using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 5.0.	This criterion will be refined in the final version of the clinical protocol submitted in the IND.
			4b-ii	The *[disease specific biomarkers]* have not been established as validated biomarkers for PA. Determining dose based on pharmacodynamic markers rather than Maximum Tolerated Dose (MTD) can result in the selection of an inadequate dose. Although your proposed approach is reasonable, you may wish to consider using MTD to determine the dose to take forward into your Phase 2/3 study(ies).	Dose escalation will no longer be determined by these measures. Secondary endpoint data will be monitored to evaluate pharmacodynamic response of the cohort 1 dose, inclusive of measures of change in *[disease related biomarkers]*. These measures may allow for future correlation to an efficacious dose of AAV9-hPCCA.
9	Clinical	Does the Agency agree with the proposed safety endpoint, and use of the described surrogate endpoints *[disease related biomarkers]* as the primary efficacy endpoint for the FIH study?	N/A	No, at this time, we do not agree with the use of a surrogate endpoint as a primary efficacy endpoint. Please provide additional rationale to support why it is necessary to use a surrogate endpoint. Additionally, you have not provided an adequate data-driven justification for the use of *[disease related biomarkers]* as surrogate biomarkers that are reasonably likely to predict clinical benefit...we note that you have not characterized the following for *[disease related biomarkers]*	The revised clinical study protocol will no longer include primary efficacy objectives. While the Sponsor acknowledges the urgency for generating efficacy data as early as practicable, for reasons described above determination of a clinically meaningful (non-surrogate) primary endpoint may not be achievable prior to initiation of a FIH study. Pharmacodynamic data will be monitored for future correlation to clinically meaningful measures, but this also may not be feasible within the timeframe of staggering between individual patient participants receiving investigational product.

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Question Number	Discipline	Question	FDA Response Number	FDA Response	NCATS Commentary
9 (continued)	Clinical	Does the Agency agree with the proposed safety endpoint, and use of the described surrogate endpoints *[disease related biomarkers]* as the primary efficacy endpoint for the FIH study?	N/A	1. Threshold for change in *[disease related biomarkers]* required to demonstrate a clinically meaningful effect 2. Consistency of response under various conditions 3. Reliability of *[disease related biomarkers]* for quantifying changes in the clinical outcome before and after treatment We recommend the following: a. Discuss the quantitative relationship between a change in *[proposed disease related biomarkers]* and a change in clinically meaningful endpoints b. Describe the reliability and consistency of *[proposed disease related biomarker]* measurement sunder variable circumstances. c. Study *[proposed disease related biomarkers]* as exploratory pharmacodynamic markers. d. Explore clinically meaningful endpoints throughout your development program	The Sponsor believes that attainment of the *[disease related biomarker assay]* levels as seen in Participants 3, 6 and 7 as shown in Figure 7 may provide evidence of a clinically meaningful outcome. Additional detail is provided below. Additional detail will be provided in the IND submission. As noted above, in addition to measures potentially collected in the Natural History study (for participants partaking in both), the proposed clinical trial will obtain at least two run-in period measures of the proposed pharmacodynamic biomarkers. These measures will help add to the totality of the evidence on both an intra- and inter-patient level. Additional detail will be provided in the IND submission. These endpoints are now proposed as secondary pharmacodynamic endpoints. Please refer to additional detail below regarding secondary and exploratory measures of clinical meaningfulness.

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Question Number	Discipline	Question	FDA Response Number	FDA Response	NCATS Commentary
9 (continued)	Clinical	Does the Agency agree with the proposed safety endpoint, and use of the described surrogate endpoints *[disease related biomarkers]* as the primary efficacy endpoint for the FIH study?	N/A	e. Consider and incorporate patient experience data in your developmental program and determination of clinically meaningful endpoints. Patient experience data provide information about the impact of a medical condition or a therapy on a patient's life, and information about the patient's preferences for treatment. Data may be collected by any persons (including patients, family members, patients' caregivers, patient advocacy organization, disease research foundations, researchers, and drug manufacturers). f. Should you decide to pursue *[the proposed disease-related biomarkers]* as surrogate endpoints, please address whether the expected clinical treatment effect size can be demonstrated in a feasible confirmatory trial given the size of the patient population.	Additional detail will be provided as the Sponsor gains clinical experience with AAV9-hPCCA.

15.2.2 Background for and Utility of Surrogate Endpoints for Proposed Clinical Trial

[The data presented in section 15.2.2 has now been published elsewhere. For most updated results and information, please refer to the articles below:

1. Manoli I, Gebremariam A, McCoy S, Pass AR, Gagné J, Hall C, Ferry S, Van Ryzin C, Sloan JL, Sacchetti E, Catesini G, Rizzo C, Martinelli D, Spada M, Dionisi-Vici C, Venditti CP. Biomarkers to predict disease progression and therapeutic response in isolated methylmalonic acidemia. *J Inherit Metab Dis.* 2023 Jul;46(4):554-572. doi: 10.1002/jimd.12636. Epub 2023 Jun 6. PMID: 37243446; PMCID: PMC10330948.

2. Aima C, Sloan J, Shchelochkov O, Ferry S, Van Ryzin C, Manoli I, Venditti, CP. P033: Integrating clinical data, biomarkers and in vivo propionate oxidation to inform genotype-based phenotype prediction in propionic acidemia. *Genetics in Medicine Open,* 2026]

As noted above, the Sponsor intends to robustly measure the proposed surrogate endpoints throughout the course of the study. To that point, the *[disease related biomarker assay]* and measures for *[two disease related biomarkers]* will be taken at least twice in the clinical trial run-in phase, including during screening (*[XX]* to *[XX]* days before IP administration) and baseline (*[XX]* to *[XX]* days before IP administration). Subsequent to IP administration, these measures will be repeated *[X]* additional times in study year *[X],* including at Day *[X]*, Week *[X]*, Week *[X]*, Week *[X]*, and Year *[X]* (all within study-defined windows). Measures will continue to be taken annually through the conclusion of the study's long-term follow-up phase at Year *[X]*. Participants enrolling into the clinical trial who also participated in the PA Natural History study may have additional pre-trial measures, as well. It is believed that this proposed testing will ultimately allow for a thorough analysis for correlation to the clinically meaningful measures also being gauged in the clinical trial (secondary, exploratory endpoints), as well as in comparison to data collected in the Natural History study.

Continuing, in addition to patients with PCCA- or PCCB-related PA, these measures have been previously assessed in patients with the organic acidopathy methylmalonic acidemia (MMA). Both are rare, autosomal recessive inborn errors of branched chain amino acid metabolism; while PA patients suffer from a defect in the propionyl-CoA enzyme, MMA results from a defect in the methylmalonyl-CoA mutase (MMUT) enzyme, or in the synthesis and transport of its cofactor, 5'-deoxy-adenosylcobalamin. Both may result in clinical manifestations due to accumulation of toxic metabolites, altered mitochondrial energy metabolism, carnitine depletion and coenzyme A sequestration. Chronic complications may include poor growth, movement disorders, cardiac dysfunction, and renal disease, among others (Fraser J et al., 2016). Patients with either PA or MMA may eventually benefit from liver transplantation to restore metabolic stability, thereby also presenting "control" comparators for the surrogate endpoint measures.

Similar to PA, MMA may also present across sub-types dependent upon the explicit gene/enzyme defect. This may manifest as complete or partial deficiency of the MMUT enzyme (*mut,* inclusive of $mut^0$and mut^- subtypes, respectively), or its cofactor 5'-deoxy-adenosylcobalamin (*cblA*, *cblB* subtypes, arising from MMAA and MMAB genes). Within these subtypes, mut^0 and cblB-type MMA are found to be more severe, correlating with decreased *[proposed disease-related biomarker]* that shows significant response post liver transplant (Manoli et al, 2023).

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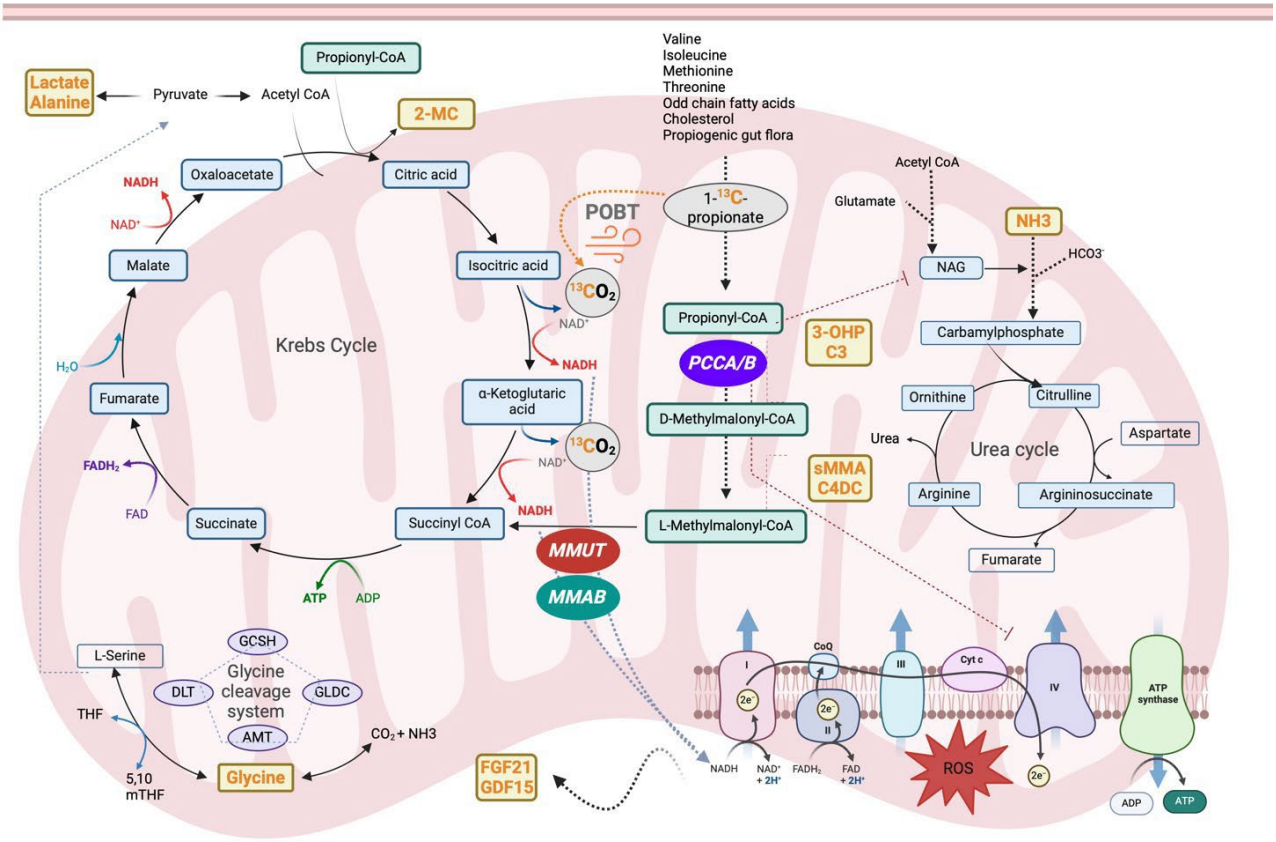
Figure 2 shows a simplified schematic of the organic acidemias in the propionate oxidation pathway, including the associated secondary dysfunction in Krebs cycle, urea cycle, electron transport chain and glycine cleavage system, resulting in characteristic biochemical abnormalities. While, as noted, these disorders differ in the affected gene/enzyme causing a metabolic block of these pathways, there are nevertheless biochemical and clinical commonalities across PCCA- and PCCB-related PA, as well as MMUT and MMAB-related methylmalonic acidemia (Manoli et al, 2023). Of specific note are the biomarkers *[disease related biomarkers]*.

Figure 2. Main biochemical pathways involved in organic acidemias



Given the conservation of these biomarkers, the similarity of metabolic and clinical manifestations across the organic acidemias, and the rarity of each disorder, supportive evidence is utilized where practicable. This is believed to be in line (if perhaps tangentially) with the tenets recently promulgated in the FDA's Draft Guidance entitled *Demonstrating Substantial Evidence of Effectiveness With One Adequate and Well-Controlled Clinical Investigation and Confirmatory Evidence (September 2023)*. While this guidance describes the utility of the "one drug, multiple related indications" paradigm, this is not feasible for a gene therapy trial where the investigational product's efficacy is directly predicated on the transcription and translation of a specific transgene (or, where the disease is caused by a single gene and/or enzyme defect).

Rather, it can be viewed that it is the pharmacodynamic principles and pathophysiology that are well understood across organic acidemias, thereby helping to provide confirmatory evidence for the appropriateness of the selected biomarkers. These biomarkers should be well-understood

pharmacodynamic endpoints, if not yet accepted by themselves as endpoints to establish effectiveness. This evidence may be further bolstered by *in vivo* studies in disease-specific relevant animal models, as well as natural history data.

Accordingly, data can and should be leveraged from related diseases and parallel programs. As detailed in literature (Manoli et al., 2021) and summarized in the pre-IND briefing package, data analyzed from the *[disease related biomarkers]* represent these types of sources.

15.2.2.1 *Biomarker Data from Natural History Studies*

In addition to the PA Natural History study ongoing at NIH, an analogous MMA natural history study has previously evaluated *[XX]* participants across the subtypes listed previously, including both before and after liver transplantation. Similar to the results of the PA Natural History study, this MMA study found that surrogate biomarkers may be of utility in distinguishing disease severity, and as such could be critical components of an interventional clinical trial. Additionally, it was noted that it may require long-term follow-up to determine whether a patient receiving the intervention improved from a "feels, functions, survives" perspective (Manoli et al, 2023).

Additional data and clarification not specifically provided at the time of the pre-IND meeting is included herein, in support of the amended FIH study design utilizing these surrogate endpoints as secondary measures to seek correlation to clinically meaningful patient parameters.

15.2.2.2 *[Disease related biomarker]*

The *[disease related biomarker assay]* has the ability to capture *in vivo* activity of the propionate metabolic pathway and acts as a relevant pharmacodynamic biomarker, which has also shown consistency across related diseases as demonstrated below (Manoli et al, 2023, Shchelochkov OA et al, 2021).

Figure 3. *[Bar graph comparing disease-related biomarker in patients with MMA and PA, with and without liver and/or kidney transplants]*

As shown in Figure 3, there are parallels between the *[disease related biomarker assay]* outputs for patients with the severe *mut*^{0} MMA (no/minimal enzyme activity, blue symbols), as compared to pre-transplant PCCA-and PCCB-related PA (orange and red symbols). Continuing, there is a statistically significant contrast as compared to the healthy control subjects (leftmost column), or patients with high enzymatic activity (*mut*$^{-}$ MMA) along with patients receiving liver or combined liver and kidney transplant and normalizing liver function (denoted by "LT" and "LKT" respectively). Kidney only transplant recipients (denoted by "KT"). have only minimal increase in enzymatic activity.

The *[disease related biomarker assay]* was also found to be reproducible. As shown in Figure 4, *[X]* healthy volunteers tested *[X]* times each over the span of *[X]* weeks.

[Evaluation of disease-related biomarker]

***DISCLAIMER**: This information is specific to AAV9-hPCCA and it does not directly apply to other investigational products. The information may no longer be applicable due to subsequent changes.*

Figure 4. Reproducibility of the *[disease related biomarker]* as shown in healthy volunteers (HV)

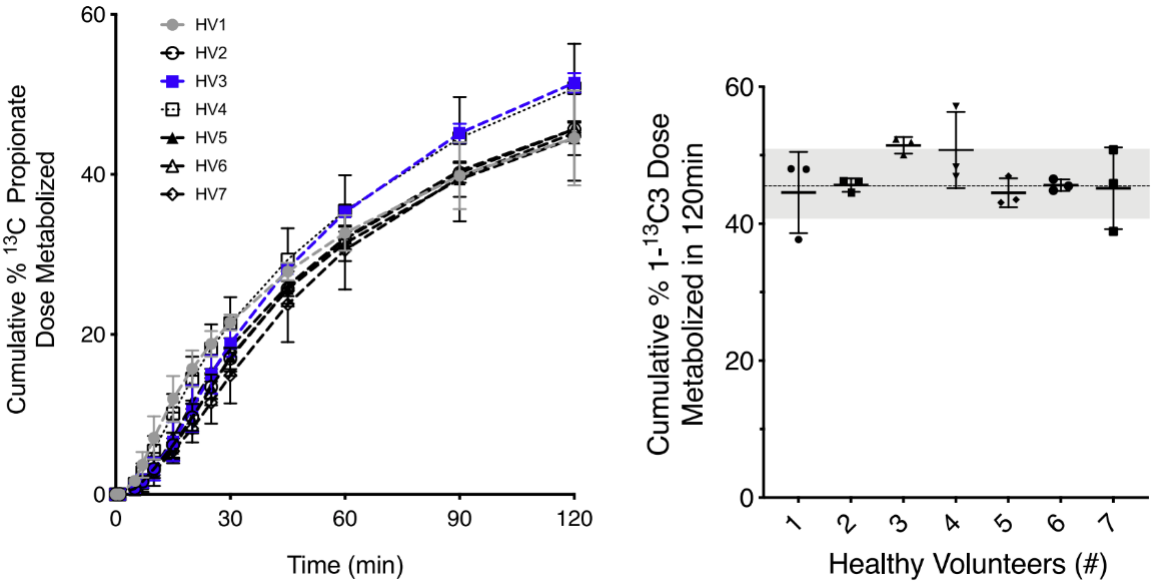
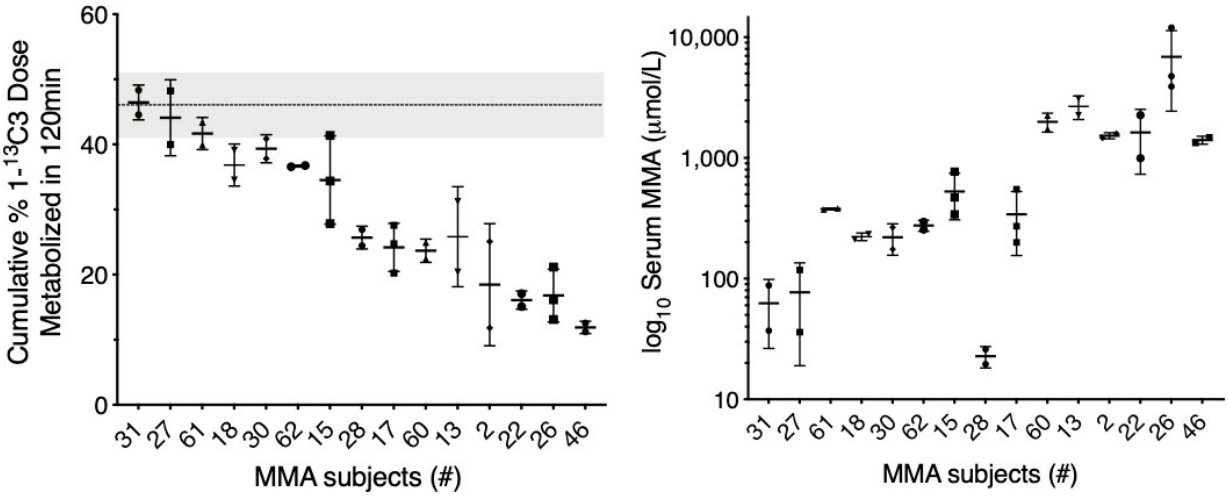


Figure 5. Comparison of the CV between *[disease related biomarker]* (left) and sMMA (right) in MMA patients

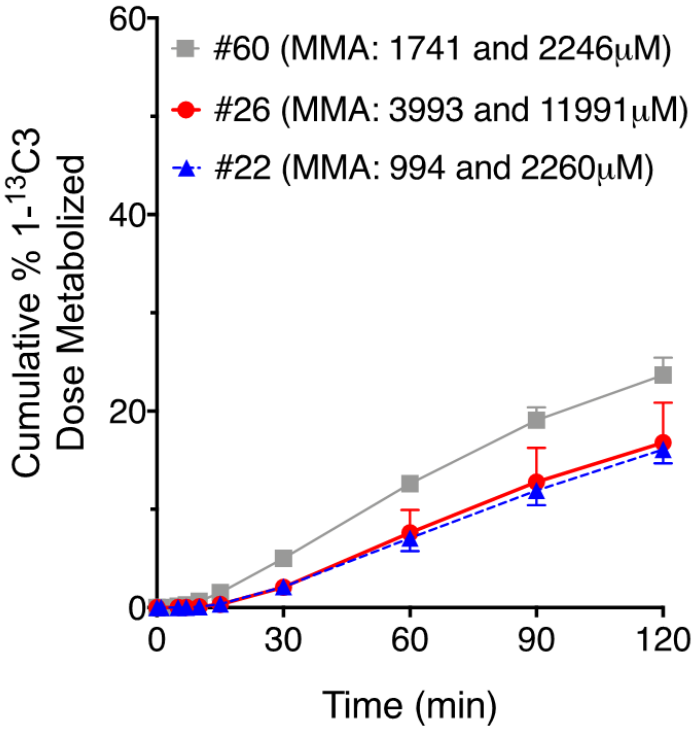


Variation between testing was higher in subjects with MMA (Figure 5), who had repeat testing at variable intervals ranging from 2 months to 4 years between their follow-up NIH visits. At 120 minutes, the CV for 1-^{13}C-propionate dose metabolized in 15 *mut* patients, who had repeated breath testing 2–3 times each, ranged from 0.39% to 50.84% with an average of 14.02 ± 12.86%. The POBT was also shown to exhibit less variability as compared to serum methylmalonic acid (sMMA) (Figure 6). Acceptable 1-^{13}C-propionate breath test CV of <15% was observed over a wide range of enzymatic activity. In contrast, CV for serum methylmalonic acid concentrations in the same subjects undergoing repeat testing was more variable, ranging from 1.5% to 75.3% with an average of 31.38 ± 24.55%.

Continuing, nearly identical POBT results were obtained despite significantly different serum methylmalonic acid concentrations, suggesting there is no significant effect of metabolite pool size and renal clearance on the POBT results. The test performs well in patients despite vastly

different metabolite pools, dietary status, and overall disease state (Figure 6).

Figure 6. *[Disease related biomarker]* results across patients with divergent sMMA concentrations



Importantly, similar results were seen in individuals with PA, with an average coefficient of variation of *[X]* (Figure 7). As can also be discerned, there is a demarcation between patients with a clinically severe phenotype of PA (patients # *[X]*), patients with more mild to moderate PA (patients #*[X]*), and patients who have restored liver function following transplant (denoted p/LT for post liver transplant).

Figure 7. *[Graph showing cumulative percentage of disease-related biomarker levels in patients with PA, pre- or post- liver transplant]*

This is shown further by the apparent bimodal distribution of this parameter in PA patients, with untransplanted patients (shaded in blue) largely occupying the left side of the figure (lower cumulative *[% disease related biomarker]* dose metabolized at *[XX]* minutes), with significantly reduced *[disease related biomarker]* compared to healthy volunteers (green shading), and post-LT PA patients (shaded in purple). As can be seen, only *[X]* untransplanted PA patient is above *[X]*%, while only *[X]* patient from the combined healthy volunteer and post-LT PA patient is below *[X]*% (Figure 8).

Figure 8. *[Bar graph showing frequency distribution of cumulative percentage of disease related biomarker levels in patients with PA, with and without liver transplant, and healthy volunteers]*

When pre-transplant PA patients are compared to a population of PA patients who have received liver transplants (healthy patient controls shown for qualitative comparison), the decreased *[disease related biomarker measure]* recovery is found to be statistically significant at both *[X]* and

[Y] minutes as compared to the latter (P values of 0.0003 and 0.0010, respectively, using the Mann Whitney test; data available upon request) (Figure 9). It is acknowledged and was discussed at the Pre-IND meeting that there exists variability amongst the PA population measured, including with PA type, patient age, and severity of disease. However, as was shown with both MMA and PA patients in the figures above, the CV for the *[disease specific biomarker]* values remained acceptable across this patient population diversity, with contrasts noted between mild manifestations as compared to post-LT PA patients and healthy volunteers.

Results are in line with those published previously, where it was noted that using machine learning (and informed by results from LT patients), *[disease related biomarker]* was found to be the most correlative surrogate endpoint in terms of discriminating between milder and severe PA (Shchelochkov OA et al, 2021), though the additional clinical biomarkers *[two disease related biomarkers]* will continue to be analyzed as secondary endpoints.

Figure 9. *[Graphs comparing disease-related biomarker levels at two different timepoints in patients with and without liver transplant, and controls]*

15.2.2.3 *[Two additional disease related biomarkers]*

While the *[disease-related biomarker]* is viewed as the measure best correlated with PA disease severity, its correlation with the biomarkers *[two other disease-related biomarkers]* has led to additional development to discern their respective predictive utilities.

[Disease related biomarker]
When compared to PA patients following liver transplant, individuals with PA have elevated *[disease related biomarker]* (nmol/mL) which is statistically significant ($p = 0.0016$; healthy control patients shown for reference) (Figure 10).

Figure 10. *[Graph comparing disease related biomarker levels in PA patients, with and without liver transplant, and controls]*

While it is acknowledged there exists a diverse range of *[disease related biomarker]* values, the *[disease related biomarker]* values differ according to disease severity as defined by the Vineland Adaptive Behavior Composite score (Mann Whitney test), as shown in Figure 11. The mean *[disease related biomarker]* value of *[X]* nmol/mL (N=*[X]*; *[X-Y]* nmol/mL) in mild PA patients shows both lower values and lesser variability compared to severe PA patients (N=*[X]*; *[X]* nmol/mL, range of *[X-Y]*). There is also a statistically significant difference ($p < 0.0001$) in *[disease related biomarker]* values between severe PA patients and those who have received LT. This is an important distinction as the severe patients represent the population likeliest to receive benefit from the proposed IP, with an effective goal of restoring liver function without transplant.

In the commentary received in advance of the Pre-IND meeting, the Agency requested the Sponsor quantify biomarker values as it relates to disease severity and clinical meaningfulness. While quantification remains an ongoing exercise, inputs into disease severity have to-date included *[disease related biomarker]* activity and the Vineland Adaptive Behavior Scales-3nd Edition (Sparrow et al 2016). While the Agency had noted the Vineland score may not be considered interpretable as a study secondary outcome assessment, it is a validated, standardized instrument that measures communication, daily living skills, socialization and motor skills, (feels, functions,

survives), and as such may be useful as a baseline measure.

Standard scores are provided for each domain and for an overall Adaptive Behavior Composite, and growth scale values are provided for 11 subdomains. The Vineland-3 growth scores, provide a sensitive metric of change in person-ability over time, compared to many of the norm-references standard cognitive measures which are primarily designed for diagnosis, and show poor reliability at the lower tail of cognitive abilities (Farmer et al, 2020). Given this, it may satisfy the need of a sensitive measure to help best discern PA disease severity, thereby providing context for future endpoint measures.

Additional information will be provided at the time of IND submission.

Figure 11 *[Graph comparing disease related biomarker levels among mild, intermediate and severe patients with PA, patients with PA who have undergone liver transplant, patients with MMA, and healthy controls]*

Notably there does exist larger variance in *[a specific disease related biomarker]* measurements in PA subjects, particularly in those with more severe disease, and as compared to post-LT PA patients as well as to the more mild to moderate PA (patients # *[X, Y and Z]*) (Figure 12). This may be due to the infrequency of measures (in some cases years apart), which could have been influenced by factors such as change in diet, microbiome, medications, as well as clinical manifestation progression.

Figure 12. *[Graph showing variation of disease related biomarker levels in patients with PA, with and without liver transplant]*

[Disease related biomarker]

On the aggregate, there does not appear to be a statistically significant difference between PA patients pre- and post-transplant (Figure 13). Pre-transplant patients, inclusive of all levels of disease severity (N=*[X]*), have a mean value of *[X]* nmol/mL (*[X-Y]* nmol/mL). Post-transplant patients (N=*[X]*) have a mean value of *[X]* nmol/ML (*[X-Y]* nmol/mL). Note that data excludes one outlier pre-transplant patient with renal failure and extremely elevated *[disease related biomarker]* level (*[X]* nmol/mL), mut MMA and healthy patient controls included for comparison.

Figure 13. *[Graph comparing disease related biomarker levels in patients with PA, with and without liver transplant, patients with MMA, and controls]*

However, when disease severity is taken into account (as measured by inputs from Vineland), the correlation becomes statistically significant ($p = 0.0003$). As shown in Figure 14, the subset of mild PA patient has a lower *[disease related biomarker]* average value of *[X]* nmol/mL with a tighter range (*[X-Y]*), while the severe PA patient population has an average of *[X]* nmol/mL (range *[X-Y]*). The mean value for the mild PA cohort approaches that of the post-transplant cohort (*[X]* nmol/mL) and healthy patient control group (*[X]* nmol/mL).

Figure 14. *[Graph quantifying disease related biomarker levels in patients with mild, intermediate and severe PA, liver transplanted PA patients, MMA patients, and controls]*

Patients with PA and MMA from an Italian cohort treated with liver transplant (LTx patients PA N=*[X]*, MMA N=*[X]*) also demonstrated a reduction of *[disease related biomarker]* (noted as MCA) compared to pretransplant (Pre-tx patients PA N=*[X]*, MMA N=*[X]*) levels p<0.001. Patients from this cohort treated by combined liver-kidney transplantation (LKTx) presented a more pronounced reduction in *[disease related biomarker]* (noted as MCA) than those by LTx or isolated KTx (Maines et al 2020).

Figure 15. Italian PA and MMA cohort demonstrated lower *[disease related biomarker]* levels after liver transplant

[For the data presented in Figure 15 please refer to Figure 4 in the article below:

Maines E, Catesini G, Boenzi S, et al. Plasma methylcitric acid and its correlations with other disease biomarkers: The impact in the follow up of patients with propionic and methylmalonic acidemia. *J Inherit Metab Dis.* 2020;43:1173–1185. https://doi.org/10.1002/jimd.12287]

Similar to *[disease related biomarker]*, there exists a larger variability in intra-patient measurements of *[another disease related biomarker]*. Notably this variability increases with increasing disease severity (i.e., larger *[disease related biomarker]* values) and is lessened in patients with mild disease (subjects # *[X, Y, and Z]*) and post-transplant patients (Figure 15). Here again these measures may have been taken years apart and with multiple factors changing similar as described for Figure 16. The proposed clinical trial will best gauge the biomarker's utility with more structured measurements.

Figure 16. *[Graph quantifying intra-patient variability of a disease related biomarker in patients with PA, with or without liver transplant]*

15.2.2.4 Correlation with Clinical Parameters

To date, *[disease related biomarker]* measured via the *in vivo* *[disease related biomarker assay]* has been found to be correlated with clinically relevant parameters including G-tube placement (presence or absence; Mann-Whitney test $p < 0.0001$, Figure 17), FSIQ ($R^2 = 0.5265$, $p < 0.0001$), left ventricular ejection fraction ($R^2=0.1884$, p=0.0166), glomerular filtration rate (GFR) based on Cystatin C ($R^2=0.5687$, p=0.0007, Figure 18), liver span ($R^2=0.2686$, p=0.0033), complete protein intake percent compared to RDA ($R^2=0.1755$, p=0.0265), lactate measurements ($R^2=0.1618$, p=0.0375, Figure 19), growth, intellectual disability, and sensorineural hearing loss.

Figure 17. *[Graph quantifying cumulative percentage of disease related biomarker at 120 minutes in patients with PA, with and without G-tube placement]*

Figure 18. *[Graphs showing correlations of a disease related biomarker with clinical parameters of cognitive, cardiac and kidney functions in patients with PA]*

As shown above, the % *[disease related biomarker]* dose metabolized at *[X]* minutes correlates with clinical parameters in PA participants (post-organ transplant data is excluded). In Tile A, the full-scale IQ (FSIQ) (healthy population mean = 100, standard deviation = 15) was obtained as part of Neurodevelopmental Assessment. The graph shows the correlation of FSIQ obtained in *[X]* PA patients, repeated measurements were not included, each dot represents one patient. Tile B shows the correlation of *[disease related biomarker]* dose metabolized at *[X]* minutes and

[X] left ventricular ejection fraction (LVEF) (%) measurements obtained from *[X]* PA patients by echocardiogram, *[X]* patients had repeated measurements obtained at follow up visits. Finally, Tile C shows the correlation of *[disease related biomarker]* dose metabolized at *[X]* minutes and *[X]* glomerular filtration rate (GFR) estimations using Cystatin C based equations in *[X]* PA patients, *[X]* patients had repeated measurements obtained at follow up visits.

Figure 19. *[Graph showing correlations of a disease related biomarker with clinical parameters in patients with PA]*

Shown above is the correlation of % *[disease related biomarkers]* dose metabolized at *[X]* minutes with clinical parameters in PA participants (post-organ transplant data is excluded). Tile A shows the correlation of % *[disease related biomarkers]* dose metabolized at *[X]* minutes with liver span obtained from abdominal ultrasound in *[X]* PA patients (*[X]* patients had two longitudinal repeated measurements). Tile B shows the correlation of % *[disease related biomarkers]* dose metabolized at *[X]* minutes with complete protein intake as a percent of age reference RDA (recommended dietary allowance) in *[X]* PA patients (*[X]* patients had two repeated measurements obtained during follow up visits). Lastly, Tile C shows the correlation of *[disease related biomarkers]* dose metabolized at *[X]* minutes and 27 lactate measurements from *[X]* PA patients, *[X]* patients had repeated measurements.

Similarly, *[disease related biomarker]* was found to have a statistically significant correlation to FSIQ (R^2= 0.3466, p< 0.0001), while *[disease related biomarker]* had a modest but still significant correlation (R^2=0.1519, p = 0.0142, Figure 20). The *[disease related biomarker]* has also been correlated to LVEF via echocardiogram (R^2= 0.4930, p < 0.0001; Figure 21) and estimated GFR using creatinine and cystatin C formulas (R^2=0.5122, p < 0.0001 and R^2=0.3935, p<0.001, respectively) in a statistically significant manner. However, the observed markedly increased *[disease related biomarker]* levels might be influenced by decreased renal clearance in some PA patients with advanced chronic kidney disease (Figure 22, right), and as such may not be an appropriate marker for the GFR biochemical outcome marker. On the other hand, *[disease related biomarker]* levels don't seem to be influenced by renal function (Figure 22, left).

Figure 20. *[Graph showing correlation of a clinical parameter to two disease related biomarkers in patients with PA prior to transplant]*

Figure 21. *[Graph showing correlation of a clinical parameter to a disease related biomarker in patients with PA prior to transplant]*

Figure 22. *[Graph showing correlation of a clinical parameter to a disease related biomarker in patients with PA prior to transplant]*

15.2.2.5 Determination of Clinical Utility (Meaningfulness) of Proposed PA Biomarkers

The Sponsor acknowledges and understands the need to choose clinically meaningful outcomes that can be reliably interpreted, including those which can function as signals of efficacy in the FIH clinical trial. Data in hand related to *[three disease related biomarkers]* provide multiple examples of statistically significant correlations with clinical parameters in PA patients, but it is understood that none have functioned as surrogate endpoints in licensed products for treatment of PA (Carbaglu is indicated solely for treatment and prevention of high amounts of ammonia in the

blood).

Continuing, FSIQ may not change with a therapeutic intervention administered during late adolescence/young adulthood, as it wouldn't be anticipated for IQ to normalize in a patient with intellectual disability (though potential improvements in functional assessments, as reflected in Vineland ABC standard scores, are possible).

Further, LVEF can be abnormal even in mild cases, such as patients who are metabolically stable or have a late-onset phenotype. There is a known rare phenotype consisting of isolated cardiomyopathy as the only symptom in PA patients presenting in their late teens or early adulthood after being previously asymptomatic (Lee et al, 2009; Laemmle et al, 2014; Riemersma et al, 2017; Grotto et al, 2019) Additionally, a founder mutation in the Amish population in *PCCB* c.1606A>G is associated with a generally mild PA phenotype but a high incidence of cardiomyopathy. (Scott Schwoerer et al, 2018; Ehrenberg et al, 2022). These patients will be excluded from the proposed clinical trial.

GFR may be a late manifestation of PA, often occurring in the 20-30 years age range in severe PA patients (Shchelochkov et al 2019). Biomarkers influenced by kidney function may not correlate to other changes, as their response is limited by decreased clearance by the kidneys, unless the kidneys are specifically targeted by the treatment.

Given these gaps in many of the clinical measures, coupled with the ongoing understanding of the PA biomarkers proposed herein, there exists a need to establish correlations with clinical endpoints such as *[clinical endpoints]*. While the Sponsor acknowledged Agency commentary at the Pre-IND meeting, long-term collection of these data may prove informative as to which clinically meaningful outcomes correlate with change in PA biomarkers.

In line with this, the Sponsor proposed the revised version of the FIH gene therapy clinical trial synopsis, in both clean and tracked changes. Amendments in line with both Agency commentary as well as the data provided herein include:

- Assessment of PA biomarkers for pharmacodynamic response is now a secondary study objective (and related endpoint)
- When reviewing participant cohort data, the DSMB will use safety data (e.g., adverse events, dose-limiting toxicities) in making determinations for subsequent IP administration, while PA biomarker data will be monitored
- The study run-in period will allow for *[X]* baseline determinations of biomarkers, and at least *[X]* for *[disease related biomarker assay]* (plus one additional if needed)
- *[Two disease related biomarker]* measures for mild PA added as exclusionary criteria
- Change in *[clinical endpoint]* added as a secondary endpoint
- Change in *[clinical endpoint]* moved to an exploratory endpoint (with additional detail)
- Quality of life measurements moved to the exploratory endpoints
- Actigraphy monitoring of *[clinical endpoint]* added as an exploratory endpoint
- *[Clinical endpoint]* score added to the *[standardized score]* measurement

- *[Disease related biomarker]* added as a parameter for change in the rate of decline of cardiac function

A wider age range may lead to differentiation in clinically meaningful outcomes across these age groups, including with neurocognitive adaptive behavior composite score changes, as well as stability in terms of renal and cardiac function (depending on the participant's baseline standing). As such, the longer-term follow-up over *[X]* years may be necessitated to best understand changes in clinically meaningful parameters.

As noted above, it is believed that the data shown in Figure 7 help quantitate a clinically meaningful outcome. If severe PA patients (low *[disease related biomarker assay]* results, ~<*[X]* %) can reach *[disease related biomarker]* noted by patients with mild PA (i.e., cumulative % *[disease related biomarker]* dose metabolized in *[X]* minutes of ~*[X]*%), this may be an indicator of clinical meaningfulness. It is not anticipated that these patients will reach the ~*[X]*% values as shown in the LT patients, but as noted in the Pre-IND meeting the effective goal of the AAV9-hPCCA program is to mitigate the need for LT and its associated risks.

This dovetails well with the study inclusion and exclusion criteria related to the proposed biomarkers. Notably they serve more to function as guardrails by excluding participants with milder forms of PA, who may not derive the same type of benefit as they may already maintain a good quality of life compared to those with severe PA patients, skewing their personal risk/benefit ratio towards risk. Based on data presented both previously and herein, it is believed this utilization of the biomarkers is appropriate.

As it relates to study inception and evolution, given the relative dearth of PA patient data and the massive unmet need for treatment, a bespoke clinical solution that establishes a tolerable risk/benefit profile and has an ability to adapt to emergent data is necessitated. Given the significant but not directly clinically meaningful PA biomarker data in hand along with the nonclinical data previously presented (and still being collected), the Sponsor is hopeful continued Agency collaboration will best harness these inputs in helping to identify next steps for establishing a clinical program tailored to this ultra-rare disease paradigm that maximizes the ability to gain interpretable safety and efficacy data as early as practicable.

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Appendix: NGS Study Report

Appendix: NGS validation summary

DISCLAIMER: This information is specific to PCCA and may not be applicable to other products or studies.

Potency Determination of a Recombinant AAV Vector

Purpose

This summary describes the proposed assay to determine the potency of a recombinant AAV vector in cell culture preparations. The assessment is made through transduction of cells with the vector followed by extraction of RNA, reverse transcription and quantification using droplet digital PCR (ddPCR).

Cell Culture and Transduction

The cell culture potion of the method is performed using 48-well cell culture plates. Approximately 100,000 *[name of cell line]* knockout cells (to be optimized) are added into the wells of a 48-well plate for 24 hours in a 37°C incubator with 5% CO_2.

The cells are transduced with a series of dilutions of viral vector (in duplicate) to allow gene transfer. The magnitude of the vector dilution is to be determined empirically based on the level of concentration of the vector material to be tested. Dilutions are performed in cell culture media and added to the plated cells. The transduced plate(s) are incubated in a 37°C incubator with 5% CO_2 for 48-72 hours (optimal time to be determined empirically).

After vector transduction, the culture medium is aspirated, and PBS is added. The cells are dislodged and are collected independently from each well, pelleted, and the supernatant is aspirated. The pellets can then be stored at -80°C or moved directly into the extraction phase.

Extraction and ddPCR

The total RNA from the thawed cell pellets is extracted using the RNeasy Kit (Qiagen). The total RNA is then quantified using a NanoDrop. The sample is diluted to ~25 ng/µL (to be optimized) and loaded into the ddPCR reaction mix. The ddPCR reaction mix consists of primers and probe for *[specific regulatory element]* and ALB (albumin) along with 1-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad). The reaction is partitioned into ~20,000 droplets using an automatic droplet generator and subjected to thermal cycling based on Bio-Rad's recommendation (to be optimized as necessary). Briefly, the thermal cycling consists of reverse transcription at 50°C for 60 minutes, enzyme activation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 30 seconds and annealing/extension temperature for 1 minute at ramp rate of 2°C, and then enzyme deactivation at 98°C for 10 minutes. The droplets will be analyzed using QX200 Droplet Reader (Bio-Rad).

Data Analysis

The amount of mRNA present in transduced cells is determined. The *[specific regulatory element]* present in the transgene is one of the targets for PCR amplification. The human albumin (ALB) housekeeping gene is also amplified, with the number of ALB copies serving to normalize the *[specific regulatory element]* copy number per cell. The quantity is expressed as the number of copies of mRNA per 1 mL of vector inoculum.

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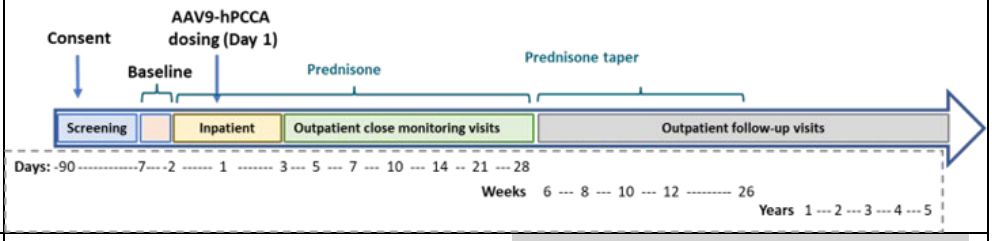
Proposed Phase 1/2 Clinical Trial Protocol Synopsis

Title	A Phase 1/2, Open-Label Safety, Dose-Escalation, Single-Center Clinical Study of AAV9-hPCCA Gene Therapy in Propionyl-CoA Carboxylase Subunit Alpha (*PCCA*)-Related Propionic Acidemia (PA)
Sponsor	NCATS
Study Phase	Phase 1/2
Study Schema	[Schematic of clinical study showing the timeline of proposed events:] 
Study Population	Pediatric and adult patients, ≥3 years of age, *[NIH Clinical Center-specific criteria]* with clinically, biochemically, and/or molecularly confirmed pathogenic variants in *PCCA* that cause PA
Number of Sites and Subjects	One clinical site; approximately 4-9 research participants.
Treatment Groups	• *Cohort 1: [starting dose]* (N=3-6) • *Cohort 2*: *[higher dose]* (N=1-3) In addition, there will be a comparator group (historical control) from the NIH natural history study of PA (NCT02890342) [1, 2].
Primary Objectives	To assess safety and tolerability of intravenous administration of AAV9-hPCCA in research participants with *PCCA* type PA
Secondary Objectives	1. To assess changes from baseline in response biomarkers, a.k.a., pharmacodynamic (PD) response, to AAV9-hPCCA 2. To assess patient outcomes as measured by the frequency and severity of specified clinical events, including metabolic crises in need of sick-day dietary modification or hospitalization and/or need for referral for liver, kidney or liver and kidney transplantation 3. To assess patient and caregiver reported outcomes (a separate consent will be presented to the caregiver)
Study Design	This is a phase 1/2, open-label, safety, dose-escalation, single-center, clinical study of AAV9-hPCCA gene therapy in research participants with *PCCA*-related PA. The study will be informed with a comparator group from an ongoing natural history study at NIH (NCT02890342; Natural History, Physiology, Microbiome and Biochemistry Studies of Propionic Acidemia). The study will consist of the following 2 cohorts: • Cohort 1: approximately 3-6 research participants, treated with a single dose of *[starting dose]* AAV9-hPCCA • Cohort 2: approximately 1-3 research participants, treated with a single dose of *[higher dose]* AAV9-hPCCA ***[Detailed study schema]***

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Title	A Phase 1/2, Open-Label Safety, Dose-Escalation, Single-Center Clinical Study of AAV9-hPCCA Gene Therapy in Propionyl-CoA Carboxylase Subunit Alpha (*PCCA)*-Related Propionic Acidemia (PA)
Study design: Cohort 1 ***[starting dose]***	The first participant dosed in this cohort will be an adolescent, ≥12 years to <18 years of age. If no eligible participant is identified, we will age de-escalate to the next eligible participant ≥3 and <18 years of age. A Data and Safety Monitoring Board (DSMB) review of available safety data will be performed at the end of 12 weeks after investigational drug administration for the first participant. If the safety review is satisfactory, dosing will proceed to the next participant (≥3 years of age), with at least a 12-week stagger separating dosing between eligible study participants. The cohort intends to enroll up to a total of six participants (age ≥3 years), pending DSMB review. After the third participant in cohort 1 has been followed for 12 weeks, the DSMB will review aggregate safety and biomarker data and advise to continue enrolling 3 additional subjects in cohort 1 or dose escalate to Cohort 2. All participants will be monitored with key metabolic biomarkers and standard clinical and laboratory evaluations for 52 weeks and then followed by collection of safety data for an additional four years. Biomarker data will be monitored to evaluate the pharmacodynamic response of this dose (trend of changes in *[disease-related biomarkers]*) [1, 3-5]. Repeat measures for all biomarkers will be obtained at screening/baseline and followed through the course of corticosteroid therapy and throughout the 5 yrs of follow up to assess magnitude and durability of change in propionyl-CoA carboxylase activity and allow future correlation to an efficacious dose.
Study design: Cohort 2 ***[higher dose]***	The first participant dosed in this cohort will be an adolescent, ≥12 to <18 years of age. If no eligible participant is identified, we will age de-escalate to the next eligible participant ≥3 and <18 years of age. The DSMB will review available safety data at the end of 12 weeks after investigational drug administration for this first participant. If the safety review is satisfactory, dosing of subsequent participants will proceed in an analogous manner to Cohort 1, up to a total of three participants ≥3 years of age (pending DSMB review of data). At least 12 weeks will separate dosing between eligible study participants. All participants will be monitored with key metabolic biomarkers and standard clinical and laboratory evaluations for 52 weeks and then followed by collection of safety data for an additional four years. At the end of Cohort 2, there will be a DSMB review of safety data for the entire cohort.
Study timeline and schedule of events (see Table A.1 below this synopsis):	• Study candidates will be evaluated for study eligibility as part of the screening period, ~90 days prior to the anticipated dosing day. Participants may co-enroll, if not already participating, in the Natural History Study of propionic acidemia conducted at NIH (NCT02890342). For screening, study candidates will undergo collection of labs, diagnostic studies, consultation with hepatology, cardiology, nephrology as needed, dietary assessment, and neurocognitive evaluation to determine their eligibility according to the inclusion/exclusion criteria outlined in this clinical synopsis. • Participants who meet the eligibility criteria during the screening will be re-evaluated seven days prior to the anticipated infusion date (+/- 2 days; baseline) to ensure they continue meeting the eligibility criteria and allow for 3 baseline determinations of biomarkers and 2 (+1) for *[disease related biomarker]*to establish a pre-treatment baseline.

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Study timeline and schedule of events (continued)	• Participants eligible for AAV9-hPCCA administration will commence prophylactic treatment with oral corticosteroid (prednisone or prednisolone): 1 mg/kg/day (not to exceed a maximum dose of 60 mg/day) started 24±4 hours prior to starting administration of AAV9-hPCCA. This dose will continue for 30 days but could be extended if there is any evidence of immunological response to AAV9, based on elevated liver function tests, and if clinically indicated, other laboratory parameters. Upon completion of the oral 1 mg/kg/day corticosteroid course, the corticosteroid dose will be tapered over the following 28 days. See Appendix A, Table A.1 for management of elevated transaminases and corticosteroid tapering. • Participants will be hospitalized for the administration of AAV9-hPCCA up to three days prior to the anticipated dosing day for baseline evaluations, pre-medications, and placement of the IV and peripherally implanted central catheter (PICC) line, as applicable. Participants will be observed at the hospital for 72 hours post-dosing prior to their discharge (see Appendix A, Table A.2 for management of infusion-related reactions). They will remain local to NIH at Children's Inn for ~2-3 months and return for monitoring visits every 2-3 days for the first two weeks, then weekly for the first month, biweekly until week 12 and then at 6 months (week 26), and 1, 2, 3, 4, and 5 years post AAV9-hPCCA administration. • Participants will be closely monitored for liver toxicity and metabolic events, such as vomiting/metabolic acidosis (see Appendix A, Table A.3 for management of vomiting & metabolic acidosis). • Participants will be closely monitored for signs of hyperammonemia (see Appendix A, Table A.4). • Participants will be closely monitored for signs of thrombocytopenia, microangiopathic hemolytic anemia, and organ damage (e.g., acute kidney injury, GI issues, or central nervous system [CNS] manifestations) of thrombotic microangiopathy (TMA), including clinical signs, frequent complete blood count (CBC) with peripheral smears/schistocyte counts, platelet counts/D-dimer and complement levels (see Appendix A, Table A.5 for management of TMA). An experienced nephrologist and expert in TMA are part of the clinical team and will guide all assessments. Participants who develop TMA will receive eculizumab or a similar drug, and if TMA progresses to severe kidney failure, hemodialysis will be implemented. Any patient weighing <40kg or aged <13 years will be transferred to a facility that can perform pediatric dialysis, such as Children's National Hospital (CNH). • Myocarditis will be managed according to grade of severity, per table below and monitoring of troponin levels (See Appendix A, Table A.6). • Participants will be monitored for anaphylaxis. • During the first 12-month follow-up after administration of AAV9-hPCCA, participants will continue taking their baseline medications and diet as advised by their healthcare providers, unless a change is warranted based on the clinical findings. • Participants will be followed up for safety and efficacy of AAV9-hPCCA for five years.
Rules for suspending the study (enrollment and investigational product (IP) administration):	The study will be *suspended* if after the administration of AAV9-hPCCA, any of the following occurs: • A participant dies • A participant develops a malignancy • A participant experiences a Grade 4 or higher adverse event (based on CTCAE v5.0) The DSMB chair or sponsor of the study determines that a medical event requires additional evaluation by the full DSMB.

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Title	A Phase 1/2, Open-Label Safety, Dose-Escalation, Single-Center Clinical Study of AAV9-hPCCA Gene Therapy in Propionyl-CoA Carboxylase Subunit Alpha (*PCCA)*-Related Propionic Acidemia (PA)
Rules for stopping the study:	The study will be *stopped* within the first 12 months after the administration of AAV9-hPCCA, if any of the following occurs: • The sponsor determines that an event or data warrant termination of the study for any reason. • A participant develops malignancy determined by the sponsor to be related to the AAV9-hPCCA administration. • A participant death is determined by the sponsor to be related to the AAV9-hPCCA administration. If the study has been stopped for any reason listed above, participants who had been dosed prior to the qualifying event, will continue to be followed as part of the long-term five-year follow-up.
Estimated Study Duration	Individual research participant involvement in the study is five years plus three months, from screening and baseline assessments to the end of the long-term follow-up. The active phase study duration is estimated to be 24 to 60 months, depending on the rate of enrollment (three vs two participants per year).
Justification for Study Population	PA is a rare, genetically and clinically heterogeneous disease *[number of patients in the NIH NHS study on PCCA-related PA]*, with affected individuals displaying a range of clinical severity and no common variant in the *PCCA* gene [6]. Many PA patients receive elective liver transplant (LT) early in life, and median ages have been reported for different patient cohorts: • 2.7 years, with a minimum of 0.6 years and maximum of 23.0 years, as reported in a European cohort using questionnaire-based data collection [7] • 3.2 years, with a minimum of 1.1 years and maximum of 9.0 years, as reported in UK and French cohorts [8] • 1.9 years, with a minimum of 0.4 years and a maximum 9.4 years, as reported in a retrospective study [9] Additional data from several literature reviews on LT in PA [10-13] highlight the need for earlier elective LT in order to avoid recurrent life-threatening metabolic decompensations with hyperammonemia, to prevent long-term complications (cardiomyopathy and end-stage renal disease) and to maximize neurocognitive function. However, it is noted that LT does not guarantee restoration of clinical function. Within the UK and French cohorts, it was noted that seven of 12 patients (58%) died within the first year after LT. Further, out of 17 LT procedures, 13 had early and severe complications [8]. Published literature suggests PA is an ultra-rare disease affecting ~1 in 243,000 individuals in the US with approximately half due to variants in the *PCCA* gene [14-16]. This highlights the challenges of enrolling a homogeneous study population in this first-in-human study. The rationale for including patients ≥3 years of age is based on the following arguments: 1. Older, adult PA patients with severe disease accumulate irreversible end-organ damage and thus are less likely to benefit from the proposed gene therapy trial. 2. Due to the largely irreversible nature of end-organ damage in PA, it is the younger patients who are more likely to benefit from the experimental AAV9-hPCCA therapy. Therefore, the study needs to include a pediatric population. 3. There is a limited number of individuals with *PCCA*-related PA, and many undergo LT in early childhood, excluding them from this AAV trial, which necessitates the inclusion of pediatric patients.

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Title	A Phase 1/2, Open-Label Safety, Dose-Escalation, Single-Center Clinical Study of AAV9-hPCCA Gene Therapy in Propionyl-CoA Carboxylase Subunit Alpha (*PCCA)*-Related Propionic Acidemia (PA)
Summary of Eligibility Criteria (*Inclusion Criteria)*:	1. Participants ≥3 years of age at the time of consent *[NIH Clinical Center-specific criteria]* (whenever applicable, assent will be obtained) 2. Individuals with the diagnosis of *PCCA*-related PA confirmed molecularly (*PCCA* gene analysis). 3. Biochemical evidence of PA (elevated 2-MC, 3-OHP in body fluids) 4. A clinical history consistent with severe PA, defined as meeting at least two of the conditions listed below: a. A history of neonatal encephalopathy with or without hyperammonemia b. A history of hemodialysis for hyperammonemia after infancy c. One or more hospitalizations, including pediatric intensive care unit (PICU) and/or emergency room (ER) visits or need for sick-day dietary adjustment for metabolic ketoacidosis in the last 24 months prior to study enrollment. d. Complete protein tolerance less than the recommended daily allowance for age and/or gastrostomy feeding-dependance for meeting caloric needs. e. One or more disease-related complications including cognitive impairment, failure to thrive (height, and/or weight, or head circumference falls lower than the third percentile), renal disease, basal ganglia injury, optic nerve disease, history of pancreatitis, bone marrow failure, cardiomyopathy. 5. Ability and willingness to comply with the scheduled study visits and procedures. 6. Complete vaccination according to the CDC vaccination schedule for age at the time of consent, with exception of live attenuated vaccines with appropriate time intervals post-immunization per accepted recommendations. 7. Females of childbearing potential who are sexually active must use at least one method of contraception. 8. Males who are sexually active must agree to use an effective barrier method (male or female condom) of contraception starting one week before and continuing until six months after gene transfer. If the participant's partner is able to become pregnant, a second form of contraception will be required for the same duration.

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Title	A Phase 1/2, Open-Label Safety, Dose-Escalation, Single-Center Clinical Study of AAV9-hPCCA Gene Therapy in Propionyl-CoA Carboxylase Subunit Alpha (*PCCA)*-Related Propionic Acidemia (PA)
Summary of Eligibility Criteria (*Exclusion Criteria)*:	1. A molecular genetic diagnosis of *PCCB*-related PA. 2. High propionate oxidative capacity, as demonstrated by *[threshold value]* as seen in PA patients with clinically mild disease or after LT. 3. Anti-AAV9 neutralizing antibody titer above *[cut-off value]* dilution. 4. Episodes of metabolic decompensation within two months prior to the scheduled dose administration 5. History of the following interventions at any point in the past: a. Gene therapy or mRNA therapy b. Solid organ transplantation c. Cell transfer therapy 6. History of investigational drugs within five half-lives of the drug before the first screening visit, whichever comes first. 7. History of malignancy or immunocompromised state, regardless of etiology 8. Left ventricular ejection fraction *[XX]* by transthoracic ECHO or other comparable diagnostic modality. 9. QTcF *[XX]* for either male or female participants. 10. Creatinine-based estimated glomerular filtration rate (eGFR) of *[XX]* as estimated by the bedside Schwartz eGFR equation (<chronic kidney disease stage *[XX]*) or ongoing dialysis for chronic kidney disease. 11. Any of the following laboratory finding at the time of the screening visit: a. Hemoglobin *[XX]* b. Absolute neutrophil count *[XX]* c. Platelet count *[XX]* d. Alanine transaminase (ALT), aspartate aminotransferase (AST), or total bilirubin *[XX]* times the upper normal limit for age e. Plasma lipase or amylase *[XX]* times the upper normal limit for age f. Plasma ammonia *[XX]* times the upper normal limit for age or clinical symptoms of hyperammonemia (e.g., lethargy, excessive irritability, vomiting) 12. Clinical or non-invasive testing indicative of advanced liver fibrosis or history of liver disease, a pre-existing diagnosis of portal hypertension. 13. History of chemotherapy, granulocyte colony stimulating factor (GCSF) or immunomodulating drugs (e.g., corticosteroids or intravenous immunoglobulin) within six months before the first screening visit. 14. Participant has received a live virus vaccine in the previous six weeks prior to screening (measles, mumps, rubella [MMR], Varicella, Rotovirus, Varivax, oral Polio). Live vaccines should not be administered within the 6 weeks prior or after AAV9-hPCCA administration. 15. Serology positive for hepatitis B virus (HBV), Hepatitis C virus (HCV), or human immunodeficiency virus (HIV), CMV, EBV, or a positive T-spot. 16. Ongoing/active infection (including current COVID-19 infection). Investigator to confirm complete resolution of infection for at least 14 days prior to dosing. 17. Use of concomitant medications to manage chronic condition(s) which interfere with the mechanism of action for AAV9-hPCCA in the opinion of the Investigator and dose(s) must not alter for at least four weeks before screening through to dosing (Day 1). 18. History of anaphylaxis. 19. History of severe allergic reactions to any components of the gene therapy product. 20. Pregnant or breast-feeding. 21. History of a medical condition or family history of a disorder (e.g., a familial cancer predisposition syndrome) which, in the opinion of Investigator, can exclude a participant from participating in the study.

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Title	A Phase 1/2, Open-Label Safety, Dose-Escalation, Single-Center Clinical Study of AAV9-hPCCA Gene Therapy in Propionyl-CoA Carboxylase Subunit Alpha (*PCCA)*-Related Propionic Acidemia (PA)
Concomitant Medications	For the first three months to one year on study, concomitant medication administration is kept unchanged, unless clinically indicated changes are required.
Drug, Drug Dosage, and Formulation	The dose of AAV9-hPCCA for the clinical study is proposed to be *[starting dose]*, and, depending on safety and efficacy signals, *[higher dose]*, as justified by the rationale described below (see **Dose Justification**). AAV9-hPCCA is a non-pyrogenic solution that will be stored per manufacturer's specifications until ready to be used. It will be thawed prior to administration as a one-time IV infusion given via peripheral intravenous (PIV) or PICC line over 30-60 minutes using a syringe pump (510(k) cleared).
Comparator Group	Historical control from the NIH natural history study of PA, which is ongoing.
Dose Justification	Proof-of-concept results showing effective rescue of the *Pcca*$^{-/-}$ mouse model from neonatal lethality with AAV9-hPCCA inform dose translation. Based on the survival data in the neonatal mouse studies, *[range]* vg/kg is the therapeutic window where we would expect to see therapeutic effects in research participants. To our knowledge, there are no reliable studies to scale AAV9 dosing from mice to humans, but there are several examples of systemic AAV9 gene therapy studies that have achieved clinical translation and are informative for our efforts. Pediatric metabolic disorders include MPSIIIa and MPSIIIb (Transpher A and B), where doses between 0.5E13 to 1.0E14 vg/kg have been proposed, and GM1 gangliosidosis (5.0E13vg/kg) [17, 18]. Considering *[XX]* vg/kg to be a high dose for PCCA deficiency, we would propose a starting dose cohort of *[XX]* vg/kg, *[relation of proposed clinical starting dose to higher dose in mice]*, as a starting point to assess safety and explore a biomarker response in PCCA-deficient participants. We propose to study up to six patients, and given the grave nature of this metabolic disorder, advocate for an initial dose of *[XX]* vg/kg that might have efficacy. If the first three participants enrolled in Cohort 1 do not show a biomarker response, including lowering of *[disease related biomarkers]*, dose escalation to *[XX]* vg/kg would be proposed and three additional participants would be enrolled, pending a safety assessment and DSMB review. In summary, an initial human dose of *[XX]* vg/kg with escalation to *[XX]* vg/kg of AAV9-hPCCA has a reasonable likelihood to be safe and to have a therapeutic benefit in participants with PA. These doses will be confirmed after the results of the six-month GLP toxicity study in wild-type mice become available.
Route of Administration	A single PIV infusion. In participants with poor or unreliable peripheral IV access, a PICC line will be used.
Dose Escalation	The study is composed of Cohort 1 (*[starting dose]*) and Cohort 2 (*[higher dose]*). Escalation is discussed above in "Study Design."
Procedures	1. PIV or PICC insertion, as needed. 2. Intravenous delivery of AAV9-hPCCA. 3. *[biomarker related procedure]*
Primary Endpoints	• Incidence of treatment-related adverse events, treatment-emergent adverse events, and serious adverse events and their relationship to AAV9-hPCCA administration based on CTCAE v5.0. • Absolute and percent change from baseline in plasma *[disease related biomarker]* at *[XX]* weeks (interim endpoint for DSMB assessment) and at the end of the *[XX]*-study period (primary endpoint).

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Secondary Endpoint	• Absolute and percent change from baseline in plasma *[disease related biomarkers]* at *[XX]* days (interim endpoint for DSMB assessment) and at the end of the *[XX]*-study period. • Change in chronic hyperammonemia and need for ammonia reducing agents (carglumic acid and ammonia scavengers). • Healthcare utilization related to PA (number of ER visits and hospitalizations/year, total days spent in the hospital/year), new or worsening complications.
Exploratory Endpoints	*[Disease-specific exploratory endpoints]* • Absolute and percent change from baseline of biomarkers such as *[disease related biomarkers]*
Statistical Considerations	Descriptive statistics

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Schedule of Clinical Activities

[Schedule of activities table]

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Appendix A

Table A.1: Management of Elevated Transaminases

CTCAE Grade	Corticosteroid treatment
Grade 2 (>2.0-5.0 x ULN) (or elevations over 1.5x baseline value if baseline close to 2.0 ULN or as deemed necessary by the treating team)	*[Management instructions per institutional SOPs/guidance]*
Persistent or Recurrent Grade 2, or Grade 3 (>5.0-20.0 x ULN) that does not plateau or resolve within 7 days of prednisolone to baseline or Grade 1	*[Management instructions per institutional SOPs/guidance]*
Grade 4 (>20.0 x ULN) with or without elevated direct bilirubin and elevated International Normalized Ratio (INR)	*[Management instructions per institutional SOPs/guidance]*

Tapering of prednisolone for subjects weighing <60kg: 1 mg/kg/day x 30 days; 0.8mg/kg/day x1 week; 0.6mg/kg/day x1 week; 0.4mg/kg/day x1 week; 0.2mg/kg/day x1 week; Off; The principal Investigator (PI) will consult with an expert pediatric endocrinologist on the taper protocol.

A three-day course of IV methylprednisolone at 10 to 20 mg/kg/day followed by 10-5-4-3-2-1 mg/kg/day taper on days 4 to 9 will be considered for Grade 4 liver toxicity, outlined for liver toxicity in other gene therapy trials [19, 20]. The intravenous course will be followed by oral prednisolone at 2mg/kg until sustained normalization of AST/ALT is achieved.

If there is no response to high-dose corticosteroid therapy, then T-cell targeted immunosuppressants will be considered at the discretion of the PI.

Synthetic function of the liver and INR will be monitored and treated in consultation with GI/hepatology consultants, who will assist with diagnostic work-up and evaluate the need for a liver biopsy.

Table A.2: Management of Infusion-related Reactions

CTCAE Grade	Treatment
Grade 1: Mild transient reaction; infusion interruption not indicated; intervention not indicated	None
Grade 2: Therapy or infusion interruption indicated but responds promptly to symptomatic treatment and prophylactic medications indicated for ≤24 hours. Investigator discretion will be utilized to determine whether to re-start dosing	*[Management instructions per institutional SOPs/guidance]*
Grade 3: Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. Investigator discretion will be utilized to determine whether to re-start dosing	*[Management instructions per institutional SOPs/guidance]*
Grade 4: Life-threatening consequences; urgent intervention indicated	*[Management instructions per institutional SOPs/guidance]*

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Table A.3: Management of Vomiting/Metabolic Acidosis

CTCAE Grade	Treatment
Grade 2, repeat vomiting episodes >3, reduced PO intake, trace or increase from baseline ketonuria	*[Management instructions per institutional SOPs/guidance]*
Grade 3, persistent vomiting, reduced oral intake associated with metabolic acidosis (pH<7.3) and ketonuria (mild to moderate)	*[Management instructions per institutional SOPs/guidance]*
Grade 4, persistent metabolic acidosis with or without elevated lactate not responding to previous interventions	*[Management instructions per institutional SOPs/guidance]*

Table A.4: Management of Hyperammonemia

CTCAE Grade	Treatment
Grade 3 hepatic failure, asterixis; mild encephalopathy, Confirmed elevated ammonia >100umol/L	*[Management instructions per institutional SOPs/guidance]*
Hyperammonemic encephalopathy/coma (Ammonia >200umol/L)	*[Management instructions per institutional SOPs/guidance]*
Hyperammonemic encephalopathy/coma (Ammonia >500umol/L), concern for metabolic stroke	*[Management instructions per institutional SOPs/guidance]*

Most common adverse reactions (≥5%) to N-carbamylglutamate are neutropenia, anemia, vomiting, electrolyte imbalance, decreased appetite, hypoglycemia, lethargy/stupor, encephalopathy and pancreatitis/lipase, amylase increased.

Table A.5: Management of Thrombotic Microangiopathy (TMA)

CTCAE Grade	Treatment
Grade 1, PLT count <150 - 75.0 x 10e9 /L	*[Management instructions per institutional SOPs/guidance]*
Grade 2-3, PLT count <75.0 - 25.0 x 10e9 /L	*[Management instructions per institutional SOPs/guidance]*
Grade 4, PLT count <25.0 x 10e9 /L	*[Management instructions per institutional SOPs/guidance]*

Table A.6: Management of Kidney Dysfunction

CTCAE Grade	Treatment
Grade 1, Creatinine increase <1.5 x baseline	*[Management instructions per institutional SOPs/guidance]*
Grade 2, Creatinine increase >1.5 - 3.0 x baseline	*[Management instructions per institutional SOPs/guidance]*
Grade 3, Creatinine increase >3.0 x baseline	*[Management instructions per institutional SOPs/guidance]*
Grade 4, Creatinine increase >6.0 x ULN	*[Management instructions per institutional SOPs/guidance]*

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Table A.7: Management of Myocarditis

CTCAE Grade	Treatment
Grade 1, Asymptomatic changes in ECG or routine echocardiogram	*[Management instructions per institutional SOPs/guidance]*
Grade 2, Symptoms with moderate activity or exertion, chest pain; elevation of heart rate above expected; ST segment changes; elevations of troponin; atrial or ventricular ectopy including single beats or couplets; or any combination of these findings	*[Management instructions per institutional SOPs/guidance]*
Grade 3, Severe with symptoms at rest or with minimal activity or exertion; atrial or ventricular tachycardia that is hemodynamically tolerated and self-terminating; mildly or moderately reduced ejection fraction of left ventricle as measured by any imaging technique; new onset pericardial effusion	*[Management instructions per institutional SOPs/guidance]*
Grade 4, Hemodynamic instability requiring more than a single inotropic agent; severely reduced ejection fraction of left ventricle; atrial or ventricular tachycardia; end-organ injury due to shock	*[Management instructions per institutional SOPs/guidance]*

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Appendix: Proposed protocol synopsis (with changes from prior version tracked)

DISCLAIMER: This information is specific to AAV9-hPCCA and does not apply directly to other investigational products or studies.

Preliminary Meeting Responses

Our Reference: *[FDA-assigned reference and meeting numbers]*

DATE: December 11, 2024 **PAGES:** 4

TO: *[name and address of sponsor representative]*

FROM: *[name and address of regulatory program manager at CBER]*

SUBJECT: Type C, Pre-IND meeting to discuss new developments since the pre-IND meeting. Sponsor seeks to obtain targeted FDA feedback on specific chemistry, manufacturing, and control (CMC), and clinical questions for the AAV9-hPCCA investigational product.

PRODUCT: Adeno-Associated Virus 9 vector expressing a functional human codon optimized cDNA encoding the Propionyl-CoA Carboxylase, alpha subunit (PCCA), under control of the *[specific promoter]* /**Product Name:** AAV9-hPCCA

PROPOSED INDICATION: Treatment of PCCA-related propionic acidemia (PA).

FDA Participants:

[List of FDA participants]

This material consists of our preliminary meeting responses to your questions and any additional comments in preparation for the discussion at the meeting scheduled for December 16, 2024. We are sharing this material to promote a collaborative and successful discussion at the meeting.

Although we continue to reserve December 16, 2024 from 1:00PM – 2:00PM EST, with you regarding this product, if you find that our attached responses and advice are sufficiently clear and complete to obviate the need for further discussion, please inform us in writing as soon as possible, and 3 calendar days from the date of receipt of FDA's Preliminary Responses, so that we may clear the meeting time. These responses would then become the official FDA responses to your questions.

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

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

Please include a reference to *[FDA-assigned reference and meeting numbers]* in your future submissions related to this product.

Preliminary Meeting Responses

CMC

Question 1:
Does the FDA agree with our risk assessment and approach to support the limits of residual host-cell DNA?

FDA Response to Question 1:
Yes, we agree with your risk assessment and approach to support the limits of residual host-cell DNA.

Question 2:
Does the FDA agree with our plans for testing and qualifying a potency assay matrix for AAV9-hPCCA?

FDA Response to Question 2:
Your proposed approach to assess product potency using a droplet digital PCR (ddPCR) assay for quantification of transgene mRNA expression and a Next Generation Sequencing (NGS) method for vector genome sequence purity for release testing is acceptable to initiate the IND. However, we have the following additional comments on your qualification of these assays:

a. In the NGS Executive Validation Summary provided in the meeting briefing package, you state that "The original validation strategy evaluated precision, linearity, accuracy, and robustness of the assay…" and you also state that "Accuracy, quantification limit, linearity and range of method were not assessed in this validation as identity testing is not considered a quantitative method". Based on those conflicting statements, we are uncertain that your NGS method is adequately qualified for its intended purpose. We re-iterate our recommendation conveyed in the Pre-IND meeting dated July 10, 2023, that you should demonstrate that the NGS method will be suitable for quantitative analysis of vector sequence variants. In other words, the NGS method should demonstrate sufficient accuracy and precision in quantifying sequence variants over a specified range. As discussed during the teleconference (dated July 10, 2023) please consider spiking the test sample with mutated sequence to assess sensitivity of the NGS assay. We recommend that you assess the accuracy, precision, range, and linearity of the NGS method during your product development.

b. We acknowledge that you intend to qualify your mRNA potency assay prior to release of the clinical material. Please submit the standard operating procedure and assay qualification report in your initial IND. You may qualify your NGS assay as part of product development and submit the qualification report after the initiation of the IND. We also acknowledge your plans to validate both methods prior to start of Phase 2.

c. We recommend that you save the retain samples from all your clinical lots to confirm the sequence purity once the NGS assay is qualified/validated. The retain samples are also helpful to facilitate future comparability analysis when any manufacturing changes are introduced.

Clinical

Question 3:
Does the Agency agree with the proposed approach for utilization of the specified surrogate biomarkers for evaluation of preliminary clinical efficacy (pharmacodynamic response) and associated measures?

FDA Response to Question 3:
Yes, we agree with evaluating the specified biomarkers in your FIH study to evaluate pharmacodynamic (PD) responses to treatment. In addition, the proposed approach to evaluate the clinical meaningfulness of changes in those PD biomarkers, including robust data collection during the clinical trial and collection of data from the ongoing natural history studies, is reasonable. We look forward to collaborating with you during clinical development to maximize the interpretability of the collected PD and efficacy data.

END

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Response to Request for Clarification

Date:	January 08, 2025
Meeting ID:	*[Type-C meeting number]*
Date of Preliminary Responses:	December 11, 2024
Meeting Type/Category:	Type C, Pre-IND
Request received:	December 20, 2024
Sponsor/Applicant:	*[name and affiliations of sponsor representative]*
Product:	Adeno-Associated Virus 9 vector expressing a functional human codon optimized cDNA encoding the Propionyl-CoA Carboxylase, alpha subunit (PCCA), under control of the *[specific promoter]* /**Product Name:** AAV9-hPCCA
Indication:	Treatment of *PCCA*-related propionic acidemia (PA).

Attached is a copy of the memorandum responding to your Request for Clarification, dated December 20, 2024, in response to the Type C, Pre-IND Preliminary Responses dated December 11, 2024.

DISCLAIMER: This information is specific to AAV9-hPCCA and does not apply directly to other investigational products or studies.

Response to Request for Clarification

Sponsor Clarification Comment for CMC question 2 (FDA comment a.):

1. In Question 2 part **a**, the adequacy of the qualification of the NGS method was questioned.

Response: The Sponsor would like to clarify that *[contractor name]*, the Sponsor's CRO for NGS characterization, has an active Type V Biologics Master File (BMF) containing validation packages and corresponding SOPs for their analytical methods, including the NGS assay. A Letter of Authorization for *[contractor name]* BMF will be included in the IND submission.

2. The FDA recommended in part **a.** of the response to Question 2 that "*the NGS method should demonstrate sufficient accuracy and precision in quantifying sequence variants over a specified range. As discussed during the teleconference (dated July 10, 2023) please consider spiking the test sample with mutated sequence to assess sensitivity of the NGS assay. We recommend that you assess the accuracy, precision, range, and linearity of the NGS method during your product development*."

Response: As part of the NGS Bioinformatics algorithm development, *[contractor name]* performed *in-silico* variant calling to assess sensitivity. Different sample iterations were assessed by combining various *[mutant types and allele frequencies]*. The data provided in the development report, which is included in *[contractor name]* Type V BMF, supports a *[cut-off value]* for variant calling (Variant Allele Frequency). The report further details the selection of the bioinformatics tools and the finalization of the *[XX]* variant callers used. The validation was then performed per ICHQ2(R2) guidance based on the *[cut-off value]* determined during development. During the validation, *[brief description of sample type, number of variants and % allele frequency]*.
The complete validation package is available in *[contractor name]* Type V BMF.

In response to FDA's recommendation to spike the test sample with mutated sequence to assess sensitivity, the Sponsor would like to highlight the following challenge with this request: analyzing data using biological spiking may be difficult because all natural controls (whether they are other virus strains or synthesized sequences) cannot be accurately quantified for variant frequencies due to natural evolution/sample divergence or errors generated during synthesis. As a result, each control of this nature would require significant characterization of itself, which could be resource or technically prohibitive. Recovery of the predicted variant frequencies

would not be probable (e.g., a predicted *[XX%]* spike may be recovered at significantly higher or lower frequencies due to the variable nature of the molecules).

We believe that the data generated during bioinformatics development and validation are sufficient to demonstrate that the NGS method is sufficiently sensitive for use as a component of the potency assay and that a separate spiking study is not required.

If a spiking study is still deemed to be required to demonstrate sensitivity, in addition to spiked samples already tested during initial validation, the Sponsor is proposing to perform an *in silico* assessment by generating representative sequences with specific types of variants *in silico* prior to 'spiking' these sequences at the desired sensitivity levels into either a fully simulated/*in silico* data set, or into an actual data set that reflects our test sample.

To be representative of the test sample, a real fastq file obtained from the sequencing of the sample can be used that will be spiked with simulated variants from the reference sequence with Illumina typical error model as a background. A set of short variants (SNPs with substitutions, deletions and insertions) and Structural Variants (SV) could be used in a similar manner as during the validation.

The recovery of those spiked sequences would then be assessed to confirm the *[cut-off value]*. This would be done exclusively at the bioinformatics level. As per ICHQ2(R2), assessment of linearity is not required for non-quantitative methods.

Does the Agency agree with removing the recommendation for a spiking study to assess the sensitivity of the NGS assay?

FDA Response to Clarification Comment for CMC question 2 (FDA comment a.):

We do not agree to remove our recommendation for a spiking study to assess the sensitivity of the NGS assay. The primary purpose of the NGS assay in your product testing plan is to ensure product potency in conjunction with the transgene mRNA expression ddPCR assay. A quantitative NGS assay that can not only detect a sequence variant but also determine the level of the sequence variant in a product lot is critical to ensure that the product lot has the intended potency for lot release. Therefore, the NGS assay should be developed as a quantitative method for sequence variants with an acceptable limit for lot release in your later phase clinical development and commercial manufacturing.

Your proposed approach of "spiking" sequence variants in silico and assessing the assay sensitivity only at the level of bioinformatics is not adequate. We recommend that you validate the NGS assay using test samples spiked with AAV vectors with mutations to assess the precision,

accuracy, linearity, and range of the assay. Because this assay should be developed as a quantitative method, you should assess the linearity or assess the precision and accuracy over the range of the assay as part of assay validation.

Please be advised that as noted above you may conduct assay validation during your product development after initiation of your proposed IND study.