National Institutes of Health (NIH), National Center for Advancing Translational Sciences (NCATS)

Adeno-Associated Virus 9 human Propionyl-CoA Carboxylase, subunit beta (AAV9-hPCCB) for the Treatment of Propionic Acidemia Caused by PCCB Deficiency

Rare Pediatric Disease Designation Request

June 14, 2024

- CONFIDENTIAL -

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List of Abbreviations

AAV	Adeno-Associated Virus
AAV9	Adeno-Associated Virus serotype 9
AAV9-hPCCB	Adeno-Associated Virus serotype 9 human Propionyl-CoA Carboxylase, subunit beta
Cas9	CRISPR associated protein 9
CoA	Coenzyme A
CRISPR	Clustered regularly interspaced short palindromic repeats
CRM	Cross Reactive Material
EF1L	Elongation Factor 1 alpha Long
ITR	Inverted Terminal Repeat
LT	Liver Transplantation
2-MC	Total 2-Methylcitrate
3-OHP	3-hydroxypropionate
MCEE	D-methylmalonyl-CoA epimerase
MCK	Murine creatine kinase
MMUT	Methylmalonyl-CoA mutase
mRNA	Messenger Ribonucleic Acid
NCATS	National Center for Advancing Translational Sciences
NHGRI	National Human Genome Research Institute
NIH	National Institutes of Health
NHS	Natural History Study
OAA	Oxaloacetic acid
P	Postnatal Day
PA	Propionic Acidemia
PBS	Phosphate Buffered Saline
PCC	Propionyl-CoA Carboxylase (EC 6.4.1.3)
Pccb	Propionyl-CoA Carboxylase, subunit beta gene (mouse)
PCCB	Propionyl-CoA Carboxylase, subunit beta protein
PCCA	Propionyl-CoA Carboxylase, subunit alpha gene (human)
PCCB	Propionyl-CoA Carboxylase, subunit beta gene (human)
POC	Proof of Concept
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic acid
ROA	Route of Administration
US	United States
TG	Transgene
vg	vector genome

WT	Wild Type

1 RARE PEDIATRIC DISEASE DESIGNATION REQUEST STATEMENT

Pursuant to Section 908 of the Food and Drug Administration Safety and Innovation Act (FDASIA),[1] the National Institutes of Health (NIH), National Center for Advancing Translational Sciences (NCATS) requests designation of Adeno-Associated Virus serotype 9 human Propionyl-CoA Carboxylase, subunit beta (AAV9-hPCCB) as a Rare Pediatric Disease (RPD) product for treatment of patients with propionic acidemia (PA) resulting from a deficiency of Propionyl-CoA Carboxylase, subunit beta (PCCB).

PA is serious and life-threatening disorder caused by deleterious mutations in either the *PCCA* or *PCCB* genes, which encode for the α and β subunits, respectively, of the multimeric enzyme propionyl-CoA carboxylase (PCC). PCC is a ubiquitously expressed enzyme that plays a role in the normal breakdown of odd chain fatty acids and branched chain amino acids. PCC deficiency leads to episodes of metabolic decompensation/acidemia resulting in acute neurological crises, cardiomyopathy, and other serious clinical manifestations. There are currently no approved therapies for the treatment of PA.

PA is a rare disease in the United States (US). There are no population-based studies of the prevalence of PA and almost all studies published in the medical literature relevant to the US population are based on birth incidence calculated from state newborn screening (NBS) data. From these studies, PA has an estimated birth incidence ranging from 0.14 to 1.2 affected infants per 100,000 births.[1-3] Based on the annual US birth rate of ~3.7 million/per year in 2022,[4] approximately 5 to 44 children are born in the US with *PCCA*- and *PCCB*-type PA each year, of which 50% of individuals will have *PCCB*-type PA. A recent study (Adhikari et al, 2020)[1] used both tandem mass spectrometry (MS/MS) NBS data from the State of California (July 2005 through December 2013) and whole exome sequencing (WES), with the results showing a birth incidence of 0.14 PA cases per 100,000 newborns (~6 cases per year) and is likely the most accurate estimate. Based on the US Census Bureau's population count in 2023 of 334,914,895 people living in the US,[5] the prevalence of PA roughly translates to, at the low end (0.14 cases per 100,000), a prevalence of around 469 to, at the high end, (1.2 cases per 100,000), 4,018 people in the US with PA, half of whom would have *PCCB*-type PA (234-2,009). In addition, there is a milder form of PA in the Amish population that is caused by a founder effect. Amish PA patients are homozygous for the PCCB c.1606G>A p.Asp536Asn mutation and the estimated prevalence of PA in this population is 1 in 4244. The Amish have an estimated population size of 385,770 with a predicted prevalence of *PCCB*-type PA of 23.5 cases per 100,000. We estimate that there are 90 Amish patients in the US with PA caused by PCCB deficiency. Please see ***Section 8*** for detailed discussion on birth incidence.

However, because PA is a life-threatening condition resulting in premature death at a young age for many patients, with some perishing in the newborn period in the setting of a neonatal crisis at presentation, the true prevalence is likely much higher. Based on our good-faith assessment from expert opinion obtained from clinical experts on PA in the Metabolic Medicine Branch of the

National Human Genome Research Institute (NHGRI) in the National Institutes of Health (NIH), led by Senior Investigator Charles P. Venditti, M.D., Ph.D., and from data from an NIH-conducted PA natural history study, we estimate there are ~50 non-Amish *PCCB*-type PA patients living in the US.

PCCB-related PA is a genetic disorder that most commonly presents within the first few days of life with hyperammonemia, poor feeding, vomiting, irritability, and lethargy.[6] Without treatment, these infants may develop neonatal encephalopathy, seizures, coma, and respiratory failure, which if left untreated can result in death. The less common late-onset form typically presents in early childhood (<1 year of age) with acute decompensation precipitated by metabolic stressors, such as infection, injury or surgery.[7] Infrequently, PA may present in late childhood with dilated cardiomyopathy or acute psychosis without prior known history of metabolic decompensations.[6] Universal NBS in the US can identify most affected patients. However, even with available treatments, mainly consisting of dietary restrictions and L-carnitine supplementation, the long-term prognosis for survival in severely affected patients is poor. Most patients will suffer from recurrent metabolic instability precipitated by periods of metabolic stress, and can develop chronic progressive multisystemic complications, including cardiomyopathy. Over the past several decades, it has been recurrently noted that PA patients with an early and severe clinical course experience increased mortality and disease associated morbidity throughout childhood and adolescence.[8]

Thus, based on available evidence, *PCCB*-related PA meets the criteria for designation as a RPD including: 1) *PCCB*-related PA is a serious or life-threatening disease in which the serious or life-threatening manifestations primarily affect individuals aged from birth to 18 years; and 2) *PCCB*-related PA is a rare disease. We therefore request that AAV9-hPCCB be designated as a Rare Pediatric Disease Product for the treatment of *PCCB*-related PA.

2 ADMINISTRATIVE INFORMATION

2.1 Sponsor

National Institutes of Health (NIH), National Center for Advancing Translational Sciences (NCATS)
9609 Medical Center Drive, Suite 1-E-162
Rockville, MD 20850

2.2 Sponsor Information and Regulatory Contacts

Regulatory Correspondent

[redacted]

Sponsor Contact

[redacted]

Principal Investigator

[redacted]

2.3 Drug Name

2.3.1 Chemical Name - Drug Substance

Adeno-Associated Virus 9 human Propionyl-CoA Carboxylase, subunit beta (AAV9-hPCCB)

2.3.2 Generic/Trade Name - Drug Product

AAV9-hPCCB is an Adeno-Associated Virus 9 (AAV9) vector expressing a cDNA encoding the Propionyl-CoA Carboxylase, subunit beta (*PCCB*), under control of the Elongation Factor 1 alpha long (EF1L) promoter. Details describing the drug product are found in ***Section 4.1***.

5'ITR	EF1L	Intron	5'UTR	hPCCB	HPRE	BGH PA	3'ITR

2.4 Manufacturer's Name and Address

GMP grade AAV9-hPCCB drug substance and drug product will be manufactured by Novartis.

Address: Kolodvorska cesta 27, 1234 Mengeš, Slovenia

3 DESCRIPTION OF RARE DISEASE OR CONDITION, PROPOSED INDICATION, AND NEED FOR THERAPY

3.1 Description of Rare Disease or Condition

Clinical Manifestations

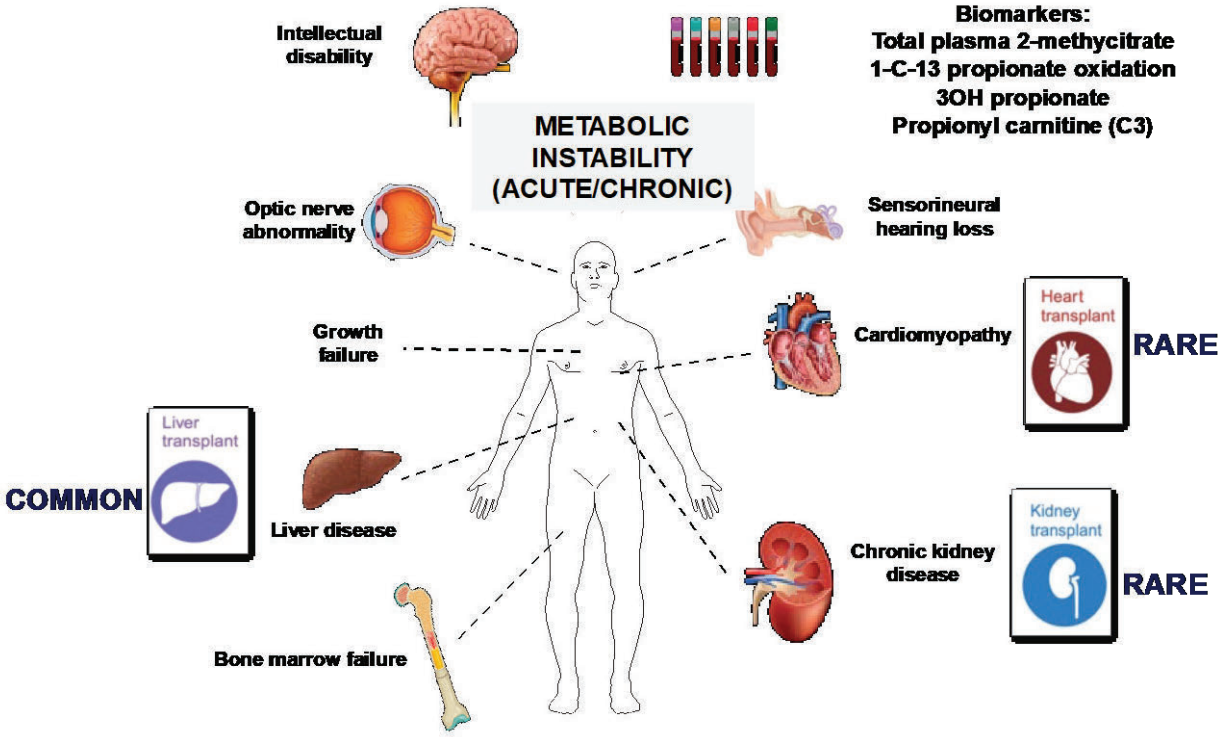
Propionic acidemia (PA) is a rare inborn error of metabolism resulting from deleterious variants in the *PCCA* or *PCCB* genes leading to impaired activity of propionyl-CoA carboxylase (PCC). PCC is a ubiquitously expressed mitochondrial enzyme whose function is closely linked to energy production. It plays a role in the metabolism of the branched chain amino acids valine, methionine, isoleucine and threonine, gut-derived propionate, and odd-chain fatty acids.

Propionic acidemia presents with a wide spectrum of clinical syndromes that can greatly vary depending on the age of the patient, and environmental stressors, such as fasting. Patients with PA are born with normal birth parameters and can be healthy in the immediate hours after birth, but typically develop progressive encephalopathy, weight loss and hyperammonemia with ketoacidosis. Without prompt and aggressive management, usually including hemodialysis, an ill baby with PA can frequently perish[7]. All patients with PA require meticulous dietary management to restrict the intake of propiogenic amino acids while insuring optimal growth, yet still are prone to develop complications including poor growth, developmental delays, seizures, intellectual disability, movement disorders, pancreatitis, hepatomegaly, cardiomyopathy, QT interval prolongation, chronic renal insufficiency, cytopenias, recurrent infections, optic nerve atrophy, hearing loss, osteoporosis, premature ovarian failure, hypotonia, and psychiatric disorders[8]. Perhaps the gravest complication of PA is the propensity to develop episodic life-threatening metabolic decompensations, especially in the first years of life, that are often precipitated by illnesses, infections, surgery, or any stress augmenting catabolism[8]. Infectious complications often accompany metabolic crises and are the major contributors to mortality. In addition to metabolic decompensations, cardiac manifestations, including cardiac failure requiring heart transplantation, and cardiac rhythm abnormalities associated with syncope, arrhythmia, and cardiac arrest remain as recalcitrant and life-threatening complications of the disorder, and can occur despite dietary management[8].

In summary, the clinical course of PA is characterized by chronic multiorgan dysfunction punctuated by episodes of metabolic instability due to metabolic acidosis, ketonuria, hyperammonemia, and hypoglycemia leading to emergency room visits and hospitalizations as summarized in ***Figure 1***. Universal newborn screening implemented in the US can identify most affected infants, but some may present and even perish in the setting of a metabolic crisis before the diagnosis is established. Infrequently, newborn screening fails to identify affected newborns, usually infants homozygous for the “mild” Amish-Mennonite *PCCB* allele

(*PCCB*:c.1606A>G, p.Asn536Asp). Such milder cases can present later in life with metabolic symptom, or a life-threatening dilated cardiomyopathy and even require heart transplantation.

Figure 1. Clinical and Laboratory Manifestations of PA



Propionic acidemia is a rare disorder with birth prevalence varying widely by region. Worldwide, the birth incidence has been reported as high as 1:1000 and as low as 1:500,000. The estimated birth incidence of PA in the general US population ranges from 0.13 to 1.2 affected infants per 100,000 births, with *PCCB* type comprising approximately 50% of total PA patients.[2-4] (Please see ***Section 8*** for detailed discussion on birth incidence).

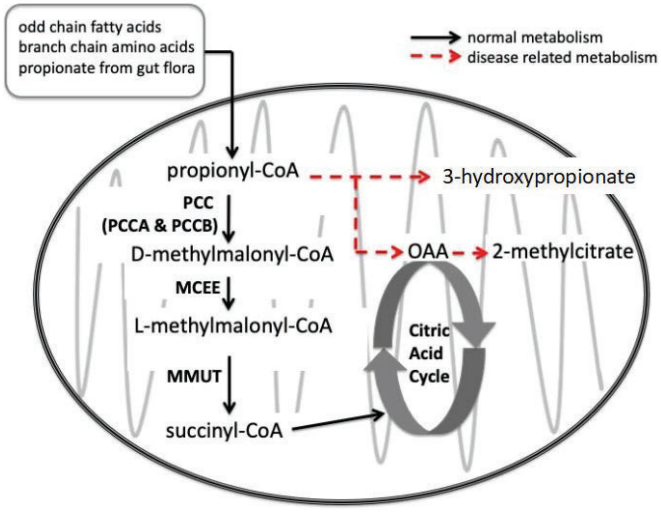
As a group, patients with PA suffer from a low quality of life, and a reduced life span. The long-term prognosis for survival in severely affected patients is poor, which was first illustrated in the 1990s by an early and relatively large (for the disease prevalence) single center study of 20 patients with PA treated at a tertiary care center.[7] This study showed that patients who presented in the first week of life (11 patients) largely perished by the age of 6 years. Even with treatment, patients who survived suffered from recurrent metabolic instability often precipitated by periods of metabolic stress, such as infections, and can develop chronic progressive multisystemic complications, including cardiomyopathy. Over the subsequent decades, it has been recurrently noted that PA patients with an early and severe clinical course experience increased mortality and disease associated morbidity.[8] The recalcitrant nature of the disorder to conventional medical management, including the dietary restriction of amino acid precursors, L-carnitine

supplementation, and administration of oral 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 disorder, successful LT in the setting of PA may improve metabolic stability and protection from early death, and therefore represents a clinical benchmark for gene replacement or other approaches that might increase hepatic PCC expression and activity.

Pathophysiology

PCC is a ubiquitously expressed, heteropolymeric mitochondrial enzyme involved primarily in the catabolism of propionogenic amino acids, particularly isoleucine, valine, methionine, and threonine, as well as odd-chain fatty acids. The enzyme is composed of α- and β-subunits encoded by their respective genes, *PCCA* and *PCCB*, and PA is equally caused by pathogenic variants in either gene. PCC catalyzes the first step in the conversion of propionyl-CoA to D-methylmalonyl-CoA in the pathway of propionyl-CoA oxidation, depicted in ***Figure 2***. The formation of disease related metabolites which serve as dynamic biomarkers of PA, 2-methylcitrate (2-MC) and 3-hydroxypropionate (3-OHP), are produced via the condensation of oxaloacetic acid (OAA) or the β-oxidation of propionyl-CoA. The downstream enzymatic steps in the pathway, including D-methylmalonyl-CoA epimerase (MCEE) and methylmalonyl-CoA mutase (MMUT), further metabolize propionyl-CoA into the citric acid (Krebs) cycle.

Figure 2. Catabolism of Propionyl-CoA.



3.2 Proposed Indication and Use of AAV9-hPCCB

The proposed clinical use of AAV9-hPCCB is for the treatment of patients with PA resulting from a deficiency of PCC due to deleterious variants in PCCB. Briefly, AAV9-hPCCB is an AAV9 vector expressing a wild-type cDNA encoding PCCB, under control of the EF1L promoter. In a newly generated *Pccb* murine models, AAV9-hPCCB demonstrated efficacy as

demonstrated by increased survival, improved growth, and lowering of disease related-metabolites 2-MC and 3-OHP by increasing PCC enzymatic activity.

3.3 Reasons Why Such Therapy Is Needed

There are currently no approved therapies for PA and novel therapies are urgently needed to prevent irreversible disease complications and improve patient outcomes. PA manifests life-threatening debilitating illness in early childhood and is poorly responsive to available medical therapy. Adult PA patients have high prevalence of severe kidney and heart disease, and irreversible disease complications in sensory organs. Current standards of care include strict adherence to low-protein diet with and without poorly palatable medical foods, and supplementation with levocarnitine. Other treatments may apply if patients develop severe complications of PA, such as, the use of cardiac medications in patients with cardiomyopathy or antiepileptic drugs in PA patients with epilepsy.[8] The long-term prognosis for survival in severely affected patients is poor as illustrated by an early and relatively large (for the disease prevalence) single center study of 20 patients with PA treated at a tertiary care center: Those who presented in the first week of life (11 patients) died by the age of 6 years.[7] Over the decades, it has been recurrently noted that PA patients with an early and severe clinical course experience increased mortality and disease associated morbidity.[8]

Some PA patients, who experience frequent hospitalizations due to metabolic instability and/or who develop renal failure or dilated cardiomyopathy may undergo liver transplantation (LT), liver-kidney transplantation or liver-heart transplantation, and then require life-long immunosuppression. 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 or addition approaches that might increase hepatic PCC expression and activity. Although the post LT state in PA remains to be fully elucidated, some patients have developed renal disease and even cardiomyopathy after successful LT. An additional and potentially fatal complication, presumed secondary to chronic exposure to immune suppression, has been the development of post-transplant lymphoproliferative disease. Therefore, while LT substantially improves the clinical and metabolic phenotypes seen in severe PA patients, it clearly carries immediate/acute (post-surgical) and chronic/subacute risks. In theory, these risks could be mitigated or perhaps eliminated by successful gene therapy with AAV9-hPCCB, which we propose as a single IV infusion aiming to restore therapeutic levels of PCCB in the liver and heart.

4 DESCRIPTION OF AAV9-HPCCB AND SCIENTIFIC RATIONALE FOR USE

4.1 Description of AAV9-hPCCB

4.1.1 Drug Product Naming Convention

Adeno-Associated Virus 9 human Propionyl-CoA Carboxylase, subunit beta (AAV9-hPCCB)

4.1.2 Drug Product Vector Design

AAV9-hPCCB is an AAV9 vector expressing a wild-type cDNA encoding *PCCB*, under control of the EF1L promoter. In humans, endogenous PCCB protein is ubiquitously expressed, therefore we designed a therapeutic transgene cassette with a constitutive promoter to enable wide expression and selected the AAV9 capsid to further enable hepatic and cardiac transduction. A schematic of the vector transgene, description of cassette features summarizing the salient features of the AAV vector and the transgene plasmid map is presented in ***Figure 3*** and ***Figure 4***. The complete sequence of the hPCCB cDNA is listed in ***Appendix A***.

Figure 3. A Schematic of the Vector Transgene, Description of the Cassette.

Figure 4. AAV9-hPCCB Vector Transgene Plasmid Map.

4.1.3 Drug Product Description, Mechanism of Action and Route of Administration

AAV9-hPCCB is a biologic modality treating PA patients with mutation(s) in the *PCCB* gene. The gene is delivered using an AAV virus, serotyped with an AAV9 capsid. The recombinant AAV virus is not pathogenic in humans but maintains its natural ability to deliver genetic material into cells. The AAV9-hPCCB will deliver a normal copy of the human *PCCB* gene and restore the catabolism of propionyl-CoA through the formation of the PCCA/PCCB heterododecamer that comprises active PCC, as described in ***Figure 2***, ***Section 3.1***. AAV9-hPCCB will be stored at -80°C and delivered in patients as an intravenous (IV) solution using the peripheral IV route of administration (ROA). The final formulation for the drug product has not been determined but the formulation for an initial batch for preclinical *in vivo* animal studies was 1.7 mM KH_2PO_4, 5 mM Na_2HPO_4, 150 mM NaCl (PBS), 5% sorbitol, 0.01% Tween 80 (pH 7.4).

4.2 Scientific Rationale for the Use of AAV9-hPCCB in the Rare Disease or Condition

PA is caused by mutations in either the *PCCA* or *PCCB* gene. It is caused by a deficiency of PCC, a ubiquitously expressed, heteropolymeric mitochondrial enzyme involved primarily in the

catabolism of propiogenic amino acids. The enzyme is composed of α- and β-subunits encoded by their respective genes, *PCCA* and *PCCB*. PCC catalyzes the first step in the conversion of propionyl-CoA to D-methylmalonyl-CoA in the pathway of propionyl-CoA oxidation, depicted in ***Figure 2***, ***Section 3.1***. Replacement of the PCCB protein to increase PCC enzyme through delivery of AAV9-hPCCB gene therapy is expected to help normalize the metabolic defects associated with PA. Of note, the recognized trophism of the AAV9 capsid toward the liver and heart further support the rationale of using a ubiquitous promoter to that will result in PCCB expression in hepatocytes as well as cardiomyocytes in the patients.

4.2.1 Clinical Efficacy of AAV9-hPCCB

To date, no clinical data have been collected with AAV9-hPCCB for the treatment of patients with PA resulting from a deficiency of PCCB protein. However, as stated previously, PA patients that have undergone successful LT demonstrate a restoration of metabolic stability and protection from early death, and therefore represent a clinical benchmark for gene replacement therapy that may lead to increased hepatic PCC expression and activity.

There is a lack of natural history longitudinally evaluating PA biomarkers and their association with outcomes corresponding to survival, functioning and disease burden. To close this gap, the Organic Acidemia Research Section in the National Human Genome Research Institute (NHGRI) initiated a Natural History Study (NHS) of the PA population (National Clinical Trial # NCT02890342)[9]. The study is being conducted at the NIH Clinical Center and has enrolled 52 participants ages 2 years and older (2.5 years – 54 years of age). The cohort features a genetically heterogeneous PA population, a high proportion of adult patients (~35%), and a subset of liver- and kidney-transplanted PA participants. A recent publication has identified candidate biomarkers in this cohort.[9]

4.2.2 Nonclinical Efficacy of AAV9-hPCCB

Summary of AAV9-hPCCB Efficacy in Murine Models of PA Caused by PCCB Deficiency

Prior to testing in mice, PCCB protein expression of AAV9-hPCCB was confirmed by an in vitro cell assay. Systemic delivery via retroorbital injection of AAV9-hPCCB at dose of $1x10^{14}$ vg/kg to neonatal lethal, juvenile lethal and hypomorphic PCCB PA mice, representative of the spectrum of genetic mutations and disease phenotypes reported in PA patients, resulted in significantly increased survival, reduced disease related metabolites, improved growth and protection against metabolic stress in comparison to the respective untreated mutant mice. Based on disease severity, murine models of PA were treated as neonates, juveniles, or adults, which is reflective of the age range of the PA patient population. PA patients experience early mortality, therefore the significant improvement in survival strongly supports the efficacy of AAV9-hPCCB therapy while the reduction of disease related metabolites observed after AAV9-hPCCB treatment provides evidence of increased PCC enzymatic activity and may be clinically

beneficial because these metabolites are thought to be toxic. Lastly, the resistance to metabolic stress in a murine model of PA is clinically relevant because patients are known to experience potentially lethal metabolic decompensation in response to stressors. The results of these studies are detailed below.

In Vitro Studies in Human Liver (HepG2) PCCB Knockout (KO) Cell Line

Infection of an engineered human liver HepG2/PCCB KO cell line incubated with varying concentrations ($5x10^4$, $1x10^5$, $5x10^5$, $1x10^6$ vg) of AAV9-hPCCB showed increasing expression of PCC enzyme by Western Blotting upon probing with a PCC specific antibody that was not seen in the control (uninfected KO cells) (***Figure 5***).

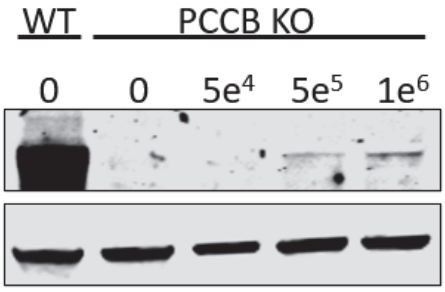


Figure 5. AAV9-hPCCB *In Vitro* Infection Studies in Human Liver (HepG2) PCCB Knockout (KO) Cell Line.

A deletion mutation in *PCCB* was engineered in a human hepatocyte derived cell line (HEPG2) to create the PCCB KO cell line and used to test the in vitro potency of AAV9-hPCCB applied at varying moiety of infections ranging from $5x10^4$ to $1x10^6$. After 48 hours, the infected PCCB KO cells were harvested and lysed, and 25 ug of total cellular protein was subjected to Western analysis using a PCCB polyclonal antibody. Beta-actin was used as a loading control. A dose response with increasing PCCB after exposure to varying doses of AAV9-hPCCB is apparent.

In Vivo Studies in Mouse Models of PA Used to Investigate Effect of AAV9-hPCCB

Gene therapy has been administered to different animal models of PA caused by PCCA deficiency: increased survival and reduction in disease related metabolite levels in the treated PA mice supported the therapeutic effects noted in these studies.[9-12] Because there are no murine models of PA caused by PCCB deficiency, we used CRISPR/Cas9 genome editing to engineer 3 different mutations that are analogous to mutations seen in the patients, such as frameshift-stop changes, in exon 7 and 14 of the murine *Pccb* gene as well as a murine Pccb p.P230L missense mutation in Exon 7, which is orthologous to the human PCCB p.P228L missense mutation which causes a milder form of PA in the homozygous state. Another severe *Pccb* mutation, caused by an 8 bp deletion in exon 7 in the *Pccb* gene, is null at the level of PCCB protein expression (cross reactive material, CRM-). *Pccb* $^{8\ bp\ del/8\ bp\ del}$ mice homozygous recapitulate the neonatal lethal form of PA in humans, and perish in the first 7 days of life, and hence, are particularly useful to assay the efficacy of AAV9-hPCCB gene therapy for the treatment of severe PA. Data from the *in vivo* studies demonstrate that administration of AAV9-hPCCB vector rescues *Pccb* ^{8}

bp del/8 bp del neonatal mice by increasing animal survival and reducing levels of pathological metabolites.

A hypomorphic model of PA caused by PCCB deficiency was also generated using an approach previously adopted to study the related disorder, methylmalonic acidemia (MMA). In brief, a rescue transgene designed to express PCCB from the murine creatine kinase promoter (*Mck*) was introduced as a germline transgene ($Tg^{Mck\text{-}Pccb}$) and bred with mice carrying a severe *Pccb* mutation, c.1495_1499, designated $Pccb^{X}$, which causes a predicted frameshift stop mutation p.Ala498Profs*2. $Pccb^{X/X}$; $Tg^{Mck\text{-}Pccb}$ are rescued from lethality due to transgene expression in the muscle but manifest growth retardation, lethality after weaning, and pronounced elevations of 3-OHP and 2-MC caused by lack of PCC in the liver. AAV9-hPCCB treatment of $Pccb^{X/X}$; $Tg^{Mck\text{-}Pccb}$ mice at weaning results in weight gain, increased survival, and reduced metabolites.

Because *Pccb* $^{8\ bp\ del/8\ bp\ del}$, $Pccb^{X/X}$; $Tg^{Mck\text{-}Pccb}$ and *Pccb* $^{P230L/8\ bp\ del}$ models have been recently generated, only small numbers of animals have been available to treat before the sunset date of the RPDD program. The results are nevertheless compelling for fundamental efficacy of AAV9-hPCCB gene therapy including rescue of -). *Pccb* $^{8\ bp\ del/8\ bp\ del}$ mice from lethality, improvement in their growth, and significant reduction of disease associated metabolites. The dose of 1e14 vg/kg in the pre-clinical studies was selected as a possible high dose for patient translation, and is slightly less than the dose of zolgesma, an approved AAV9 gene therapy, given to safely to infants with spinal muscular atrophy, type 1.

Detailed Experimental Design and Results

For definitive testing of AAV gene therapy vectors for human translation, we created mice using CRISPR/Cas9 genome editing to engineer mutations that are compatible with those seen in the patients, such as frameshift-stop changes. One mutation in Exon 7 of the *Pccb* gene is severe and null at the level of protein expression (CRM -). Please see ***Table 1***.

Table 1. ***Pccb*** $^{8\ bp\ del/8\ bp\ del}$ **Neonatal Lethal Mouse Model**

Pccb **exon Targeted**	**Pccb Mutation**	**Homozygous phenotype**
7	8 base pair deletion, frameshift stop	Neonatal lethal CRM negative Increased 2-MC and 3-OHP

Building on many years of previous work with AAV gene therapy in related mouse models of methylmalonic acidemia, where early lethality is a uniform characteristic of the homozygous mutant phenotype, we initiated our studies to determine the therapeutic efficacy of AAV9-hPCCB delivered by retro-orbital plexus injection in newborn *Pccb* $^{8\ bp\ del/8\ bp\ del}$ mice. Because the untreated *Pccb* $^{8\ bp\ del8/8\ bp\ del}$ mice experience 100% lethality in the newborn period (***Figure 6***), we injected AAV9-hPCCB via retro-orbital plexus to the systemic circulation, to recapitulate IV delivery, the anticipated ROA in humans.

Survival of AAV9-hPCCB Treated *Pccb* $^{8\ bp\ del/8\ bp\ del}$ Mice

Heterozygous *Pccb* $^{8\ bp\ del/+}$ breeding units yielded litters and all the pups obtained in the litter were treated with AAV9-hPCCB within a few hours after birth (at P1). For the survival study in the *Pccb* $^{8\ bp\ del/8\ bp\ del}$ mice, we used a dose of $1x10^{14}$ vg/kg administered via a retro-orbital injection. To minimize stress on the newborns and mothers, the pups were not weighed before AAV9-hPCCB administration.

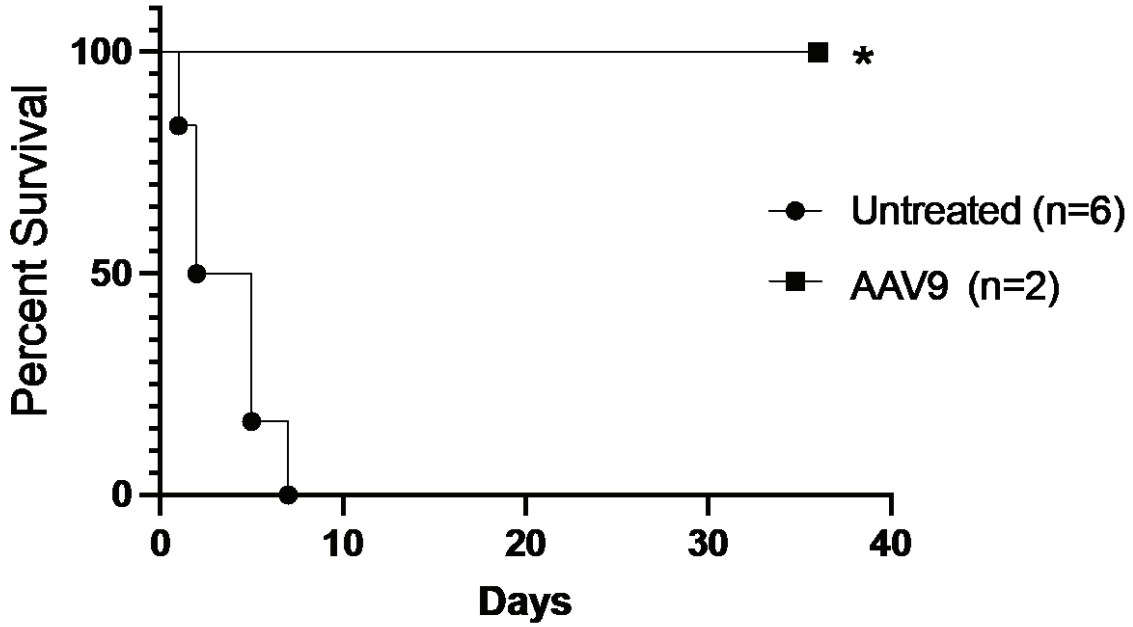
Table 3 below summarizes the proof-of-concept neonatal rescue study in homozygous *Pccb* $^{8\ bp\ del/8\ bp\ del}$ neonatal animals. At D45, survival was intact and the experiment is ongoing.

Table 3. List of AAV9-hPCCB Treated Neonatal *Pccb* $^{8\ bp\ del8/8\ bp\ del}$ Pups

Genotype	Treatment	Dose (vg/pup)	Dose (vg/kg)	Sex	Number of Mice
Pccb $^{8\ bp\ del8/8\ bp\ del}$	None	N/A	N/A	Not Available	6
Pccb $^{8\ bp\ del8/8\ bp\ del}$	AAV9-hPCCB	$1.4x11^{11}$	$1x10^{14}$	male	2

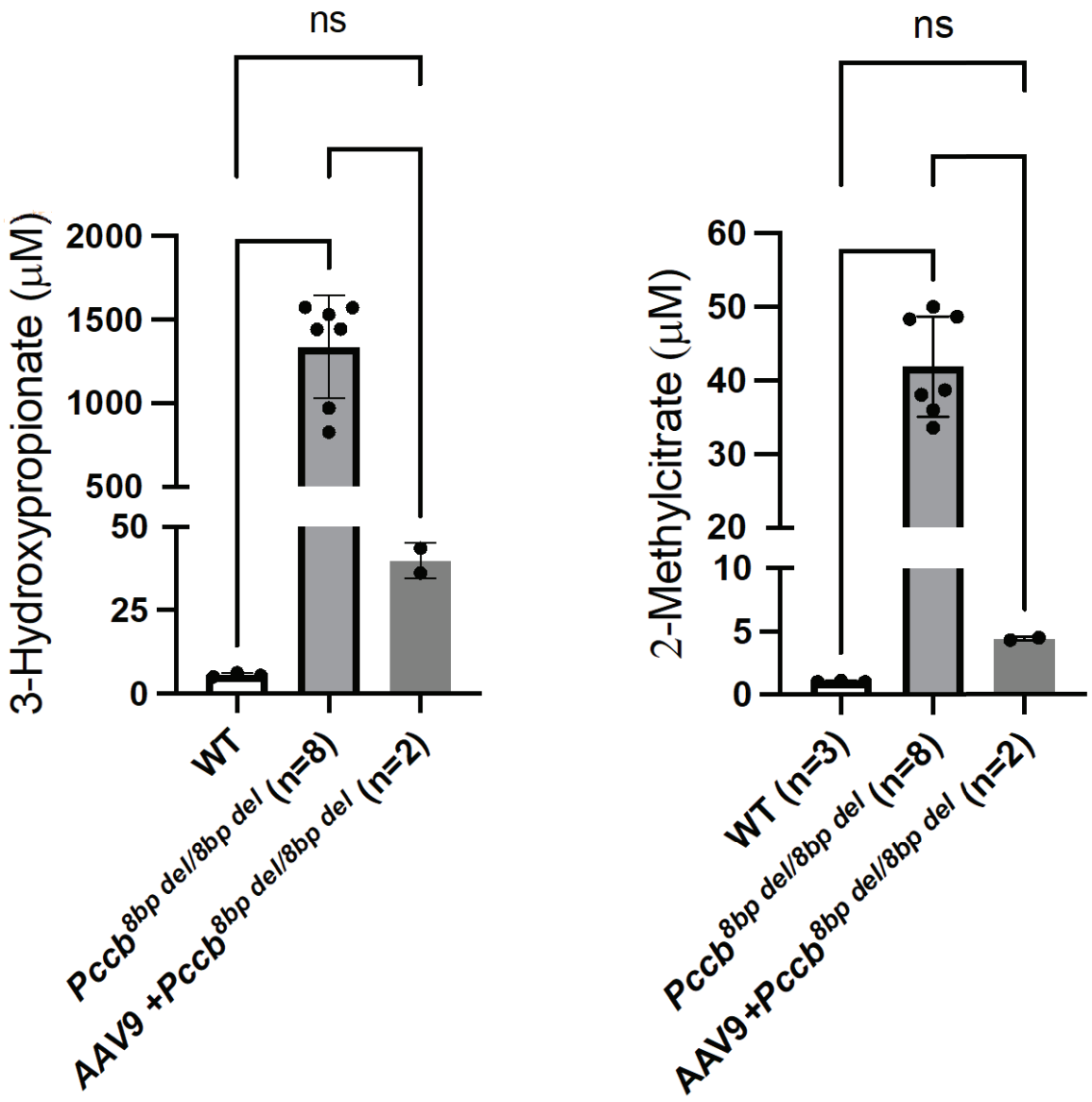
The survival of the AAV9-hPCCB treated *Pccb* $^{8\ bp\ del/8\ bp\ del}$ mice are presented in ***Figure 6***. A dose of $1x10^{14}$ vg/kg provided rescue from neonatal lethality. In the same mice, the disease related metabolites, 3-OHP and 2-MC, which serve as metabolic biomarkers reflective of PCC activity restored in the liver from AAV9-hPCCB expression, were measured and compared to the levels in untreated *Pccb* $^{8\ bp\ del8/8\ bp\ del}$ mice (N=6). The AAV9-hPCCB treated *Pccb* $^{8\ bp\ del8/8\ bp\ del}$ mice (N=2) had a substantial reduction in 3-OHP ($P<0.05$) and 2-MC ($P<0.001$) at D30 post injection. The results are presented in ***Figure 7***.

Figure 6. Survival Curve of AAV9-hPCCB Treated and Untreated *Pccb* $^{8\ bp\ del8/8\ bp\ del}$ Mice.



Pccb $^{8\ bp\ del8/8\ bp\ del}$ **mice were treated AAV9-hPCCB at P0-1. The dose was 1x10^{14} vg/kg. No treatment (untreated) were used as a control. The graph depicts the percent survival of different cohorts of animals, AAV9=AAV9-hPCCB, * *P*<0.05 compared to survival of untreated vs treated *Pccb*** $^{8\ bp\ del8/8\ bp\ del}$ **mice. *P* values were calculated with GraphPad Prism software using a Log-rank (Mantel-Cox) test.**

Figure 7. Plasma 2-MC and 3-OHP in AAV9-hPCCB Treated *Pccb* $^{8\ bp\ del8/8\ bp\ del}$ Mice.



3-OHP and 2-MC levels in the plasma were measured by GC-MS in AAV9-hPCCB *Pccb* $^{8\ bp\ del8/8\ bp\ del}$ mice 30 days posttreatment, age matched untreated wildtype mice and untreated *Pccb* $^{8\ bp\ del8/8\ bp\ del}$ *one-day old pups.* AAV9=AAV9-hPCCB, WT=wildtype, ns=not significant, * *P*<0.001 **** *P*<0.0001. *P* values were calculated with GraphPad Prism software using one-way ANOVA.**

AAV9-hPCCB Treatment of *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ Mice

Pccb$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice represent an early childhood model of PCCB deficiency and can be treated at weaning. In a proof-of-concept study, 2 *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice received AAV9-hPCCB at a dose of $1x10^{14}$ vg/kg delivered via retroorbital injection on D25.
Survival, growth, and the levels of 2-MC and 3-OHP were measured in the treated mice on D10 and D30 compared to historic untreated controls sampled on D25.

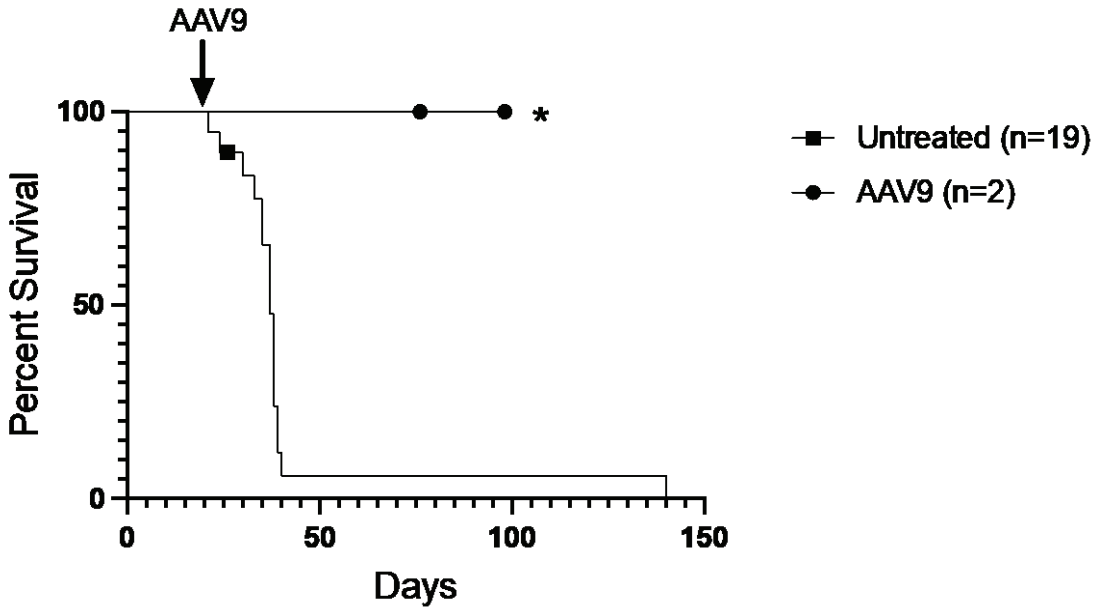
Table 4 below summarizes the proof-of-concept treatment study in *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice.

Table 4. List of AAV9-hPCCB Treated Juvenile *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ Mice

Genotype	Treatment	Dose (vg/kg)	Sex	Number of Mice
Pccb$^{X/X}$; Tg$^{Mck\text{-}Pccb}$	None	N/A	7 males 12 females	19
Pccb$^{X/X}$; Tg$^{Mck\text{-}Pccb}$	AAV9-hPCCB	1x10^{14}	2 females	2

The survival of the AAV9-hPCCB treated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice are presented in ***Figure 8.*** A dose of 1x10^{14} vg/kg significantly increased survival compared to untreated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ controls.

Figure 8. Survival Curve of AAV9-hPCCB Treated and Untreated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ Mice.

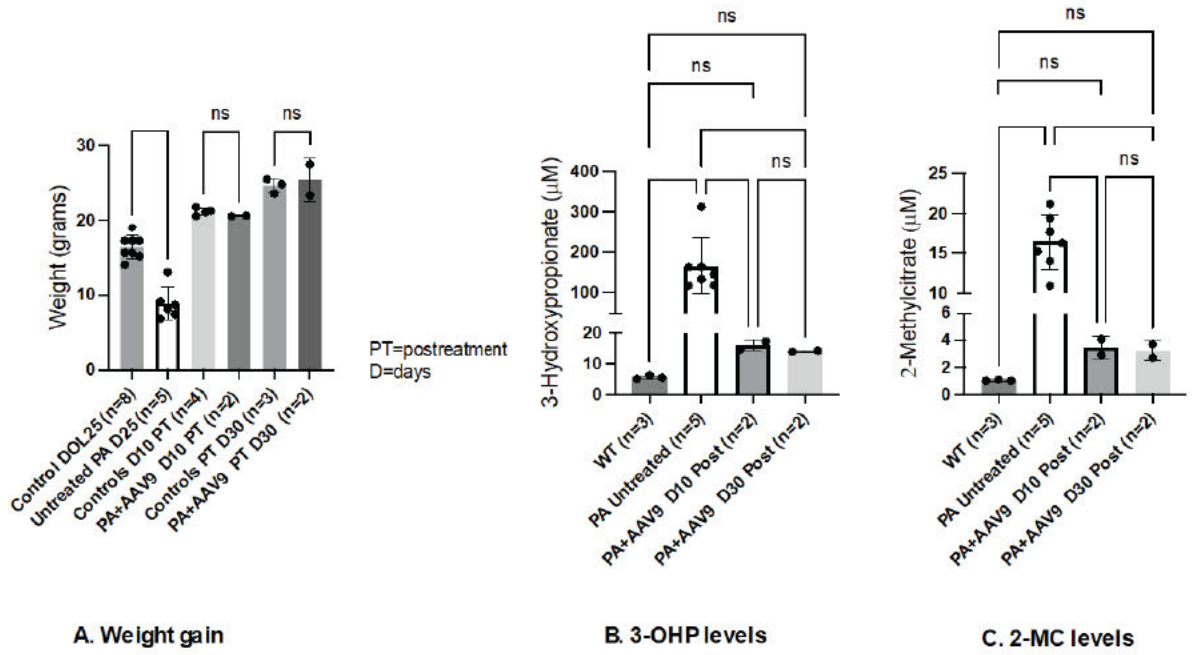


***Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice were treated AAV9-hPCCB at weaning as indicated by the black arrow. The dose was 1x10^{14} vg/kg. No treatment (untreated) was used as a control. The graph depicts the percent survival of different cohorts of animals. AAV9=AAV9-hPCCB, * *P*<0.05 compared to survival of untreated vs treated *Pccb*$^{8\ bp\ del8/8\ bp\ del}$ mice. *P* values were calculated with GraphPad Prism software using a Log-rank (Mantel-Cox) test.**

Measurement of Growth, Plasma 2-MC and 3-OHP in AAV9-hPCCB Treated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ Mice

Animals were weighed on D25 and again on D35 after AAV9-hPCCB treatment alongside wild type controls (N=8) and compared to untreated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice weighed on D25 (n=5). After 10 days, the AAV9-hPCCB treated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice achieved the same weight as wild type untreated controls (P=N.S.). In the same mice, the disease related metabolites, 3-OHP and 2-MC, which serve as metabolic biomarkers reflective of PCC activity restored in the liver from AAV9-hPCCB expression, were measured and compared to the levels in untreated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice (N=5). The AAV9-hPCCB treated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$ mice had a substantial reduction in 3-OHP ($P<0.05$) and 2-MC ($P<0.001$) at both D10 and D30 post injection in comparison to untreated *Pccb*$^{X/X}$; Tg$^{Mck\text{-}Pccb}$. The results are presented in ***Figure 9***.

Figure 9. Growth, Plasma 2-MC and 3-OHP in AAV9-hPCCB Treated *Pccb*^{XX}; Tg$^{Mck\text{-}Pccb}$ Mice



A. Weight gain **B. 3-OHP levels** **C. 2-MC levels**

***Pccb*^{XX}; Tg$^{Mck\text{-}Pccb}$ mice were treated AAV9-hPCCB at weaning. The dose was 1x10^{14} vg/kg. Weight gain (A), 3-OHP (B), and 2-MC (C) levels in the plasma were measured by GC-MS. No treatment (untreated) was used as a control. AAV9=AAV9-hPCCB, ns=not significant, * *P*<0.05 **, *P*<0.01, *** *P*<0.001. *P* values were calculated with GraphPad Prism software using one-way ANOVA.**

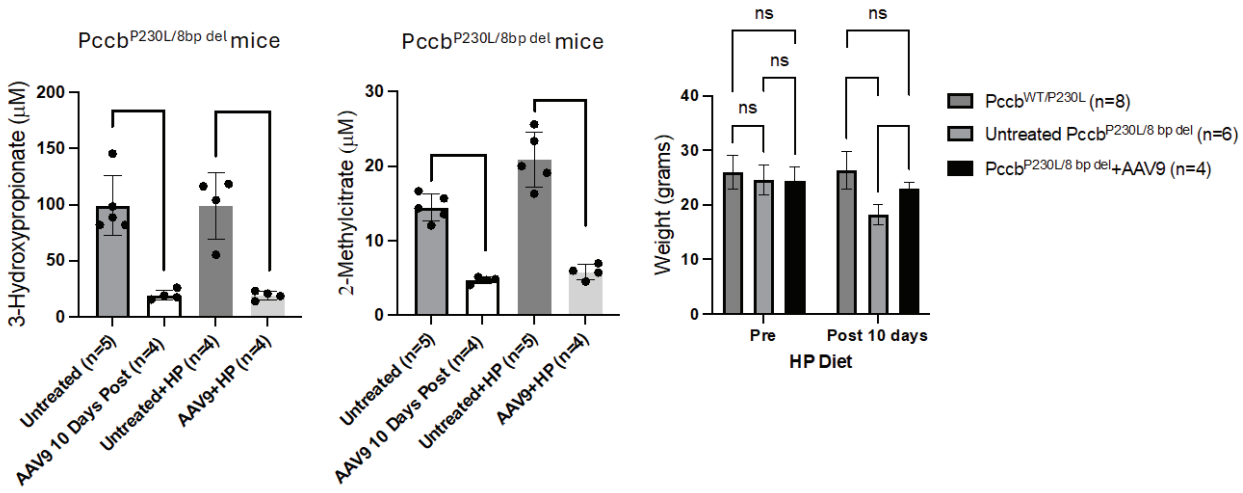
AAV9-hPCCB Treated *Pccb*$^{P230L/8bp\ del}$ Mice Exhibit Reduced Metabolites and Resistance to Metabolic Stress in Comparison to Untreated *Pccb*$^{P230l/*bp\ del}$ Mice

The *Pccb*$^{P230L/8bp\ del}$ murine model is a compound heterozygote, harboring a missense mutation and frameshift stop allele, a genotype combination frequently noted in PA patients. *Pccb*$^{P230L/8bp\ del}$ mice were treated AAV9-hPCCB at 2 months of age at a dose of 1x10^{14} vg/kg. The disease related metabolites, 3-OH and 2-MC, were significantly reduce 10 days post AAV9-hPCCB treatment presented in ***Figure 10A &B***. Untreated and AAV9-hPCCB treated *Pccb*$^{P230l/8bp\ del}$ mice were then placed on a high protein diet to induce metabolic stress. The AAV9-hPCCB treated *Pccb*$^{P230l/8bp\ del}$ mice maintained their weight and appeared healthy during the 10-day exposure to high protein while the untreated *Pccb*$^{P230l/8bp\ del}$ mice became lethargic and lost a significant amount of weight ***Figure 10C.*** The high protein diet was discontinued after 10 days of feeding because of the weight loss and poor health of the untreated *Pccb*$^{P230l/8bp\ del}$ animals. The unaffected *Pccb*$^{WT/P230L}$ mice did not lose weight while on the high protein diet, indicating that the stress caused by the diet was disease related.

Table 5. List of AAV9-hPCCB Treated Adult *Pccb* $^{P230L/8\ bp\ del}$ Mice

Genotype	Treatment	Dose (vg/kg)	Sex	Number of Mice
Pccb $^{P230L/8\ bp\ del}$	None	N/A	3 males 3 females	6
Pccb $^{P230L/8\ bp\ del}$	AAV9-hPCCB	$1x10^{14}$	2 males 2 females	4

Figure 10. AAV9-hPCCB Treated *Pccb*$^{P230L/8bp\ del}$ Mice Followed by High Protein Challenge



A. 3-OHP levels **B. 2-MC levels** **C. Protein Challenge**

Pccb$^{P230L/8bp\ del}$ mice were treated AAV9-hPCCB at 2 months of age at a dose of $1x10^{14}$ vg/kg. 3-OHP (A), and 2-MC (B) levels in the plasma were measured by GC-MS were measured in AAV9 treated and age match controls 10 days post AAV9 treatment and 10 days after being place on a high protein diet. No treatment (untreated) was used as a control. (C) Protein Challenge show mouse weights just prior to being placed on a high protein diet and after 10 days on a high protein diet. AAV9=AAV9-hPCCB, ns=not significant * *P*<0.05, * *P*<0.001, **** *P*<0.0001. *P* values were calculated with GraphPad Prism software using one-way ANOVA.**

5 ORPHAN DRUG STATUS

AAV9-hPCCB is not designated as an orphan drug or product for the treatment of patients with PA resulting from a deficiency of PCCB, and AAV9-hPCCB is currently not designated as an orphan drug or product in the United States for any other indication.

6 PATIENT SUBSET CONSIDERATIONS AND MEDICAL PLAUSIBILITY OF THE CHOSEN SUBSET

The prevalence of PA does not exceed 200,000. Therefore, subset considerations and medical plausibility of a subset are not applicable to the proposed indication of patients with PA resulting from a deficiency of PCCB in this request.

7 REGULATORY STATUS AND MARKETING HISTORY

NCATS is currently in late phase pre-clinical development of AAV9-hPCCB,

AAV9-hPCCB is not currently marketed or approved for use in any country, including the United States. In accordance with section 526(a)(1) of the Federal Food, Drug, and Cosmetic Act [21 USC 360bb(a)(1)]; 21 CFR 316.20(b)(7); and 21 CFR 316.23(a), NCATS has not previously submitted a marketing application to FDA for the same active moiety in AAV9-hPCCB for the same rare disease or condition prior to submission of the AAV9-hPCCB Rare Pediatric Disease designation request.

8 DOCUMENTATION OF PATIENT POPULATION SIZE

PA is a rare disease in the United States (US), which includes PCC enzyme deficiency from deleterious variants in either the *PCCA* or *PCCB* genes. The *PCCA*- and *PCCB*-types appear to occur with equal distribution and have similar disease severity and manifestations.[8] There are no population-based prevalence studies, and most of the data used to estimate prevalence are from birth incidence from identification of affected babies using tandem mass spectrometry based newborn screening (NBS) for propionylcarnitine. Overall, from these sources, PA in the US is a very low incidence/prevalence rare disorder, with birth incidence ranging from approximately 0.14-1.22 per 100,000 in the US, regardless of region or time period examined (Table 4). Based on the annual US birth rate of 3.7 million/per year,[5] approximately 5-45 children are born in the US with both *PCCA*- and *PCCB*-type PA each year, with around 50% of individuals having *PCCB*-type PA (approximately 3-23 infants per year).

Studies identified from the medical literature are summarized as follows:

- The most recent study was performed and published by Adhikari et al in 2020,[2] which reported the experience of the NBSeq project that evaluated whole-exome sequencing (WES) as a new methodology for NBS. In this study, the authors obtained archived residual dried blood spots (DBS) and data for nearly all inborn errors of metabolism (IEM) cases (n=1,728 DBSs) identified using tandem mass spectrometry (MS/MS) from 4.4 million screened infants born in California between July 2005 and December 2013. They then compared the MS/MS with the results from WES. This was the largest study to date on sequencing efforts of an entire population of IEM-affected cases identified from NBS, inclusive of 48 different IEMs and the 78 genes associated with these disorders, representing 1,190 newborns: 805

IEM-affected newborns and 385 MS/MS false positives. There were 237 affected individuals with an organic acid disorder, of which 6 out of 4.4 million newborns were shown to have been diagnosed with PA, which approximates to a birth incidence of 0.14 per 100,000 or 1 in 733,333.

- Therrell, 2014[16] reported on the largest newborn screening study in the US, comprising all states performing tandem mass spectrometry for propionylcarnitine during the study period 2001-2011, identified 105 cases of propionic acidemia in >25 million infants screened for a predicted birth prevalence of 1 in 240,181 or 0.42 per 100,000 births.
- Chapman et al, 2018,[3] conducted a study on NBS results for 3 disorders, maple syrup urine disease, PA, and methylmalonic aciduria (MMA), from 3 geographic regions around the world, including state screening labs in the US, the southwest region of Germany, and Kuwait. The US data was collected and analyzed from the US NBS database from 1991 to 2000, and positive results from the diseases in questions were compared to the on-line reported birth rates per US states. Some states reported MMA and PA together, given the marker C3 being common to both. For PA alone, there were a total of 12 cases identified from a total of 2,912,901, for a birth incidence of 1:242,741, or 0.41 cases per 100,000. For MMA and PA together, there were 147 cases in 7,544,243 births, for a birth incidence of 1:50,709 (MMA alone birth incidence was 1:69,354, 3.5 times the incidence of PA alone).
- Almasi et al, 2019,[4] conducted a systematic literature review and meta-analysis on the worldwide epidemiology of PA, which estimated point prevalence of PA per 100,000 births calculated separately by region (North American, Europe, Asia-Pacific, Middle-East and North Africa (MENA)), and in 2 time periods 1981-2000, and 2001-present. They identified 43 studies included in the qualitative synthesis on epidemiology, and 31 studies included in the quantitative synthesis. The vast majority of articles reported on newborn screening programs (NBS) providing estimates on the birth prevalence of the disease, defined as the number of affected newborns divided by the total population screened, 8 of which described results from NBS programs in the US. There were 6 studies used for the North America (NA) point estimate. The results showed that for NA, birth prevalence ranged from 0.20 (California, US) to 1.35 (Ontario, Canada) per 100,000 newborns, with the pooled point estimate of 0.33 per 100,000 newborns in NA. Similar rates were noted in Europe, and higher rates in Japan and MENA. Pooled point estimates remained below 1 per 100,000 newborns in all regions except MENA, which were significantly higher (>3 per 100,000). The authors additionally noted a scarcity of studies in PCCA- and PCCB-subtypes but are reported to be approximately equally distributed,[8] and that broadly targeted population-based prevalence studies are not available. Their overall conclusions were that OA is an “ultra-rare” disorder; however, ultra-rare was not specifically defined.

Table 4: Brief Summaries of the US Studies Included in the Almasi Meta-Analysis[3]

Lead Author, Year of Publication	Estimated Birth Incidence per 100,000 Newborns	Brief Description
Feuchtbaum, 2012[14]	0.2	Study to describe birth prevalence of genetic disorders among different racial/ethnic groups through analysis of population-based NBS data from 2005-2010, inclusive of 2,282,138 newborns screened. Calculated birth incidence 0.2 per 100,000 newborns screened.
Frazier, 2006[15]	0.33	Report of North Carolina's 8-year experience with MS/MS NBS, and incidence of disease for selected amino acid, fatty acid and organic acid disorders, 1997-2005. Calculated incidence of PA was 1:300,000 births.
Therrell, 2014[16]	0.42	Tabulation of 10-year NBS data for selected IEM from 2001-2011 from the National Newborn Screening Information System (NNSIS). Identified 105 PA cases in 25,026,374 newborns screened, for a birth incidence of 1:238,346 (0.42 per 100,000)
Weisfeld-Adams, 2009[17]	0.5	Retrospective NBS data analysis in a 4-year period (2005-2008) in NY. Total number of infants screened = 1,006,325; 5 cases of PA were confirmed (1:201,265).
Comeau, 2004[18]	0.6	Study summarizes the New England Newborn Screening Program's (NENBSP) approach and experience, from Jan 1999- Jan 2003. PA clustered into panel testing, and not separately broken out within the paper. Birth incidence estimated to be around 0.6 per 100,000.
Naylor, 1999[19]	0.71	Study reporting results of NBS using MS/MS of more than 700,000 newborns from Pennsylvania, Ohio, North Carolina and Louisiana, which detected 163 IEMs. 32 patients were identified with organic acidemias, of which 5 had PA (0.71 per 100,000).
Chace, 2001[20]	~0.7	Study report on the validation of MS/MS analysis for MMA and PA from DBS obtained from 908,543 newborns from NBS from

Lead Author, Year of Publication	Estimated Birth Incidence per 100,000 Newborns	Brief Description
		approximately 1992-2001. Results showed 1:64,896 with either MMA or PA by MS/MS, but birth incidence was not broken out by MMA or PA individually.
Zytkovicz, 2001[21]	1.2	Study summarizes 2-year MS/MS results for amino acid and fatty and organic acid disorders from DBS from the New England Newborn Screening Program from Feb 1999 to Feb 2001, inclusive of 164,000 newborns. 36 infants were identified for C3, of which 2 were PA patients, for an estimated birth incidence of 1.2 per 100,000.

A genomics-based analysis of variants in *PCCB* using publicly available databases (gnomAD 2.1 and 4.1) was additionally employed to estimate the prevalence of *PCCB* deficiency. Based on an analysis of the sum of minor allele frequencies for all pathogenic and likely pathogenic variants in *PCCB* and using the Hardy-Weinberg equation, we estimate the global prevalence of *PCCB* PA to be about 1/468,324[21]. We note a that a founder variant in the Amish population, c.1606G>A p.Asp536Asn, is more prevalent than in other populations [minor allele frequency (q) of 0.015], predicting a prevalence of PCCB deficiency in the Amish population of 1 in 4244 (q^2). PA caused by homozygosity of the PCCB c.1606G>A p.Asp536Asn causes a mild form of PA. The estimated population of the Amish of North America (adults and children) in June 2022 is 373,620, which increased by approximately 12,150 since the previous year[22]. Assuming an Amish population growth at the same rate since 2022, the Amish are now estimated to have a population size of 385,770 and a predicted prevalence of PCCB deficiency of 23.5 cases per 100,000. We therefore estimate that there are 90 Amish patients in the US with PA caused by PCCB deficiency.

Thus overall, these findings are notable for the following:

First, there are no population-based prevalence studies for PA for the US population in the medical literature.

Second, all of the studies identified were based on birth prevalence calculated from state NBS results in various regions around the US and in different time periods, mainly after 1999 when screening based on propionylcarnitine (C3) levels detected by MS/MS became more broadly available. Birth incidence ranged from 0.14 to 1.22 per 100,000 newborns, and the results were largely similar across the US and over time. This translates to about 5-45 children born with PA per year, with 50% of these expected to be *PCCB*-type PA (3-23 cases per year). The most recent

analysis conducted by Adhikari et al, 2020,[2] compared MS/MS with WES, which likely addressed false positives and is likely the most accurate. This study showed a birth incidence of 0.14 cases of PA per 100,000 newborns (6 cases per year), half of which are expected to be *PCCB*-type PA.

Third, based on the US Census Bureau's population count in 2023 there were 334,914,895 people living in the US.[24] This roughly translates to a PA prevalence ranging from, at the low end (0.14 cases per 100,000) of around 469 people in the US having PA to, at the high end, (1.2 cases per 100,000) of 4,018 people with PA, 234-2009 of whom would have *PCCB*-type PA. However, because PA is a life-threatening condition resulting in premature death at a young age for many patients, the true prevalence is likely lower than this. Based on our good-faith assessment from expert opinion obtained from world's experts on PA (Charles P. Venditti, M.D., Ph.D., NIH, NHGRI), and from data from an NIH-conducted PA natural history study,[9] we estimate there are ~50-100 non-Amish *PCCB*-type PA patients living in the US, and perhaps as many as 90 additional Amish patients with *PCCB*-type PA.

In conclusion, the *PCCB*-type PA population amenable to therapy with AAV9-hPCCB gene therapy is a very low incidence/prevalence rare disease, with an estimated number of patients ranging from 234-2,009 patients in the US in the general population and as many as 90 patients of Amish extraction.

9 RARE PEDIATRIC DISEASE DESIGNATION JUSTIFICATION

Based on the evidence presented above, pursuant to Section 908 of FDASIA,[1] and consistent with the Rare Pediatric Disease Priority Review Vouchers Guidance for Industry,[25] we believe that AAV9-hPCCB qualifies as a Rare Pediatric Drug Product for the treatment of *PCCB*-related PA. Specifically:

1) *PCCB*-type PA is a serious or life-threatening disease in which the serious or life-threatening manifestations primarily affect individuals aged from birth to 18 years.

Most PA patients are identified at birth through NBS. Despite this early recognition and initiation of best-available therapies, including dietary restrictions and supplements, and in some cases LT, severely affected patients continue to experience serious or life-threatening complications of their disease. The majority of non-Amish patients will manifest irreversible serious end-organ damage, such as kidney, cardiac or CNS impairment, during childhood and adolescence, and there is a need for better treatments to prevent, mitigate or ameliorate these disease-related complications.

2) *PCCB*-related PA is a rare disease, with an estimated prevalence of fewer than 4,000 patients in the US.

We additionally note that AAV9-hPCCB is intended as a gene therapy specifically for gene replacement due to mutations in the PCC enzyme subunit beta and has no foreseeable use in the

treatment of any other disease or a different adult indication. AAV9-hPCCB is currently in late pre-clinical phases of testing at NIH, with planned progression to clinical trials within the next few years. The likely initial patient population will be children or adolescents.

We therefore request that AAV9-hPCCB be designated as a Rare Pediatric Disease Product for the treatment of the *PCCB*-related PA.

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APPENDIX A

The nucleotide sequence of the human PCCB cDNA contained in the therapeutic transgene.

ATGGCGGCGGCATTACGGGTGGCGGCGGTCGGGGCAAGGCTCAGCGTTCTGGCGAG
CGGTCTCCGCGCCGCGGTCCGCAGCCTTTGCAGCCAGGCCACCTCTGTTAACGAACG
CATCGAAAACAAGCGCCGGACCGCGCTGCTGGGAGGGGGCCAACGCCGTATTGACG
CGCAGCACAAGCGAGGAAAGCTAACAGCCAGGGAGAGGATCAGTCTCTTGCTGGAC
CCTGGCAGCTTTGTTGAGAGCGACATGTTTGTGGAACACAGATGTGCAGATTTTGGA
ATGGCTGCTGATAAGAATAAGTTTCCTGGAGACAGCGTGGTCACTGGACGAGGCCG
AATCAATGGAAGATTGGTTTATGTCTTCAGTCAGGATTTTACAGTTTTTGGAGGCAG
TCTGTCAGGAGCACATGCCCAAAAGATCTGCAAAATCATGGACCAGGCCATAACGG
TGGGGGCTCCAGTGATTGGGCTGAATGACTCTGGGGGAGCACGGATCCAAGAAGGA
GTGGAGTCTTTGGCTGGCTATGCAGACATCTTTCTGAGGAATGTTACGGCATCCGGA
GTCATCCCTCAGATTTCTCTGATCATGGGCCCATGTGCTGGTGGGGCCGTCTACTCCC
CAGCCCTAACAGACTTCACGTTCATGGTAAAGGACACCTCCTACCTGTTCATCACTG
GCCCTGATGTTGTGAAGTCTGTCACCAATGAGGATGTTACCCAGGAGGAGCTCGGTG
GTGCCAAGACCCACACCACCATGTCAGGTGTGGCCCACAGAGCTTTTGAAAATGAT
GTTGATGCCTTGTGTAATCTCCGGGATTTCTTCAACTACCTGCCCCTGAGCAGTCAGG
ACCCGGCTCCCGTCCGTGAGTGCCACGATCCCAGTGACCGTCTGGTTCCTGAGCTTG
ACACAATTGTCCCTTTGGAATCAACCAAAGCCTACAACATGGTGGACATCATACACT
CTGTTGTTGATGAGCGTGAATTTTTTGAGATCATGCCCAATTATGCCAAGAACATCA
TTGTTGGTTTTGCAAGAATGAATGGGAGGACTGTTGGAATTGTTGGCAACCAACCTA
AGGTGGCCTCAGGATGCTTGGATATTAATTCATCTGTGAAAGGGGCTCGTTTTGTCA
GATTCTGTGATGCATTCAATATTCCACTCATCACTTTTGTTGATGTCCCTGGCTTTCT
ACCTGGCACAGCACAGGAATACGGGGGCATCATCCGGCATGGTGCCAAGCTTCTCT
ACGCATTTGCTGAGGCAACTGTACCCAAAGTCACAGTCATCACCAGGAAGGCCTAT
GGAGGTGCCTATGATGTCATGAGCTCTAAGCACCTTTGTGGTGATACCAACTATGCC
TGGCCCACCGCAGAGATTGCAGTCATGGGAGCAAAGGGCGCTGTGGAGATCATCTT
CAAAGGGCATGAGAATGTGGAAGCTGCTCAGGCAGAGTACATCGAGAAGTTTGCCA
ACCCTTTCCCTGCAGCAGTGCGAGGGTTTGTGGATGACATCATCCAACCTTCTTCCA
CACGTGCCCGAATCTGCTGTGACCTGGATGTCTTGGCCAGCAAGAAGGTACAACGTC
CTTGGAGAAAACATGCAAATATTCCATTGTAA.