A phase 2 study of a vitamin metabolite for PKAN

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Clinical Trial Protocol
V 5.0

A phase 2 study of a vitamin metabolite for PKAN

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Investigator Agreement

The trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the FDA Terms of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: Penelope Hogarth, M.D.

Signed: _____ Date: _____

Protocol Synopsis

Protocol Title	**A phase 2 study of a vitamin metabolite for PKAN**
Sponsor	NIH (NICHD)
Additional Funding Support	Spoonbill Foundation
Investigators	Penelope Hogarth, MD (PI, Oregon Health & Science University) Susan Hayflick, MD (Co-I, Oregon Health & Science University) David Stone, PhD (Co-I, Director - Food Innovation Center, Oregon State University) Brian French, PhD (Co-I, Psychometrician, Washington State University)
Project Manager	Allison Gregory, MS, CGC (Oregon Health & Science University)
Study Center	Single-site (remote clinical trial design involving US and Canadian participants) Oregon Health & Science University
Per Participant Study Period	**Primary Cohort**: 6 months on placebo / product + 18 months on open-label product + up to 4 months for screening and follow-up **Open-label Cohort**: Subjects' participation will end shortly after the final subject in the primary cohort completes all study activities in September, 2022.
Total Trial Period	5 years
Study Design	This will be a single site, phase 2, safety and tolerability trial of 4'-phosphopantetheine (4'-PPT), a downstream product of intermediary metabolism in children and adults with pantothenate kinase-associated neurodegeneration (PKAN), an inborn error of vitamin B5 metabolism. There will be two cohorts: For the "**Primary Cohort**", an initial 6-month, dose-ranging, parallel-group, randomized, double-blind, placebo-controlled phase will be followed by an 18-month, single-dose, open-label phase. During the double-blind phase, participants will be randomly assigned to receive one of three doses (5mg/m^2 or 15mg/m^2 or 20mg/m^2) or placebo in a 1:1:1:1 distribution. All participants will be assigned to a single dose (15mg/m^2) during the subsequent open-label phase. The primary data analysis will be based on the Primary Cohort dataset. Once the target enrollment of the Primary Cohort is met in the double-blind arm, new participants entering the study will be enrolled directly into an open-label arm that will last until the final subject in the Primary Cohort has completed their participation, including up to 4 additional mos for screening and follow-up. This will be designated the "**Open-label Cohort**". Because the rolling enrollment into the Open-label Cohort will result in variable duration of participation, the safety and biomarker data from

	the Open-label Cohort will not be used for the primary data analysis, although a secondary analysis of these data may be used for regulatory filings. The study product will be taken once daily but may be split into divided doses if the participant does not tolerate a single daily dose. Note that the double-blind phase of the study is not intended to be dose-finding: all doses are predicted to be many-fold below toxicity and all doses are predicted to correct the metabolic defect in PKAN, albeit with slightly differing time courses. The range of doses to be used during the double-blind phase is employed purely to test our hypotheses regarding the pharmacodynamic profile of an exploratory 'proof of concept' biomarker, CoASY. The mid-dose of 15mg/m^2 is based on the minimum effective dose in the mouse and converted to the human equivalent dose using an FDA algorithm and other guidances.
Study Objective and Specific Aims	The primary objective of this study is to evaluate safety and tolerability of the study product in adult and pediatric participants with PKAN. The primary aims are: • To compare safety or tolerability profiles of the study product between active and placebo groups over the 6-month double-blind parallel group phase of the study; and • To examine the long-term safety and tolerability profile of the compound over the 18-month (placebo group) -24-month (active group) active treatment period The primary hypothesis is that there will be no clinically significant difference in safety or tolerability profiles between active and placebo groups during six months of double-blind parallel study, nor between initial dose groups over 18-24 months of treatment with the study product. The secondary aim is: To evaluate the peripheral pharmacodynamic response of one component of a PKAN "molecular signature" (CoASY mRNA expression levels) in response to the study product. The secondary hypotheses are that: • CoASY mRNA expression levels will initially rise in all the study product-treated participants after 3 days ±2 days of treatment compared with placebo-treated participants; and • CoASY mRNA expression levels will subsequently decline in all participants after up to 18 months of study product treatment, consistent with secondary down-regulation of CoASY activity in the presence of excess substrate, (i.e. feedback inhibition); and

	• There will be no difference in CoASY mRNA expression between initial treatment groups after up to 18 months of the single-dose active study product treatment. In a third exploratory aim, with the goal of informing future studies of the study product or other putative disease-modifying therapeutics, we will assess the feasibility of applying a latent growth curve (LGC) approach to natural history data collected before and during an intervention to detect disease-modifying effects.
Number of Participants	Approximately 64 participants (allowing for 4 dropouts to provide 60 evaluable participants) in the Primary Cohort; up to 86 additional participants in the Open-label Cohort. Participant recruitment will not be limited by PKAN phenotype but participants will be stratified to ensure approximately equal distribution of 'classic' and 'atypical' participants in each group. We anticipate about half of the participants will have classic disease. • Classic participants are defined as onset motor sx before age 5 and (if over 10 at enrollment) loss of independent ambulation by age 10. Goal is n=30 classic participants. • Atypical participants are defined as all other PKAN participants who don't meet classic disease criteria. Goal is n=30 atypical participants.
Main Eligibility Criteria	**Inclusion Criteria** To be included in this trial, participants must: • Have a diagnosis of PKAN confirmed by: a) genetic testing confirming 2 pathogenic or likely pathogenic mutations, or (b) typical findings on exam and brain MR imaging with only one pathogenic mutation +/- a second likely pathogenic or Variant Of Uncertain Signficance (VOUS) in *PANK2*, or (c) typical findings on exam and brain MR imaging with a single likely pathogenic or VOUS in *PANK2,* or (d) be a symptomatic sibling of a proband subject meeting a, b, or c. • Be between age 3 months and age 89 years at the time of screening. • Be able to take the study product by mouth or via gastrostomy tube. • Be willing and able to complete study procedures / telephone visits / blood draws independently, OR have a caregiver / parent willing and able to assist with these tasks. • Be enrolled or willing to enroll in the PKANready natural history study (eIRB 10832) • Be resident in North America for the duration of the trial. **Exclusion Criteria** To be included in this trial, participants must NOT:

	• Have had exposure to a putative *PANK2* 'bypass' therapeutic agent in the 30 days prior to screening. • Be concurrently enrolled in another interventional clinical trial. • Have concurrent medical or other conditions expected to preclude completion of study procedures or confound the assessment of clinical and laboratory measures of safety.
Primary Outcome	Our primary endpoints are: • *Safety* outcome measures will include treatment-emergent adverse events and abnormalities on standard laboratory tests. TEAE will be collected at 10 time points over 24 months of treatment in the Primary Cohort and collected at up to 7 time points over up to 18 months of treatment in the Open-label Cohort. Laboratory tests will be collected at 9 time points in the Primary Cohort and up to 6 time points in the Open-Label Cohort over the same respective periods. • *Tolerability* outcomes will be assessed via participant retention in the study and adherence to the study product regimen.
Secondary Outcomes	Our secondary endpoint is: CoASY mRNA expression levels measured at 8 time points in the Primary Cohort and at up to 6 time points in the the Open-label Cohort.
Safety Assessments	Treatment-emergent Adverse Events Baseline and at intervals throughout treatment (10 time points for the Primary Cohort and up to 7 timepoints for the Open-label Cohort) • Treatment-emergent adverse events will be classified according to Medical Dictionary for Regulatory Activities (MedDRA) terminology. • Severity of the AEs will be graded according to the NCI Common Terminology Criteria for Adverse Events, Version 4.0 Clinical safety laboratory studies Baseline and at intervals throughout treatment (9 time points for Primary Cohort; up to 6 time points for the Open-label Cohort) • Complete metabolic profile (Includes NA^+, K^+, CL^-, CO2, BUN, Creat, Ca^{2+}, Glu, Alb, T. Protein, Alk Phos, T. Bili, AST, ALT) • Complete blood count with auto-differential • Creatine kinase (CK)
Tolerability Assessments	Participant retention • During treatment and follow up • Number of participants retained in each arm at each of the follow up time points Participant adherence to study product regimen as measure of tolerability • During treatment and follow up • Mean percent of study product consumed

Sample Size Considerations	Because PKAN is an ultra-rare disease, we are limited to 64 participants for the Primary Cohort (15 in each arm + 4 potential dropouts allowing for 5% loss to follow-up) whom we will recruit from our established research registry, (prioritizing those participating in our "PKANready" ongoing natural history study) as well as by methods described within the protocol. When our double-blind enrollment goal is met, enrollment of up to 86 additional participants will shift to an open-label arm, designated the Open-label Cohort. Our primary objective is to estimate effects of the study product and we will prioritize effect sizes over statistical power in interpreting results. Still, we wish to include sufficient numbers to estimate effects in our secondary and exploratory analyses of CoASY mRNA expression levels with some precision. In preliminary cross-sectional data, we found that the mean CoASY delta Ct (dCt) measures were 1.4 standard deviations higher in classic PKAN children (n=8) than age-matched healthy controls (n=6) on the $\log_2$ scale, where higher dCt indicates lower CoASY activity (mean=17.39 vs 16.85, SD=0.39). We anticipate that participants at all three dose levels will resemble healthy controls after 3±2 days, while the placebo group will show no change. We simulated this change from baseline for 12 participants per group. For the standard deviation, we performed one set of simulations using the upper limit of the 60% CI for the standard deviation (0.34-0.48) as a conservative estimate. We regressed the dCt value on four indicator variables for study arm after treatment, with the intercept representing the common mean baseline value, and included a random intercept to induce correlation between paired measurements. We then used a Wald test for $\beta_{placebo1} - (\beta_{low1} + \beta_{mid1} + \beta_{high1})/3 = 0$ at α=0.05. Pooling over 1000 simulated datasets, we find that we reject the null in **85-95%** of simulations, approximating power to detect this difference at one month between the active and placebo groups.

Statistical Methods	A **modified Intention-to-Treat (ITT)** analysis dataset of all randomized participants who consumed at least one dose of the study product. This is also the **Safety Analysis** dataset. The **Per-Protocol** dataset will include participants who consume at least 75% of assigned study product. **General approach** The proposed study takes place over 28 months in the Primary Cohort. A screening period of up to 2 months will be followed by an initial 6-month double-blind, placebo-controlled parallel trial of the study product during which participants will be assigned to one of three dose groups [5mg/m^2, 15mg/m^2, 20mg/m^2]; and then by a further 18 months of open label study during which in which all participants will be assigned a 15mg/m^2 dose. Participants will be followed for up to two months beyond the end of the open-label period. The primary data analysis will be based on the Primary Cohort dataset. Once the target enrollment in the Primary Cohort is met, we will shift to direct enrollment into an Open-label Cohort. A screening period of up to 2 months will be followed by up to 18 months during which all participants will be assigned a 15mg/^{m2}

dose and follow a similar pattern of procedures as the Primary Cohort during the open-label phase of the study. Participants will be followed for up to two months beyond the end of their open-label period. Because the rolling enrollment into the Open-label Cohort will result in variable duration of participation, the safety and biomarker data from the Open-label Cohort will not be used for the primary data analysis, although a secondary analysis of these data may be used for regulatory filings.

Descriptive statistics will be presented as counts and percentages for categorical variables, counts and ranges for events, and means with standard deviations, medians, and ranges for continuous variables. For safety and tolerability measures, we will consider clinical, rather than statistical, significance. Initial determinations will be made by the independent medical safety monitor physician familiar with the study population and blinded to treatment allocation.

For modeling and inferential tests, we will consider a p-value less than 0.1 to be significant if the magnitude and precision of the estimated effect support that conclusion. Covariates will be specified in the description below. We will perform extensive exploratory data analysis and checks of model fit and will transform variables with obvious departures from normality.

Analysis of primary endpoints

Safety Analysis

Comparisons of participants experiencing treatment-emergent adverse events and clinically-significant abnormalities on clinical labs will be reviewed for clinical significance in the Safety Analysis dataset, which includes all participants who consume at least one dose of the study product. Events expected as part of the usual disease course in PKAN, whether present at trial enrollment or not, will not be considered or captured as adverse events.

Frequencies of participants experiencing at least one adverse event (AE) will be displayed by body system and preferred term according to *Medical Dictionary for Regulatory Activities* (MedDRA) terminology. Detailed information collected for each AE will include a description of the event; duration; whether the AE meets criteria as a Serious Adverse Event (SAE); severity / grade of the event; relationship to study product; changes made to the study product in response to the AE /SAE; and clinical outcome. Severity of the AEs will be graded according to the NCI *Common Terminology Criteria for Adverse Events*, Version 4.0. If the event meets criteria as an SAE, the nature of the SAE will be classified using standard FDA criteria.

The number and percentage of patients who experienced any serious AE (SAE), treatment-emergent AE, and treatment-emergent SAE will be summarized, as well as the total number of events per arm. AE data will be presented for months 0-6 and 7-37 and cumulatively, as appropriate. Emphasis in the analyses will be placed on AEs classified as treatment-emergent.

Clinically-significant abnormalities on clinical labs will be tabulated similarly to AEs. The number and percentage of patients experiencing clinically-significant abnormalities will be compiled in summary tables to examine the distribution by collection month [0-1-3-6-7-9-12-18-24 (25) months for the Primary Cohort; and 0-1-3-6-12-18 (19) months for the Open-label Cohort].

Graphic displays may be provided to illustrate the results over time. Initial review of group differences and determination of clinical significance will be performed by an unaffiliated physician familiar with the study population and blinded to treatment arm.

Tolerability Analysis

We will compare the number of participants retained in each arm at each of the follow-up time points [0-(0+3 days)-1-3-6-(6M+3 days)-7-9-12-18-24-25 months for the Primary Cohort; and 0-(0+3 days)-1-3-6-9-12-18-19 for the Open-label Cohort], taking into account whether dropout seems plausibly related to study participation and the mean percent of study product consumed. High retention in the study and product consumption per-protocol will be considered evidence of tolerability.

Other analyses

Analysis of secondary endpoints

We will **estimate a CoASY pharmacodynamic profile** in response to different doses of the study product in the Per-Protocol dataset. CoASY mRNA expression levels are continuous, measured in normalized differences in cycle times (ΔCt) on the $\log_2$ scale. Lower ΔCt indicates higher expression levels. We will use regression models controlling for PKAN type to estimate mean levels by treatment arms, including mixed effects when modeling over multiple time points.

To estimate the **change in expression levels after 3±2 days of treatment compared to placebo**, we will use a mixed effects regression model of the form $CoASY_{ijt} = \beta_0 + \beta_{jt} I(group = j) I(time = t \; and \; t > 0) + \alpha I(cPKAN) + b_i$, j = placebo, low, mid, high; t = 1. Each individual contributes two observations (baseline and after); b_i represents the random intercept for subject. Baseline, represented by $\beta_{0,}$ is constrained to be the same for all groups because of randomization. Our primary approach will treat time as binary (0=baseline, 1=treated), though this can later be expanded to capture differences between days 1-5 of treatment and include more time points. The β_{jt} represent mean changes from baseline for the three dose groups and placebo. An indicator for PKAN type (cPKAN or aPKAN is included to reflect its role in stratified randomization. We will review regression coefficients as estimates of the magnitude of effect and $\beta_{\text{placebo1}} - (\beta_{\text{low1}} + \beta_{\text{mid1}} + \beta_{\text{high1}})/3 = 0$ with a two-sided alpha of 0.1 to determine whether CoASY mRNA expression levels rose in all the study product-treated participants after initiation of treatment compared to placebo.

Similarly, we will check for **treatment response when the placebo group initiates treatment after month 6** by recoding their observations to use months 6 as 0

	(baseline). We will use the same regression model but expect $\beta_{placebo1}$ to differ from zero. Further, because PKAN types may differ in response, we will test for an **interaction effect between PKAN type and treatment** averaged over dose levels as $CoASY_{ijt} = \beta_0 + \beta_1 I(aPKAN\ type) + \beta_2 I(time = post) + \beta_{12} I(aPKAN) I(post) + b_i$, where indicator variables represent active vs placebo, aPKAN vs classic, and β_{12} is the coefficient for their interaction. 50% of participants are expected to have aPKAN. **At six months**, we expect CoASY mRNA expression levels to have decreased somewhat, and that **the group with the highest dose will show the lowest expression**. In addition to repeating the comparisons outlined for initiation of treatment above, we will test $\beta_{low1} > \beta_{mid1} > \beta_{high1} > \beta_{placebo1}$. As a final step, we will develop a **longitudinal model to characterize CoASY mRNA expression profiles over the entire 24-month study treatment period**. We plan to use a mixed effects model with random intercept and slope to account for correlation between measurements and to allow individuals to vary from the group mean. We will explore a number of possible parameterizations of the time effect, including fractional polynomials and splines. We will allow for different trajectories by (a) dose group and (b) PKAN type in separate models with a group x time interaction. This model will take the general form $CoASY_{ij} = \beta_0 + \beta_1 t_{ij} + \beta_2' group + \beta_3 t_{ij} \times group + b_{1i} + b_{2i} t_{ij}$, where t_{ij} represents time on treatment in months (including partial months as decimals) and group indicates dose group or PKAN type, depending on the model. Latent growth curve modeling plan for the exploratory aim will not be described here but is described in full in the protocol.

1. **Protocol Title: A phase 2 study of a vitamin metabolite in PKAN**

2. **Objectives**

The *primary objective of this study* is to assess the safety and tolerability of 4'-phosphopantetheine (4'-PPT), a product of intermediary metabolism, in pantothenate kinase-associated neurodegeneration (PKAN), an inborn error of vitamin B5 metabolism. The study product meets the FDA statutory definition for consideration as a medical food and is being developed as such for the nutritional management of PKAN.

The *primary hypothesis* is that there will be no clinically significant difference in safety or tolerability profiles between active and placebo groups during six months of double-blind parallel study, nor between dose groups over up to 18 months of treatment with 4'-PPT.

The *primary aims* are:

- To compare safety or tolerability profiles of 4'-PPT between active and placebo groups over the 6-month double-blind parallel group phase of the study; and
- To examine the long-term safety and tolerability profile of the compound over up to 18 months of open-label period.

The *primary endpoints* are:

- Safety outcome measures will include treatment-emergent adverse events and abnormalities on standard laboratory tests collected over up to 24 months of treatment.
- Tolerability outcomes will be assessed via participant retention in the study and adherence to the study product regimen.

The *secondary objective of this study* is to assess the pharmacodynamic profile of an exploratory, 'proof-of-concept' molecular biomarker (CoASY mRNA expression) in response to the study product.

3. **The *secondary hypothesis is that* CoASY is an inducible enzyme that will respond rapidly to exogenous** substrate (4'-PPT) and subsequently decline in activity as a result of excess substrate and feedback inhibition; and that that these changes will be detectable in peripheral *CoASY* mRNA expression levels. We anticipate that:

- *CoASY* mRNA expression levels will initially rise in all 4'-PPT-treated participants after 3 days ±2 days of treatment compared with placebo-treated participants; and
- *CoASY* mRNA expression levels will subsequently decline in all participants after completing treatment with 4'-PPT treatment, consistent with secondary down-regulation of *CoASY* activity in the presence of excess substrate, (i.e. feedback inhibition) and there will be no difference in *CoASY* mRNA expression between groups at 18-24 months.

The *secondary endpoint* is CoASY mRNA expression levels measured at 8 time points in the Primary Cohort and up to 6 timepoints in the Open-label Cohort.

In addition, in a *third exploratory aim*, with the goal of informing future studies of 4'-PPT or other compounds, we will assess the feasibility of applying a latent growth curve (LGC) approach to natural

history data collected before and during an intervention to capture a disease-modifying effect. The natural history data is being collected under a separate IRB-approved protocol eIRB 10832.

4. Background

a. PKAN is an ultra-rare inborn error of metabolism that disproportionately affects children and causes severe disability

Pantothenate kinase-associated neurodegeneration (PKAN) is an inborn error of vitamin B5 metabolism. Children with the classic form of the disease suffer from a progressive neurodegenerative disorder that begins in the first few years of life, characterized by a severe movement disorder, cognitive and neuropsychiatric abnormalities, and pathologic iron accumulation in the basal ganglia of the brain. Children typically lose independent ambulation before age 10 and many die in the first decade of life. Those individuals who have disease onset later in life tend to have less severe manifestations but nonetheless suffer profound disability[1,2]. Pathologically, the disease shows remarkable regional specificity in the brain, selectively affecting the globus pallidus, with very limited involvement of other regions[3]. PKAN is an orphan disease, with a prevalence of 1-2 cases per million in the general population[4], with higher prevalence reported in a few small populations where geographic, socio-economic, cultural or religious factors have resulted in founder mutations[4,5]. Treatment options are currently limited to symptomatic management: there are no proven disease-modifying agents available.

b. PKAN Patients cannot metabolize vitamin B5 normally to make coenzyme A

PKAN is caused by mutations in the *PANK2* gene, which encodes one of four pantothenate kinase (PANK) enzymes in the human that serve to phosphorylate vitamin B5 in the rate-limiting step in coenzyme A (CoA) metabolism [Figure 1]. CoA is essential for the tri-carboxylic acid cycle, fatty acid oxidation and synthesis, amino acid metabolism and neurotransmitter synthesis, serving more than 9% of mammalian biochemical reactions[6].

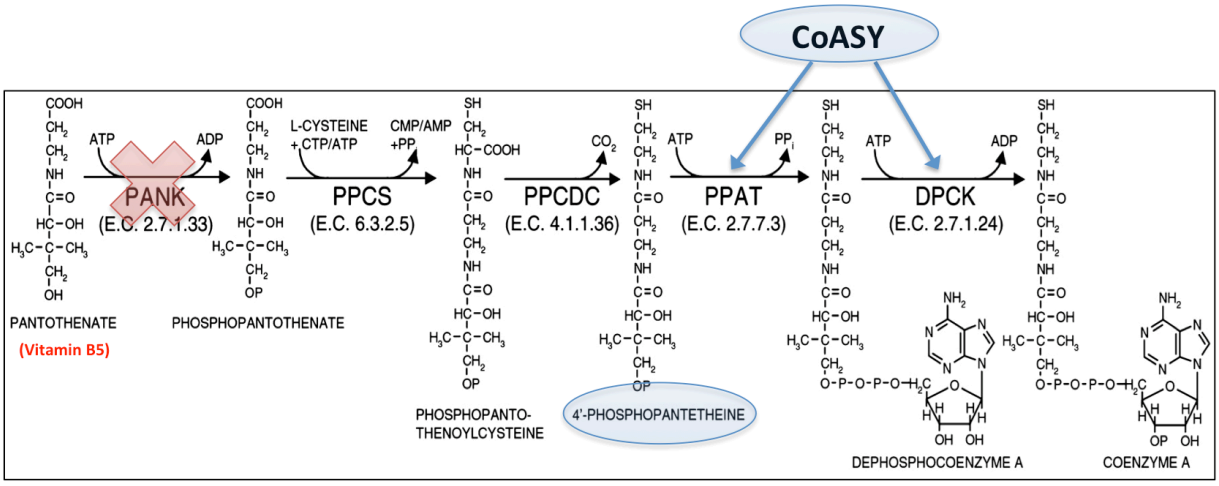

Figure 1: ***Coenzyme A metabolic pathway.*** *Patients with mutations in the PANK2 gene lack an enzyme to phosphorylate vitamin B5 (pantothenate) in selected regions of the brain in the key regulatory step in the coenzyme A (CoA) biosynthetic pathway. PPAT and DPCK together comprise the bi-functional enzyme CoASY. 4'-phosphopantetheine is a product of intermediary metabolism that is converted by CoASY into CoA.*

c. Molecular and functional evidence of the disease is seen in the PANK2 knock-out mouse

Using a mouse model of PKAN, we have recently discovered a set of biomarkers that reflect defects in CoA metabolism, iron homeostasis and dopamine neurotransmission, abnormalities that are recapitulated in the human disease[7]. We engineered a mouse knock-out (KO) of the murine

pantothenate kinase 2 gene ortholog, *Pank2*[8]. With the knowledge that PKAN is an exquisitely circumscribed disorder in human brain, selectively affecting the globus pallidus[3], we sought to investigate gene expression changes in the analogous brain regions of the KO mouse with the goal of identifying differences that could be attributed specifically to defective Pank2. We dissected mouse brain into three regions, one containing the globus pallidus (GP; entopeduncular nucleus in mouse), one containing substantia nigra (SN), and one containing cerebellum. Confidence in the specificity of the dissection method was established using histological and transcriptional analyses of tissue from each region. The structures were visualized with region-specific immunohistochemical stains and published gene expression differences, confirming the integrity and identity of each region. After dissecting brain into 'disease-containing' region (GP) versus 'disease-protected' regions (SN and cerebellum), we analyzed the expression of genes relevant to CoA synthesis, iron homeostasis and dopamine neurotransmission.

Compared to litter-matched wild-type controls (WT), the brains of homozygous *Pank2* KO animals show statistically-significant regional differences in CoA-synthetic gene expression. Decreased expression of the genes encoding enzymes downstream of pantothenate kinase, Ppcs and Coasy, is found only in the GP region in KO animals; wild type levels are found in the SN and cerebellum. Down-regulation of genes in response to decreased substrate is a well-recognized phenomenon for proteins that are transcriptionally regulated. In addition to these CoA synthetic differences, we observed decreased expression of genes encoding two iron homeostasis proteins, again only in GP region in the KO animals.

Rather unexpectedly, we also observed a dopamine-related gene expression defect in only the GP region of our KO mouse model of PKAN. Dopamine involvement in the human disease has been uncertain. We see clinical signs of dystonia-parkinsonism but few patients benefit from L-DOPA administration, and single case reports or small series of SPECT and PET imaging conflict in their results or are contaminated with cases that are likely not PKAN. In GP region only, we see marked up-regulation of two genes encoding dopamine receptors D1a and D2 in our mice. Receptor up-regulation is a common response to decreased availability of ligand, whether from synthesis, transport, sequestration or another mechanism. Expression of GABA receptors is not different from wild type, indicating that this is a dopamine-specific change.

Finally, we have found statistically-significant defects in mitochondrial function (complex I and extra-cellular acidification rate, ECAR) in the Pank2 KO mouse brain compared to WT, demonstrating a defective biochemical correlate to the mRNA expression changes described above.

d. Circulating and cultured cells from human PKAN patients demonstrate the same abnormal phenotype seen in the mouse

At least one of the pharmacodynamic mRNA biomarkers found in KO mouse GP region is recapitulated in peripheral cells from people with PKAN, providing not only translational evidence for the relevance of the mouse model, but also a readily-accessible potential disease biomarker in the human population. We analyzed both primary and transformed cells (circulating lymphocytes, and transformed lymphoblasts and fibroblasts) from children and adults with PKAN, and found statistically-significant derangements in mRNA expression of the gene encoding CoASY, a key enzyme downstream of PANK2, when compared with age-, gender- and passage-matched control cells. In addition, we see derangements in mitochondrial function (Complex I and ECAR) in cultured fibroblasts

from PKAN patients compared to controls, mirroring the findings in the murine model. These transcriptional and functional differences are robust, yet they seem to be tolerated by these cell types both *in vitro* and *in vivo*. This phenomenon is not surprising, given that most cells in the human body, including most cells in brain, seem to tolerate a defect in *PANK2* without significant impairment. The basis for this selective vulnerability is not yet fully understood. We have not yet analyzed iron and dopamine mRNA expression biomarkers in human cells; however, we suspect that iron analytes will prove to be influenced more by systemic iron status rather than disease state.

e. The study product ameliorates the deficits in the mouse model and in cultured human cells.
Single gene disorders of intermediary metabolism offer the most direct path to rational therapeutics. By bypassing the enzyme block, alternate substrates can resolve the primary defect near to its source and avert development of downstream sequelae. In the Pank2 KO mice, we have found that oral administration of the study product, 4'-phosphopantethine (4'-PPT), normalizes CoA-, iron-, and dopamine-related changes in brain. Fourteen-day feeding of 4'-PPT leads to a dose-responsive correction in the expression of all perturbed biomarker genes, suggesting that 4'-PPT normalizes CoA metabolism in the KO mouse. The secondary correction of iron and dopamine defects resulting from normalizing CoA metabolism is strong evidence that these perturbations arise as a direct sequela of the CoA defect and, importantly, that they are reversible at the level of both transcript and cellular metabolism. When the study product is withdrawn, the levels return to the aberrant pre-treatment levels. Importantly, the study product also corrects the mitochondrial functional defects observed in transformed fibroblasts from PKAN patients.

These pre-clinical data provide a compelling rationale for advancing 4'-PPT to clinical studies. Further, because the study product is identical to a naturally-occurring product of intermediary metabolism and will be used in very low doses intended to mimic physiologic levels, we can justify the first clinical studies being in the target population, including vulnerable child populations.

5. Study Design

This will be a single site, phase 2, safety and tolerability trial of 4'-phosphopantetheine (4'-PPT), a downstream product of intermediary metabolism in children and adults with PKAN, a inborn error of vitamin B5 metabolism. An initial 6-month, dose-ranging, parallel-group, randomized, double-blind, placebo-controlled phase will be followed by an 18-month, single-dose, open-label phase. There will be up to 2 months for screening and up to 2 months for follow up for up to a total of 28 months participation for this Primary Cohort. During the double-blind phase, participants in the Primary Cohort will be assigned randomly to one of three dose groups or placebo, with a 1:1:1:1 distribution. Once we meet our enrollment target for the Primary Cohort, we will shift additional enrollment directly into a single-dose open-label arm that will last up to 18 months per participant including up to 2 months for screening and up to 2 months for follow up. Our primary hypothesis is that there will be no clinically significant difference in safety or tolerability profiles between active and placebo groups during six months of double-blind parallel-group study, nor between dose groups over up to 18 months of treatment with 4'-PPT. Safety measures will include treatment-emergent adverse events collected at 10 time points and abnormalities on standard laboratory tests collected at 9 time points over 25 months (including follow up) for Primary Cohort participants. For the Open-label Cohort, treatment-emergent adverse events will be collected at up to 7 time points and safety labs at up to 6 time points over up to 20 months (including follow up). Participants will be stratified by PKAN type: classic or atypical, to ensure approximately equal distribution in each arm.

As a secondary, "proof of concept" aim, we will assess the pharmacodynamic profile of CoASY mRNA expression in the peripheral blood of participants receiving 4'-PPT or placebo. We will measure CoASY mRNA expression at 8 time points for the Primary Cohort and at up to 6 timepoints for the Open-label Cohort to examine four secondary hypotheses:

a. **CoASY mRNA expression levels will rise in all 4'-PPT-treated participants after 1 month of treatment** compared with placebo-treated participants.

b. There will be **no difference in CoASY mRNA expression between 4'-PPT dose groups after 1 month of treatment**. Statistical tests will fail to reject the null and the magnitude of the difference will be small compared to the one-month change.

c. **After 6 months of treatment, CoASY mRNA expression levels will vary inversely with 4'-PPT dose**, and

d. There will be no **difference between initial dose groups [placebo/active] after up to 18 months on the same active dose**, consistent with down-regulation of CoASY activity in the presence of excess substrate, (i.e. feedback inhibition).

6. Study Product

The study product is 4'-phosphopantetheine (4'-PPT) formulated in a fruit-flavored syrup suitable for administration by mouth or via G-tube. Several factors contribute to the prediction that 4'-PPT is of very low toxic potential[9]. First, it is a synthetic version of a naturally-occurring product of intermediary metabolism in the vitamin B5 pathway [See Figure 1]. Second, the compound has shown no evidence of toxicity when given to mice in doses far in excess of those used in this trial. Third and perhaps most important, it will be used in very low doses intended to mimic physiologic levels: all doses employed in the study are based on a range tested in the mouse model as the 'minimum effective', rather than the more usual 'maximum tolerated' doses. Although derived from mouse studies, the dose range to be used in this trial turns out to be remarkably close to the molar equivalent of the Recommended Daily Allowance (RDA) of pantothenate, the upstream vitamin the study product aims to replace, which itself shows little toxicity even in doses far in excess of the RDA, with no upper limit established by the Institute of Medicine[10].

We are developing the study product as a ***medical food*** for the nutritional management of PKAN, an inborn error of vitamin B5 metabolism. Medical foods are a unique regulatory category of food product that were originally developed for inborn errors of metabolism. A medical food is defined under the 1988 amendments to the Orphan Drug Act as "a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements… are established by medical evaluation". Formulated 4'-PPT fits all five key criteria of the agency's statutory definition of a medical food[11]:

a. It is a specially formulated and processed product (as opposed to a naturally occurring foodstuff used in its natural state) for the partial or exclusive feeding of a patient by means of oral intake or enteral feeding by tube, meaning a tube or catheter that delivers nutrients beyond the oral cavity directly into the stomach or small intestine. (*4'-PPT will be synthesized and specially formulated into a food product – a fruit-flavored semi-liquid syrup that we are calling 'CoA-Z' - and provided to PKAN*

patients by mouth or gastrostomy tube).

b. It is intended for the dietary management of a patient who, because of therapeutic or chronic medical needs, has limited or impaired capacity to ingest, digest, absorb, or metabolize ordinary foodstuffs or certain nutrients, or who has other special medically determined nutrient requirements, the dietary management of which cannot be achieved by the modification of the normal diet alone. *(Patients with PKAN have impaired capacity to metabolize vitamin B5, an essential dietary nutrient, and this deficiency cannot be managed by modification of the normal diet alone).*

c. It provides nutritional support specifically modified for the management of the unique nutrient needs that result from the specific disease or condition, as determined by medical evaluation. *(Formulated 4'-PPT will provide nutritional support for the PKAN patient's unique nutrient deficiency resulting from mutations in the PANK2 gene).*

d. It is intended to be used under medical supervision. *(Formulated 4'-PPT is intended to be used only under medical supervision, either in a clinical trial or clinical care setting).*

e. It is intended only for a patient receiving active and ongoing medical supervision wherein the patient requires medical care on a recurring basis for, among other things, instructions on the use of the medical food. *(Formulated 4'-PPT is intended only for PKAN patients receiving active and ongoing medical supervision, whether in a clinical trial or clinical care setting).*

Medical foods are not regulated as drugs, and are not subject to the same pre-market approval process as drugs. The components of a medical food either need to be approved food additives or to be "Generally recognized as safe" (GRAS). Currently, our plan is to seek approval for 4'-PPT as a food additive, with the intended use being the dietary management of PKAN. A food additive can be tested in humans prior to approval, including in vulnerable populations, and the human data can be submitted as part of the food petition. Clinical trials of medical foods do not need to be conducted under an IND, unless the medical food is seeking to "diagnose, prevent, treat, mitigate or cure" a disease. The FDA distinguishes between "treatment" claims and "management" of a disease in its Guidance for Clinical Investigators, Sponsors, and IRBs on Investigational New Drug Applications (INDs) — Determining Whether Human Research Studies Can Be Conducted Without an IND[12] Although nutritional management of PKAN or any inborn error of metabolism might be expected to have an impact on the long-term neurologic outcomes of the disease, we are intentionally limiting the goals of this study to an examination of the safety, tolerability, and exploratory "proof of concept" pharmacodynamic biomarker profile of formulated 4'-PPT in PKAN, with the specific goal of keeping our product strictly within the agency's statutory definition of a medical food. The FDA's Center for Food Safety and Applied Nutrition (CFSAN, the FDA office that oversees medical foods) has thus far been supportive of our plan to develop the study product as a medical food. A cross-agency group at FDA has reviewed this study protocol and determined that an IND is not required for the conduct of human studies; this communication was previously reviewed by the IRB and is available in the study documents.

GMP 4'-PPT, the study base substance, will be manufactured in a GMP facility by one of the world's largest process chemistry organizations in the pharmaceutical industry, in a facility that was inspected by the FDA in 2018 without issuance of a Form 483. The 4'-PPT base substance will be shipped to the OHSU Research Pharmacy, which in turn will dispense in batches to the Food Innovation Center, an urban agricultural

experiment station in Portland, Oregon that is part of Oregon State University. The FIC serves the Pacific Northwest food industry as well as local, national and international food business communities, providing services for product and process development, sensory and consumer testing, and food safety. The FIC is an FDA-registered food facility and is licensed to manufacture and sell food. In collaboration with the study team, FIC has developed a fruit-flavored semi-liquid gel suitable for oral intake or G-tube administration as the formulation for the GMP medical food product. The formulated study product will be called "CoA-Z". The FIC will have conducted shelf-life testing of the formulated product per FDA guidance prior to initiation of this clinical study. The formulated study product will be a 1 mg / mL solution packaged and processed in sealed medicine bottles with a child-safe cap and in-neck adapters for accurate dispensing using a syringe, using materials and a process developed in consultation with Dr. Mark Daeschel, Oregon State University's consultant FDA food process authority. The study base substance will be formulated in batches throughout the study, with the timing based on FDA guidance arising from stability data from the shelf-life studies. The study product will be delivered by FIC to the OHSU Research Pharmacy for dispensing during the study, following all applicable Research Pharmacy policies and procedures.

Note that the double-blind phase of this study is not intended to be dose-finding: all doses are predicted to be many-fold below toxicity and all doses are predicted to correct the metabolic defect in PKAN, albeit with slightly differing time courses. The range of doses to be used during the double-blind phase is employed to test our hypotheses regarding the pharmacodynamic profile of an exploratory biomarker, CoASY. The mid-dose of 15mg/m^2 that will be given to all participants during the open-label phase is based on the minimum effective dose in the mouse and converted to the human equivalent dose using an FDA algorithm and other guidances[13,14]. The actual single daily dose in mL to be taken by the study participant will be calculated by the research pharmacist based on the participant's body surface area. If a subject has difficulty taking the volume of the single dose, then at the Principal Investigator's discretion the dose may be divided into 2 equal volumes and taken twice a day.

7. Study Population

a. Number of Subjects

We will screen up to 150 individuals with the goal of enrolling 64 participants, to achieve 60 participants completing the double-blind arm of the study as the Primary Cohort. We are conservatively allowing for 4 dropouts (5% loss) based on our experience in one other interventional clinical trial with this clinical population; however, if participant loss is higher than anticipated during the projected 24 month recruitment phase , we will strive to replace all dropouts to maximize our chances of achieving 60 participants completing the study. Once enrollment into the Primary Cohort is met, we will shift to enrolling up to 86 additional participants in the direct to open-label arm (Open-label Cohort).

Classic PKAN participants are defined as those with onset of motor symptoms before age 5 and (if over 10 at enrollment) loss of independent ambulation by age 10. Goal is n=30 classic participants.

Atypical PKAN participants are defined as all other PKAN participants who don't meet classic disease criteria. Goal is n=30 atypical participants.

b. Inclusion and Exclusion Criteria

For inclusion in this trial, subjects must:

- The subject has a diagnosis of PKAN confirmed by: a) genetic testing confirming 2 pathogenic or likely pathogenic mutations, or (b) typical findings on exam and brain MR imaging with only one pathogenic mutation +/- a second likely pathogenic or Variant Of Uncertain Significance (VOUS) in *PANK2*, or (c) typical findings on exam and brain MR imaging with a single likely pathogenic or VOUS in *PANK2,* or (d) be a symptomatic sibling of a proband subject meeting a, b, or c.
- Be between age 3 months to age 89 years at the time of screening.
- Be able to take the study product by mouth or via gastrostomy tube.
- Be willing and able to complete study procedures / telephone visits / blood draws independently, OR have a caregiver / parent willing and able to assist with these tasks.
- Be enrolled or willing to enroll in the PKANready natural history study (eIRB 10832).
- Be resident in North America for the duration of the trial.

For inclusion in this trial, subjects must NOT:

- Have been exposed to a putative *PANK2* 'bypass' therapeutic agent in the 30 days prior to screening.
- Be concurrently enrolled in another interventional clinical trial.
- Have concurrent medical or other conditions that in the opinion of the investigators are expected to preclude completion of study procedures or confound the assessment of clinical and laboratory measures of safety.

Dr. Caleb Rogers, OHSU clinical geneticist, will serve as an independent eligibility reviewer for the study. He will review whether each subject meets the inclusion criteria before they may be consented.

c. Vulnerable Populations

This study will include vulnerable populations as a necessary part of the research. In particular, children will be included because PKAN disproportionately affects children. Those with classic PKAN are affected in the first few years of life and tend to have more severe and rapidly-progressive disease, and it is this sub-population in whom dietary management is ultimately expected to have the greatest impact.

The project may also include decisionally-impaired adults. PKAN does not cause severe cognitive impairment, but some adults with so-called 'atypical' PKAN as well as 'classic' patients surviving to adulthood may have limited decisional impairment that may be slowly progressive (as defined by the National Bioethics Advisory Commission – see 'Decisionally Impaired Adults Supplement' in eIRB). In our experience, most adult PKAN patients have a health care proxy in place (usually a parent, occasionally a spouse or sibling) and would expect and seek this person's involvement in the consent process. The inclusion of PKAN adults with limited decisional impairment is considered essential for understanding differences between the atypical and classic disease populations in biochemical and clinical safety response to the study product.

d. Setting

This single-site study will be conducted as a remote clinical trial. The burden for clinical trial participation is especially high for families affected by orphan diseases: the study participant, usually accompanied by a parent or other caregiver, must travel to a study site that is often a great distance from their home. The travel often takes a physical and psychological toll. Our experience with PKAN patients in particular suggests that the stress of travel may significantly exacerbate symptoms, especially in those with more advanced disease, who may suffer dystonic crises, sometimes requiring hospitalization, following travel. Thus, travel not only increases the risk of harm in a clinical trial for this population, but also may skew the clinical assessments and thus confound the research results.

Therefore, this study will be conducted using a novel, home-based approach that will minimize the risks of travel to participants, reduce the burden to families, and contain the costs of trial execution. Study assessments (mainly adverse event evaluation) will be conducted via scheduled telephone or OHSU approved teleconferencing service. Blood draw kits for safety and biomarker labs along with return mailers will be sent to participants and arrangements made with a local lab or provider for blood draw. Participant height and weight will be obtained at set intervals throughout the study for dose calculation. Study product will be shipped to participants and left-over product returned by the participant via pre-paid mailer for product accountability / compliance assessment. With participants' consent, local clinical providers will be notified of their patient's participation in the study and provided contact information for the trial investigators, whom they will be encouraged to contact with any concerns at any point in the study.

While this long-distance approach is unconventional and would not be appropriate for a high-risk drug intervention, it is justified in this study because of the low toxic potential posed by 4'-PPT, a normal metabolic constituent of the body being used at doses intended to mimic physiologic levels. The patient and family community have enthusiastically endorsed the trial design and plan for study conduct.

e. Recruitment Methods

OHSU is the leading center in the world for research and clinical care for PKAN and other genetic disorders of brain iron accumulation. Most participants in this trial will be recruited from those in our established PKAN research registry / data and specimen repository (eIRB 7232) who have consented to be contacted for future research opportunities. Most registrants are also active participants in our PKAN natural history study (eIRB 10832) and many are known to us clinically. The clinical trial will be posted on the clinicaltrials.gov website. We will also develop informational and trial recruitment materials for dissemination by the patient advocacy organization and via our own website NBIAcure.org prior to study launch: these materials will be submitted for IRB approval in a future modification to this protocol.

f. Consent Process / Modifications to the Consent Process

Most participants will be known to us because they will be already enrolled in the PKAN natural history study eIRB 10832; in these cases, we will have a signed consent and HIPAA authorization for that study with complete medical record / genetic / imaging / medication data already on file to determine eligibility. Patients contacting us via the NBIAcure website, via clinicaltrials.gov, via the family organization or other means will be screened by phone to determine eligibility using an IRB-approved phone script. When indicated, we will obtain a signed HIPAA authorization to obtain records outside

OHSU and review medical records, including confirmation of genetic diagnosis and, if needed, imaging studies, before enrollment. A Waiver of Authorization is requested to allow phone screening of these subjects and any subsequent communication by email or fax. Data from subjects failing screening will be retained until the end of the study, then destroyed.

The consent document will be provided to potential participants / families in advance of the consent process. The consent will be available as an online document that can be signed electronically, or as a conventional hard-copy document. The consent process for the study will be conducted by a clinically-trained designee and the PI, together or separately; the clinical Co-PI will be available as a backup if neither are available. For the majority of participants, the consent process will start by viewing a pre-consent video from home, then will be completed by phone or via OHSU-approved conferencing service, directly following the material in the approved consent document, which participants / families will be asked to have in front of them at the time of the telephone consent process. Following the consent discussion, the participant and the consenting researcher may sign the consent document electronically using a secure system for electronic / digital signature that provides an encrypted identifiable 'signature' which under OHRP guidance is considered an 'original' for the purposes of recordkeeping. Alternatively, the participant can sign a hard copy of the consent and fax or mail to the site, where the consenting researcher will sign, with a fully-executed copy then returned to the participant by mail. Participants being seen at OHSU for clinical care will be consented face-to-face with a hard copy of the consent which both parties will sign. Consent from a parent or legally authorized representative will be sought for any child younger than 15yo and for any adults or children 15-17yo with decisional impairment. Assent will be sought from children 7-14yo who are deemed to have the capacity to understand the research at an age-appropriate level, as well as from any older participants with limited decisional impairment. All participants will consent to genetic research.

Non-English-speaking participants may be enrolled; in these cases, a short form will be submitted to the IRB for approval prior to enrollment and OHSU or contracted translator services will be used for the initial consent and subsequent study visits.

8. Procedures Involved

The schedules of activities for the two cohorts are summarized in the tables on pages 23-26. Note that participants in this clinical trial are required to be concurrently enrolled in the PKANready natural history study (eIRB 10832); procedures for that study are described in that protocol and will not be described here.

a. Screening visit

Following the informed consent process and receipt of the signed consent and HIPAA authorization at the site, study staff will obtain participant demographics, medical history and concomitant medications via phone. A medical records release will be obtained from the participant if we do not already have one on file. PKAN diagnosis will be confirmed, obtaining corroborating clinical and imaging studies from records if genetic confirmation is lacking per inclusion criteria outlined. Once these data are in hand, the participant will be sent a kit for blood draw of safety labs and biomarker studies, with arrangements for the blood draw and measurement of height and weight made through a CLIA-approved laboratory or provider local to the participant's home (in most cases, this will be Quest Diagnostics with whom we have a national contract for this study). Safety lab results and height / weight results will be faxed to the study site by the laboratory, and the biomarker sample will be

shipped overnight to the site by the lab doing the blood draw using a pre-paid mailer. If all eligibility criteria are met, the participant will be randomized, using a randomization scheme managed by the study biostatistician and research pharmacist. The first period's study product, a dosing diary, blood draw kits, and pre-paid shipping materials for return of left-over study product will be sent to the participant, and an appointment made for a Baseline visit phone call.

b. Baseline visit

The Baseline visit will be conducted by phone. Concomitant medications, adverse events, study product dosing and administration, and instructions for the visit 1 and 2 blood draws will be reviewed with the participant. The timing of the first product administration will be mutually confirmed between the participant and the study staff; this will determine the study schedule going forward. Participants will be instructed in the logistics and importance of getting safety and biomarker labs drawn on schedule, completing the dosing diary, documenting the timing and nature of any adverse events, and returning left-over study product at the end of each dosing period as each new batch of product is received.

c. Follow-up visits

Follow-up visits will be conducted by phone, with the timing and activities as detailed in the Schedule of Activities table on p. 23-26. Study product, blood draw kits and pre-paid return shipping materials for return of study product will be sent to the participant at intervals throughout the study. The timing and frequency of study product shipments will be dictated not only by the study schedule but also likely by FDA feedback on stability data from the ongoing shelf-life studies. Participants will always receive more study product than needed to supply them to the next shipment date or study visit date to ensure there is no interruption in dosing due to shipment delays. Study staff will arrange for the blood draws and height / weight measurement with a CLIA-certified lab local to the participant's home as per the Schedule of Activities. Participants will return left-over study product to the study site using pre-paid shipping materials at the end of each dosing interval as new product is received.

d. End-of-study visit

The Primary Cohort participants will take their final dose of study product at Month 24 visit, ideally on the same day as the Month 24 scheduled blood draw for safety and biomarker labs. A final study visit will take place at Month 25 (+ 1 month window allowed for final follow up); at a minimum, this will include AE and Concomitant Medications assessment; safety and / or biomarker labs may be added at investigator discretion, for example if any safety lab value at the Month 24 visit shows clinically significant abnormalities or if a sample was compromised.

The duration of participation for the Open-label Cohort will not be fixed, but rather will vary based on when they enter the study in its overall timeline. This means that the timepoint of the final dose and end-of-study visits for the Open-label Cohort will also vary. Scheduling of the final two study visits for the Open-label Cohort will begin concurrently with those of the last-subject-last-visit for the Primary Cohort. Every effort will be made for the final on-product visit to coincide within window of a scheduled study visit, which will follow the assessment schedule of the Month 18 visit. A final study visit will take place up to two months after the final on-product visit; at a minimum, this will include AE and Concomitant Medications assessment; safety and / or biomarker labs may be added at investigator

discretion, for example if any safety lab value at the prior visit shows clinically-significant abnormalities or if a sample was compromised.

e. Suspension of Study Product / Early termination

Because this clinical trial is being conducted remotely, every effort will be made to ensure that participants complete at least the scheduled safety labs and telephone visits within the study visit windows. A participant-specific schedule of visit dates will be generated at enrollment, and study staff will send visit reminders via phone calls or emails in advance of scheduled blood draws. Per-participant compliance will be closely tracked: participants will not be allowed to complete more than 3 required visits more than 7 days out of window over the life of the study, with a 'required' visit defined as one involving safety lab blood draw and / or adverse event assessment. If compliance drops below this standard during the double-blind phase, the Primary Cohort participant will be terminated from the study, and required to be re-screened and re-randomized as a new participant in order to re-enroll in the study. If compliance drops below this standard during the open-label phase, the study product supply will be suspended but the Primary Cohort or Open-label Cohort participant will still be considered enrolled in the study and ongoing attempts will be made to have the participant complete assessments. If the participant subsequently succeeds in completing the most recent set of missed assessments, study product supply and dosing may be resumed. A repeat episode of non-compliance with the study schedule during the open-label phase will result in the participant's termination from the study. Exceptions may be made on a case-by-case basis, with the input of the medical monitor, DSMB and IRB as needed.

If the participant elects to withdraw from the study prematurely, or if the investigator and / or the participant's medical providers feel it is in the participant's best interests to withdraw from the study early, a Primary Cohort participant will be asked to complete the Month 24 and Month 25 activities and an Open Label participant will be asked to complete the Month 18 and Month 19 activities, if possible. The reason for premature withdrawal will be recorded. Every effort will be made to ensure any remaining study product is returned to the study site.

f. Unscheduled visits

An unscheduled visit may be conducted at investigators' discretion at any point in the study in response to an adverse event or other clinically-significant event. Such a visit will always include assessment of adverse events and concomitant medications; safety labs and biomarker labs may be added at the investigator's discretion.

Schedule of Activities for Primary Cohort Participants

Procedures Primary Cohort	Screening[#] Month -2	Baseline[#] Month 0	Visit 1 Month 0 + 3 days +/- 2d	Visit 2 Month 1 +/- 5d	Visit 3 Month 3 +/- 5d	Visit 4 Month 6 -10 to 0d	Visit 5 Month 6 + 3 days +/- 2d	Visit 6 Month 7 +/- 5d	Visit 7 Month 9 +/- 5d	Visit 8 Month 12 +/- 5d	Visit 9 Month 18 +/- 5d	Visit 10 Month 24 +/- 5d	Visit 11 Month 25 + 1 month/- 5d
Informed Consent	X												
Demographics	X												
Medical history / Confirmation of PKAN diagnosis	X												
Randomization	X												
Complete metabolic profile[A,B]	X			X	X	X		X	X	X	X	X	
CBC with differential [B]	X			X	X	X		X	X	X	X	X	
Biomarker labs [B]	X		X	X		X	X	X		X		X	
Height / Weight [C]	X					X				X	X	X	
Study product reconciliation				X	X	X		X	X	X	X	X	
Concomitant Medications Log	X	X		X	X	X		X	X	X	X	X	X
Evaluate for Adverse Events		X		X	X	X		X	X	X	X	X	X

[A] = Standard CMP includes Na^+, K^+, Cl^-, CO2, BUN, Creat, Ca^{2+}, Glu, Alb, T. Protein, Alk Phos, T. Bili, AST, ALT. CK may be ordered separately.

[B]= If a technical error occurs drawing, running, or shipping a lab, it may need to be repeated. Minor variations in safety lab components allowable at PI discretion.

[C]= Height and weight will be measured more frequently at the PI's discretion for children based on age and apparent growth rate

An unscheduled visit may be conducted at investigators' discretion at any point in the study in response to AE or other clinically-significant event. Such a visit will include AE assessment and concomitant medications assessment. Safety labs and biomarker labs may be added at investigator discretion. For subjects who are hospitalized, were recently discharged, or are too sick to leave home for safety labs, it is acceptable to use clinical labs collected up to 14 days before or after the study window.

Schedule of Activities for Participants Enrolling Directly into Open-Label Arm (Rolling enrollment; therefore, actual SoA completed by a given participant will vary depending on timing of enrollment in overall timeline of the study)

Procedures Open-label Cohort	**Screening[#]** **Month -2**	**Baseline[#]** **Month 0**	**Visit 1** **Month 0 + 3 days +/- 2d**	**Visit 2** **Month 1 +/- 5d**	**Visit 3** **Month 3 +/- 5d**	**Visit 4** **Month 6 +/- 5d**	**Visit 5** **Month 12 +/- 5d**	**Visit 6** **Month 18 +/- 5d**	**Visit 7** **Month 19 + 1 month/- 5d**
Informed Consent	X								
Demographics	X								
Medical history / Confirmation of PKAN diagnosis	X								
Complete metabolic profile[A, B]	X			X	X	X	X	X	
CBC with differential[B]	X			X	X	X	X	X	
Biomarker labs[B]	X		X	X		X	X	X	
Height / Weight[C]	X					X	X	X	
Study product reconciliation				X	X	X	X	X	
Concomitant Medications Log	X	X		X	X	X	X	X	X
Evaluate for Adverse Events		X		X	X	X	X	X	X

[#]Screening procedures may be completed anytime up to 60 days before Baseline; OR Screening and Baseline may be combined into single visit if participant is seen on site at OHSU and all eligibility criteria are met.

[A] = Standard CMP includes Na^+, K^+, Cl^-, CO2, BUN, Creat, Ca^{2+}, Glu, Alb, T. Protein, Alk Phos, T. Bili, AST, ALT. CK may be ordered separately.

[B]= If a technical error occurs drawing, running or shipping a lab, it may need to be repeated. Minor variations in safety lab components allowable at PI discretion.

[C]= Height and weight will be measured more frequently at the PI's discretion for children based on age and apparent growth rate

An unscheduled visit may be conducted at investigators' discretion at any point in the study in response to AE or other clinically-significant event. Such a visit will include AE assessment and concomitant medications assessment. Safety labs and biomarker labs may be added at investigator discretion. For subjects who are hospitalized, were recently discharged, or are too sick to leave home for safety labs, it is acceptable to use clinical labs collected up to 14 days before or after the study window.

9. Data and Specimens

a. Handling of Data and Specimens

Data sources for this project include medical record and imaging data to determine eligibility (including demographics, medical history and concomitant medications); data gathered during telephone visits (including adverse events and concomitant medications); study product dosing diaries; and clinical and research biosample data.

Clinical safety lab biosamples will labeled and results may be stored by the local CLIA-approved lab responsible for drawing and running the clinical safety labs, in accordance with their own internal standard clinical testing protocol. (In most cases, the CLIA-certified lab will be Quest Diagnostics with whom we have a national contract for this study). Clinical safety lab results will be faxed by the CLIA-approved lab to a secure OHSU fax machine located within the study team's badge-access only office suite. Faxed paper copies of the lab results will be stored in the participant's study binder in the same office suite. Research biomarker samples will be shipped by the local CLIA-approved lab drawing the sample to the study site using an overnight commercial courier such as FedEx and stored in the Hayflick lab freezer. Study staff will be responsible for receipt of all specimens and data, whether by courier, fax, or other means. Study data will be entered by study study staff into a 21 CFR Part 11-compliant REDCap Cloud database, a web-hosted application hosted by nPhase (located in Encinitas, CA, an approved system that has been reviewed by OHSU security). Additionally, summary electronic files of safety lab data will be downloaded from Quest Diagnostics's secure portal periodically onto a secure OHSU server as a CSV or Excel file for upload to our REDCap Cloud database. Data and specimens will be stored at the site indefinitely.

b. Sharing of Results with Subjects

Upon request, safety lab results performed by CLIA-certified labs may be shared with participants, and with their explicit consent, also with their medical providers. In particular, if a safety lab yields an abnormal or concerning result, this will be shared with the subject and, upon subject request, their provider. No other participant-specific results will be shared. All research results are at an early stage and individual results are not expected to yield any information significant to subject's health.

c. Data and Specimen Banking

All data and any remaining biosamples will be stored in a separate stand-alone repository created in association with this study. Data and samples will be stored indefinitely, and may be used for future research, including genetic research. The repository protocol (eIRB 19422) describes the details of storage and access.

10. Data Analysis

a. Sample size calculation

Because PKAN is an ultra-rare disease, we are limited to 64 participants (15 in each arm + 4 potential dropouts allowing for 5% loss to follow-up) whom we will recruit from our established research registry, (prioritizing those participating in our "PKANready" ongoing natural history study) as well as by methods described within the protocol. This will be designated the "Primary Cohort". When our double-blind enrollment goal is met, enrollment of up to 86 additional participants will shift to an open-label arm; this will be designated the "Open-label Cohort". Enrollment into this cohort will be rolling, and thus the duration of follow up will vary based on the point of entry in the overall life of the

study. Our primary objective is to estimate effects of the study product and we will prioritize effect sizes over statistical power in interpreting results. Still, we wish to include sufficient numbers to estimate effects in our secondary and exploratory analyses of CoASY mRNA expression levels with some precision. In preliminary cross-sectional data, we found that the mean CoASY delta Ct (dCt) measures were 1.4 standard deviations higher in classic PKAN children (n=8) than age-matched healthy controls (n=6) on the $\log_2$ scale, where higher dCt indicates lower CoASY activity (mean=17.39 vs 16.85, SD=0.39). We anticipate that participants at all three dose levels will resemble healthy controls after 3±2 days, while the placebo group will show no change. We simulated this change from baseline for 12 participants per group. For the standard deviation, we performed one set of simulations using the upper limit of the 60% CI for the standard deviation (0.34-0.48) as a conservative estimate. We regressed the dCt value on four indicator variables for study arm after treatment, with the intercept representing the common mean baseline value, and included a random intercept to induce correlation between paired measurements. We then used a Wald test for $\beta_{placebo1} - (\beta_{low1} + \beta_{mid1} + \beta_{high1})/3 = 0$ at α=0.05. Pooling over 1000 simulated datasets, we find that we reject the null in 85-95% of simulations, approximating power to detect this difference at one month between the active and placebo groups.

b. Statistical plan for primary and secondary aims

Safety Analysis

Comparisons of participants experiencing treatment-emergent adverse events and abnormalities on clinical labs will be reviewed for clinical significance in the Safety Analysis dataset, which includes all participants in the primary cohort who consume at least one dose of the study product. Events expected as part of the usual disease course in PKAN, whether present at trial enrollment or not, will not be considered or captured as adverse events.

Frequencies of participants experiencing at least one adverse event (AE) will be displayed by body system and preferred term according to *Medical Dictionary for Regulatory Activities* (MedDRA) terminology. Detailed information collected for each AE will include a description of the event; duration; whether the AE meets criteria as a Serious Adverse Event (SAE); severity / grade of the event; relationship to study product; changes made to the study product in response to the event; and clinical outcome. Severity of the AEs will be graded according to the NCI *Common Terminology Criteria for Adverse Events*, Version 4.0[15]. If the event meets criteria as an SAE, the nature of the SAE will be classified according to standard FDA criteria.

The number and percentage of patients who experienced any serious AE (SAE), treatment-emergent AE, and treatment-emergent SAE will be summarized, as well as the total number of events per arm. AE data will be presented for months 0-6, 7-25 and cumulatively, as appropriate. Emphasis in the analyses will be placed on AEs classified as treatment-emergent.

Clinically-significant abnormalities on clinical labs will be tabulated similarly to AEs. The number and percentage of patients experiencing clinically-significant abnormalities will be compiled in summary tables to examine the distribution by collection month [0-1-3-6-7-9-12-18-24 +/-25 months for the Primary Cohort; and 0-1-3-6-9-12-18 +/- 19 for the Open-label Cohort].

Graphic displays may be provided to illustrate the results over time. Initial review of group differences and determination of clinical significance will be performed by an unaffiliated physician familiar with the study population and blinded to treatment arm.

Tolerability Analysis

We will compare the number of participants retained in each arm at each of the follow-up time points [0-(0+1week)-1-3-6-(6M+1week)-7-9-12-18-24-25 months for the Primary Cohort; and 0-(0+1week) -1-3-6-9-12-18-19 for the Open-label Cohort], taking into account whether dropout seems plausibly related to study participation and the mean percent of study product consumed. High retention in the study and product consumption per-protocol will be considered evidence of tolerability.

Analysis of secondary endpoints

We will estimate a CoASY pharmacodynamic profile in response to different doses of the study product in the Per-Protocol dataset. CoASY mRNA expression levels are continuous, measured in normalized differences in cycle times (ΔCt) on the $\log_2$ scale. Lower ΔCt indicates higher expression levels. We will use regression models controlling for PKAN type to estimate mean levels by treatment arms, including mixed effects when modeling over multiple time points.

To estimate the **change in expression levels after 3±2 days of treatment compared to placebo**, we will use a mixed effects regression model of the form $CoASY_{ijt} = \beta_0 + \beta_{jt} I(group = j) I(time = t \; and \; t > 0) + \alpha I(cPKAN) + b_i$, j = placebo, low, mid, high; t = 1. Each individual contributes two observations (baseline and after); b_i represents the random intercept for subject. Baseline, represented by β_0, is constrained to be the same for all groups because of randomization. Our primary approach will treat time as binary (0=baseline, 1=treated), though this can later be expanded to capture differences beween days 1-5 of treatment. The β_{jt} represent mean changes from baseline for the three dose groups and placebo. An indicator for PKAN type (cPKAN or aPKAN) is included to reflect its role in stratified randomization. We will review regression coefficients as estimates of the magnitude of effect and test $\beta_{placebo1} - (\beta_{low1} + \beta_{mid1} + \beta_{high1})/3 = 0$ with a two-sided alpha of 0.1 to determine whether CoASY mRNA expression levels rose in all the study product-treated participants after initiation of treatment compared to placebo.

Similarly, we will check for **treatment response when the placebo group initiates treatment after month 6** by recoding their observations to use month 6 as 0 (baseline). We will use the same regression model but expect $\beta_{placebo1}$ to differ from zero. Further, because PKAN types may differ in response, we will test for an **interaction effect between PKAN type and treatment** averaged over dose levels as $CoASY_{ijt} = \beta_0 + \beta_1 I(aPKAN \; type) + \beta_2 I(time = post) + \beta_{12} I(aPKAN) I(post) + b_i$, where indicator variables represent active vs placebo, atypical PKAN vs classic, and β_{12} is the coefficient for their interaction. 50% of participants are expected to have aPKAN.

At six months, we expect CoASY mRNA expression levels to have decreased somewhat, and that **the group with the highest dose will show the lowest expression**. In addition to repeating the comparisons outlined for initation of treatment above, we will test $\beta_{low1} > \beta_{mid1} > \beta_{high1} > \beta_{placebo1}$.

As a final step, we will develop a longitudinal model to characterize CoASY mRNA expression profiles over the entire 24-month study period. We plan to use a mixed effects model with random intercept and slope to account for correlation between measurements and to allow individuals to vary from the group mean. We will explore a number of possible parameterizations of the time effect, including fractional polynomials and splines. We will allow for different trajectories by (a) dose group and (b) PKAN type in separate models with a group x time interaction. This model will take the general form $\boldsymbol{CoASY_{ij} = \beta_0 + \beta_1 t_{ij} + \beta_2' group + \beta_3 t_{ij} \times group + b_{1i} + b_{2i} t_{ij}}$, where t_{ij} represents time on treatment in months (including partial months as decimals) and group indicates dose group or PKAN type, depending on the model.

c. Statistical plan for exploratory aim

Sample Size and Statistical Power Issues

We acknowledge that our sample sizes will be small given the low incidence of PKAN in the general population. Thus, our focus will be on estimating the magnitude of effects to capture disease progression and reaction to intervention. We will explore correction techniques for small sample size, such as bootstrapping, shown to work with similar models with small sample sizes (e.g., N = 33), as well as Bayesian analyses.We will handle missing data with full information maximum likelihood estimation, which will lessen the risk of bias and loss of power due to missing data.

Data analysis

Our clinical experience with PKAN has informed our approach. We expect to observe differences from one subject to another in their initial functional status and in the rate of change in functional status after intervention. Furthermore, we know that children with PKAN will first gain and then lose function over time. The use of LGC models allows for both variable-centered and subject-centered elements in the analysis. This is critical given our prediction that subjects will demonstrate different patterns of disease progression and may have individual differences in responding to treatment. Our modeling strategy does not rely on an assumption of uniformity in progression rate or treatment effects and allows for flexible multivariate growth curve models to be constructed, allowing us to capture subtleties in disease progression and influences (e.g., a disease-modifying intervention) on it that might be masked by traditional analyses. This approach will allow us to examine change at the group level while modeling individual differences in initial functional status and change in function over time. We will be able to examine trajectories of motor, cognitive, social, language and adaptive functioning to track developmental milestone attainment and loss and determine if these are linear or non-linear. We will be able to determine whether a putative disease-modifying agent alters the natural history of the disease. In addition, we will assess the sensitivity of individual measures (e.g., upper extremity function score) compared to a combination of measures (e.g., global mobility score) to detect change. This will inform refinement of models and measures to

capture accurately fast vs slow disease progression monitoring and responses to intervention levels.

Constructing the model

We will use the Mplus software (v.8) to estimate LGCs including robust standard errors and corrected chi-square values to handle non-normality and missing data as needed. The model parameters of interest will be the overall level of skill or ability (i.e., intercept) and amount of change (i.e., slope) for the domain assessed. The basic model, a starting point, is defined as:
$\mathbf{Y_{it}} = \boldsymbol{\alpha}_{\mathbf{yi}} + \boldsymbol{\beta}_{\mathbf{yi}}\boldsymbol{\lambda}_{\mathbf{t}} + \boldsymbol{\varepsilon}_{\mathbf{it}}$,
where $\mathbf{Y_{it}}$ is assumed to represent the repeated measures (motor domain), where i stands for a person, t represents the time-ordered measurements, $\boldsymbol{\alpha}_{\mathbf{yi}}$ is defined as the initial functioning status on a given domain for a person's trajectory and $\boldsymbol{\beta}_{\mathbf{yi}}$ is the slope representing change. In addition, $\boldsymbol{\lambda}_{\mathbf{t}}$ represents the measured time points and $\boldsymbol{\varepsilon}_{\mathbf{it}}$ represents the residual for each person. This base model allows one to develop more complex models of linear or non-linear trajectories, including covariates (e.g., age, mutation, intervention). Several indices will be used to evaluate model fit, including indices such as: chi-square (χ^2), Comparative Fit Index (CFI), and the Standardized Root Mean Squared Residual (SRMR). For example, a CFI value greater than or equal to 0.95 with a SRMR value at or below 0.05 indicate excellent fit.

Three broad steps will define model building and trimming. First, trajectories of the domains of interest will be examined in unconditional LGCs. We will explore both linear and non-linear models to determine what best captures change and compare these models statistically for best fit. Next, we will combine the single models into a multivariate unconditional model across domains where the parameter estimates are obtained controlling for other variables in the model. For example, the estimates of baseline status of cognitive functioning in the unconditional multivariate model will control for the baseline levels of the motor functioning. This will allow for the correlation between the initial levels of functioning and change in these in functioning to be estimated, enabling us to understand the shape and level of change. Finally, we will add covariates (e.g., intervention group) to models. It is the conditional multivariate LGC where the influence of - or differences due to - these covariates on initial status and rates of disease progress can be estimated. We will also use recent work in this area to enable re-parameterization of such models to make the results more meaningful in terms of the parameter estimates reflecting quantities of interest to daily functioning. This modeling process will allow us to trace the disease progression over various patient functioning variables, the influence of the intervention of the change trajectory, and begin to understand how disease progression of PKAN occurs at the aggregate and individual level. The product of this effort will be a robust model and parameter estimates of the natural history of PKAN with key outcomes that can inform the variability in the intervention effects while controlling for other variables. Model estimates will be essential for the planning of larger scale studies of compounds with putative disease-modification effects such as 4'-PPT.

d. Other analyses

Adherence and retention analyses

Adherence will be defined as the proportion of product consumed in each study time period, averaged over all periods with weighting to compensate for uneven spacing of collection points.

Subjects will be instructed to return all unused study product to the site at the end of each study time period, with pre-paid mailers provided along with blood draw supplies and the next time period's study product supply. Adherence to the study product regimen will be assessed by calculating actual product consumed / expected product consumption. In the event that the unused portion is lost, the study team will ask the participant to approximate. We will compare means and standard deviations among the four study arms during the first six months and among the three dose groups in the Primary Cohort in months 7-36.

Retention will be tabulated as the number participating at the end of each month from baseline to month 37 (including the final follow up visit), grouped by study arm and annotated with reason(s) for study exit. Exit may occur if the participant is lost to follow up or indicates that s/he is no longer willing or able to participate.

Baseline descriptive statistics

Intervention groups (4 groups of 15 participants each) and the direct to open-enrollment group will be compared on baseline characteristics, including PKAN type, age, gender, laboratory measurements, and CoASY mRNA expression levels using descriptive statistics. Inferential statistics will not be used.

Additional sub-group analyses

Age will be evaluated as a covariate in secondary analyses.

Tabulation of individual response data

Safety and tolerability events will be listed for individual participants by measure and time point. Individual CoASY expression profiles over time will be plotted for visual inspection.

e. Procedures to minimize bias

Measures to minimize bias

Randomization/masking procedures

Study statisticians will prepare randomization lists stratified by PKAN type and deliver these to the research pharmacy. The study team will remain blinded to treatment assignment throughout the study. The Independent Medical Monitor can instruct the Research Pharmacy to break the blind for a given subject if deemed medically necessary, or under conditions outlined in the DSMP. The research pharmacy will distribute the randomization scheme for data analysis purposes following standard procedures.

Evaluation of the success of blinding

We will assess the success of blinding in at six months, before unblinding, by asking participants or their proxies to which treatment allocation they think they were assigned. Possible responses will fall in five categories of "Strongly believe the treatment is active", "Somewhat believe the treatment is active", "Somewhat believe the treatment is placebo", "Strongly believe the treatment is placebo" and "Don't know." We will calculate and compare blinding

indices (BI) in each arm using the method of Bang et al. We will not use statistical inference testing because of the limited size of the study.

11. Privacy, Confidentiality, and Data Security

As noted, most participants will be recruited from those in our established repository (eIRB 7232) and our PKAN natural history study (eIRB 10832). Only participants who have already provided consent to be re-contacted about future research opportunities will be contacted. Other potential subjects may contact our team after seeing an IRB-approved post about the study on our website, via clinicaltrials.gov, or other means. When any potential participants are reached by phone, if they feel they do not have sufficient privacy, study staff will offer to call them back at a better time. Study staff will not leave any sensitive information in messages left on voicemail.

Consent phone calls will be pre-arranged at a designated time when potential participants can ensure their privacy. Following the consent discussion, a secure link to the online consent form in REDCap Cloud will be sent via the OHSU email system. Rarely, a paper consent form will instead be mailed for signatures with a return envelope. Participants being seen at OHSU for clinical care will be consented face-to-face in a private exam room at OHSU.

All study visits will be done remotely by phone. Again, if a participant feels they do not have sufficient privacy, study staff can offer to call them back at a later time that day. Safety labs and research blood samples will be collected by a designated lab local to each subject. In most cases, samples will be collected at Quest clinical draw stations that meet standard privacy requirements. A small number of participants in more remote locations may have blood collected at their local physician's clinical offices, which should provide a similar level of privacy.

Blood samples sent to OHSU for research biomarker testing will be received by study staff and re-labeled with a code number that does not contain any part of the 18 HIPAA identifiers. Study staff will generate a key to the codes that will be maintained by the study coordinator. This key will be restricted to the PI and study staff and will be kept secure on a restricted OHSU network drive in a limited access folder. Hayflick Lab staff will only have access to subject code numbers and will not be able to link back to names or other identifiers. Samples will be stored in the Hayflick lab freezer. The lab requires badge-access and is only accessible to those with permission. Samples will be stored until the study is complete. At that point, leftover samples will be transferred into the associated research repository established for this purpose and stored indefinitely.

Study data will be entered and stored electronically in a 21 CFR Part 11-compliant REDCap Cloud database, a web-hosted application hosted by nPhase (located in Encinitas, CA), an approved system that has been reviewed by OHSU security. Electronic files of safety lab data will be downloaded from Quest Diagnostics's secure portal periodically onto a secure OHSU server as a CSV or Excel file for upload to our REDCap Cloud database. Paper copies of safety labs and/or study product dose logs completed by subjects faxed or sent to the study site will be stored in a locked filing cabinet in an office that requires badge access. Upon receipt, standard institutional practices will be followed as described in the OHSU Information Security and Research Data Resource Guide to maintain the confidentiality and security of the data collected in this study. Study staff will be trained with regard to these procedures.

12. Provisions to Monitor the Data to Ensure the Safety of Subjects

See also separate Data Safety Monitoring Plan document. This study will have multiple levels of monitoring, including investigator-monitors, a medical safety monitor, and a full Data Safety Monitoring Board.

The neurologist PI (Dr. Penelope Hogarth) and pediatrician–medical geneticist Co-I (Dr. Susan Hayflick) who will act as real-time *investigator-monitors* in this clinical trial each have many years of experience conducting clinical studies in the vulnerable populations involved in the study. Their clinical and research careers have been dedicated to children and adults affected with inherited diseases. Dr. Cary Harding, a medical geneticist with extensive research and clinical experience in the management of children and adults with genetic and metabolic diseases, will serve as the *independent medical safety monitor* for this study. Dr. Harding will also serve as Chair of the Data Safety and Monitoring Board that will be convened prior to the start of the study, and which will meet on a scheduled and, if needed, *ad hoc* basis to review summary reports of the study progress and any adverse events that have occurred and will make formal recommendations based on these data as to whether to suspend, terminate or continue the research. The study team will follow OHSU's policy and procedures for inclusion of decisionally-impaired adults at: (http://www.ohsu.edu/xd/about/services/integrity/policies/upload/di.pdf).

The PI will review all adverse events in real-time, taking immediate action as needed in consultation with the clinical Co-I and Medical Safety Monitor. In addition, the PI will make a formal determination of seriousness and relatedness for each adverse event for data recording purposes and to meet reporting requirements, again consulting with the Medical Safety Monitor as needed.

13. Risks and Benefits

a. Risks to Subjects

Because it has not been studied in humans before, the study product poses unknown risks. However, our expectation is that toxicity will be very low for this high-purity compound that is identical to a naturally-occurring product of intermediary metabolism; which has shown no toxicity at all at high doses in mouse studies completed to date; and which is being dosed at "minimum effective" rather than the more usual "maximum tolerated" doses. The "minimum effective" dose derived from mouse studies turns out to be remarkably close to the molar equivalent of the recommended daily allowance (RDA) of the source vitamin pantothenate, which itself shows little toxicity even in doses far in excess of the RDA[9]. The dose to be used in this study is within usual dietary intake ranges of the parent vitamin, pantothenate.

All clinical procedures involved in this research (blood draws) are judged to pose minimal risk to even the youngest of participants. There also exists a small risk of loss of confidentiality of the data collected; these data may include sensitive genetic information.

b. Potential Benefits to Subjects

Although the aims of this study are to examine safety, tolerability, and pharmacodynamic response of a vitamin metabolite for PKAN, the nutritional management of the disease with the study product may afford clinical benefits for participants over the study participation period.

c. Importance of Knowledge to be Gained

If the study product is found to be safe and well-tolerated and exploratory pharmacodynamic measures provide "proof of concept" for its use for the nutritional management of PKAN, the product will offer an alternative to drugs in development that may carry higher risks of toxicity and be less palatable. While we of course hope that the proposed exploratory analysis will demonstrate a beneficial effect on disease progression, we suspect that this may require that the food be initiated early in disease, before substantial neurodegeneration has already taken place.

We hope that our novel remote trial design, if successfully executed, will provide the impetus to consider a similar approach for future studies in this and other disease populations, especially where the interventions are low risk and the barriers to research participation and disease burden are high.

Any drug alternative is likely to be much more expensive to patients, families and insurance companies than this medical food, which is being developed by sister non-profit organizations in the US and Netherlands committed to keeping the cost as low as possible to enable universal access to all families regardless of health insurance coverage or financial resources. We hope this non-profit approach will serve as a model for therapeutics development in other disorders and ultimately promote equitable access to potentially life-saving interventions.

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