APPROVAL

Principal Investigator: Lori-anne Schillaci

Title: The effectiveness of synthetic BH4 (saproterin dihydrochloride or “Kuvan”) in Amish PKU patients
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Type of Review: Full

The IRB reviewed this submission.

 Per Federal regulation, changes MAY NOT be made to any element of the current research without prior IRB approval, except to eliminate an immediate and apparent hazard to subjects enrolled in the study. Per Federal regulation, the research may not continue beyond the Approval End date. You must submit a continuing review form 6-8 weeks before this Approval End date in order to maintain IRB approval. Failure to maintain IRB approval is human subjects non-compliance. Please note that even if your study falls into a category that does not require an Approval End date, the institution may require a yearly “checkin” to confirm the status of the study.

***Approval by the IRB does NOT mean that you have permission to start your study. Prior to starting your study, you may be required to obtain (1) a coverage analysis for studies that involve patient care, regardless of source of funding, and/or (2) a contract with the Sponsor of your study or an agreement with any third-party collaborator that

The UH IRB operates under HHS Federalwide Assurance (FWA) number 00003937 and IRB registration numbers 00000684, 00001691 and 00008600. The CWRU IRB operates under DHHS FWA00004428 and IRB registration number 00000683.

PROPOSED STUDY: The effectiveness of high-dose synthetic BH4 (saproterin dihydrochloride or KuvanTm) in Amish PKU patients

Phenylketonuria (PKU) is caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH). PAH is responsible for the rate limiting step in phenylalanine catabolism. This reaction is dependent on tetrahydrobiopterin (BH4). BH4 is a catalytic cofactor for the enzyme PAH. Supplementation with synthetic BH4 (saproterin dihydrochloride or KuvanTm) has been shown to activate residual PAH activity and lower Phe levels in certain patients. It may also have a chaperone effect by binding abnormal PAH protein and decreasing its degradation. Saproterin is an approved oral drug developed to reduce blood Phenylalanine (Phe) levels and increase Phe tolerance in BH4 responsive patients with hyperphenylalaninemia. Data from BH4 loading tests indicate an incidence of BH4 responsiveness of >40% in the general PKU population and >80% in mild PKU patients (6). This therapy is most effective in patients with mild PAH deficiency because they are more likely to have some residual PAH activity. However, there have been a small number of patients with less then 1% residual PAH activity that have been identified as BH4 responsive (2,8).

Certain genotypes have been found to be good predictors of Saproterin responsiveness; however, these correlations are imperfect. Therefore the standard of care is to perform a trial of Saproterin therapy to assess responsiveness in every PAH patient regardless of genotype. Currently this recommendation does not include patients with two null mutations.

The purpose of our study is to determine if Amish patients with PKU show responsiveness after a high dose, prolonged Saproterin trial. Our population of interest has a high frequency of a specific splice site mutation, the 1066-11G>A mutation. This splice site mutation activates a cryptic splice site resulting in an in frame insertion of 9 nucleotides preceding exon 11. This leads to protein conformational changes and abrogation of function. Previous studies of this genotype have indicated <1% residual activity of the PAH enzyme and an insignificant responsiveness to Saproterin. However, in this specific study Phe levels were evaluated only over 24 hours after a single-dose BH4 challenge at the standard dose of 20mg/kg (2).

Recently, observation in several Amish patients suggests some promise that these patients may be responsive to Saproterin after a longer trial of the medication (Personal comment - Samuel Yang, MD). In addition, studies evaluating PAH enzyme activity in these patients, have found evidence that this genotype may need higher cofactor concentrations for optimal enzyme activity and therefore may require a higher dose of Saproterin then the currently recommended 20mg/kg (9). To date, no formal clinical trial has been done.

To answer the question of whether this genotype is responsive to Saproterin at higher doses, over a prolonged period, we propose a randomized clinical trial comparing blood Phe levels in patients treated

with standard-dose Saproterin to those treated with highdose Saproterin . Responsiveness (primary outcome) will be defined as a 20% reduction

in baseline Phe levels on a stable diet or a 20% increase in Phe tolerance while taking Saproterin. As a secondary outcome, executive functioning will be evaluated using the Behavior Rating Inventory of Executive Function (BRIEF), which was previously identified as part of the "uniform assessment method for PKU" (10). Appropriate versions of the BRIEF will be used for each age group. In addition to executive functioning, we will measure quality of life through the previously validated PKU-QOL survey (11).

The results of this study could reveal a new population of Saproterin responsive PKU patients. This could call into question other null mutations of the PAH gene and their responsiveness to Saproterin. This may lead to the investigation of genotypes previously thought to be unresponsive to Saproterin. Further, diet treatment alone has proven to be difficult for the Amish population for cultural and economic reasons and Saproterin may help improve Phe control in responsive patients.

Hypothesis: Based on new clinical information, we hypothesize that if given a prolonged trial of Saproterin at a higher dose, Amish patients with PKU, and more specifically those homozygous for the c.1066-11G>A mutation, will have a significant reduction in Phe levels or an increase in Phe tolerance and/or improvement in executive functioning and quality of life.

PROPOSED METHODS:

This study will include Amish patients from three local centers. All patients will be enrolled through University Hospitals Case Medical Center. Consent will be obtained in person at UHCMC. These centers include: - University Hospitals Case Western Reserve/Rainbow Babies and Children's Hospital/Center for Human Genetics - Akron Children's hospital in Akron, Ohio - DDC center for children with special needs, Middlefield, Ohio

IRB approval: University Hospitals Case Western Reserve IRB

Inclusion Criteria: - Current diagnosis of PKU with the following: - Age of at least 2 years or older - Baseline Phe level of > 360 umol/L - Willing to maintain a stable diet - Patient or guardian are willing and able to provide written, signed informed consent after the nature of the study has been explained and prior to any researchrelated procedures - Are willing to comply with all study procedures - Have any two identifiable mutations found on PAH gene sequencing OR have at least one copy of the splice site mutation of interest (c.1066-11G>A)

- Any patients already taking Saproterin (or have taken in the past), must have a treatment end date at least 14 days prior to Day 1 of the study.

Exclusion Criteria: - Any patient currently taking Saproterin who has taken the medication at any point in the 14 days prior to Day 1 of the study - Under 2 years of age - Unwilling to maintain a stable diet - Patients with baseline Phe levels < 360 umol/L - Patients unable to comply with all study procedures - Patients unable to provide written, signed informed consent

Recruitment:

Will be performed through the following ways:

 Amish patients will be identified through their primary metabolic physicians/genetic counselors at three different sites – University Hospitals, DDC clinic in Middlefield, Ohio and Akron Children's hospital. Despite, recruiting patients from the three centers above, throughout the study, all patients will be seen at University Hospitals for study visits. UH will be the only clinic site involved in this study. The study team will only have access to electronic records from the UH site, for patients of people on the study team. Letters will be mailed to each patient thought to meet inclusion criteria Follow-up phone calls will be made to these patients Patients will be recruited in-person by their primary metabolic physicians or genetic counselors as they are seen in each of the above clinics for follow-up

Informed consent process:

The majority of patients will be transported to University Hospitals Center for Human Genetics to discuss the study and review the informed consent. For patients that are our regular clinic patients, we will see them following a scheduled clinical visit to discuss the study and review the informed consent. At the request of the patient, we will arrange to travel to their community clinic or home to discuss the study and review the informed consent. Informed consent for patients will consist of: 1. The research project goals, risks and benefits will be explained to each patient in person by either myself (Dr. Lori-Anne Schillaci) or Dr. Shawn McCandless. 2. Written informed consent will be obtained by either myself (Dr. Lori-Anne Schillaci) or Dr. Shawn McCandless from any patient wishing to participate, prior to the initial blood draw is performed. For minors, written informed consent will be obtained from one parent.

Study patients will present to their preferred clinic for 5 office visits. - Visit at beginning of part 1 (initial visit) - Visit at beginning of part 2 - Visit at conclusion of part 2 - Visit at the beginning of part 3 - Visit at the conclusion of part 3

Primary Outcome: - Reduction of baseline Phe levels by 20% OR an increase in dietary Phe tolerance by 20%

Secondary Outcome: - Improved executive functioning and/or quality of life based on BRIEF and PKUQOL surveys as described above.

Initial testing/Part 1: length 4 weeks

Recruited patients will present to clinic for part 1 of the study. After informed consent is obtained, molecular genetic testing will be performed. Testing will include sequence analysis of the PAH gene. This testing is necessary to identify the specific genotypes present in each patient. We expect the majority of this population to be homozygous or heterozygous for the above described splice site mutation. Any patients found not to meet inclusion criteria after PAH gene sequencing will not be able to continue in the study past part I. Genotypes will be grouped and analyzed separately at the conclusion of the study. Patients who do not wish to undergo genetic testing will not be included in the study.

Each patient will also have an initial blood Phe level drawn in the form of a dried blood spot on filter paper card that will be supplied by the testing company. All Phe testing will be performed in a CLIA certified laboratory at The Clinic for Special Children in Strasburg, PA.

Baseline assessment tools (BRIEF, PKU-QOL) will also be performed at this initial visit. Patients will be given a unique study number by our investigational drug pharmacy and will begin part 1 of the study as described below.

In part 1 of the study, patients will not receive any medication. Each patient will be treated with diet alone. They will maintain a stable, Phe restricted diet (including formula) that is consistent with their diet at the time of enrollment. This will be monitored by food diaries kept for 3 days of each week. Specifically, patients will be expected to keep diaries for the 3 consecutive days leading up to blood draw. Based on these diaries, average weekly Phe intake and Phe tolerance will be calculated and recorded. Patients will also complete a food frequency tool, which will be reviewed at part 1, 2 and 3's initial visit. We will use all of the tools above, plus allow patients regular access to our dietician in order to encourage the maintenance of a stable diet.

Blood Phe concentration will be measured weekly (each Monday) by blood spot cards. Patients will not require weekly clinic visits to have Phe levels drawn. Blood spot cards will be performed at home and then mailed to the reference laboratory. In order to teach and enable patients to become comfortable performing their own blood spots at home, nursing staff (from the DDC clinic) will travel to each patient's house weekly for part 1 of the study (total of 3 home blood spots). For parts 2 and 3 of the study, those patients and families who are able, will perform their own blood spots at home. Those families who remain uncomfortable performing their own blood spots will continue to have their blood spots collected by home nursing.

Initial visit: - Venous blood draw for PAH gene sequencing - Baseline Phe level by dried blood spot card in office - Urine pregnancy testing in females of reproductive age - Executive functioning and quality of life assessment tools (BRIEF, PKU-QOL) given - Baseline diet information collected and recorded.

Average weekly Phe calculated, Phe tolerance calculated. - Food frequency tool completed and reviewed.

Blood Phe values and Phe tolerance from part 1 of the study (on diet treatment alone) will be compared to blood Phe values and Phe tolerance from parts 2 and 3, on low-dose and high-dose treatment.

Part 2: length - 4 weeks

Study numbers will be randomized into “standard-dose Saproterin” or “high-dose Saproterin” groups (treatment group A or treatment group B). The investigational drug pharmacy will provide study patients with their medication in kits, at the beginning of each treatment period. Goal high-dose Saproterin dosing will be 40mg/kg (rounded up to the nearest 500mg), provided in the form of pre-packaged 500mg packets of powder. Labeled dosing on these packets will be covered by the investigational drug pharmacy and unidentifiable to patients. Standard-dose Saproterin will be 20mg/kg (rounded to the nearest 100mg) provided in the form of 100mg tablets. Dosing of the tablets will be unidentifiable to patients. Medication will be given orally once daily.

Initial part 2 visit: after 4 week diet only period - Food diaries collected for the previous 4 weeks (3 per week). Average weekly Phe calculated, Phe tolerance calculated. - Food frequency tool completed and reviewed. - Baseline Phe level drawn by dried blood spot card in office - Urine pregnancy testing in females of reproductive age - Executive functioning and quality of life assessment tools (BRIEF, PKU-QOL) given - Provided with medication kits (high or standard-dose, 4 week supply)

Final part 2 visit: after 4 weeks of treatment - Food diaries collected for the previous 4 weeks (3 per week). Average weekly Phe calculated, Phe tolerance calculated. - Executive functioning and quality of life assessment tools (BRIEF, PKU-QOL) given - Empty medication bottles/packet kits collected from each patient to assess compliance - Review of any potential adverse events

This is the end of part 2. We will then have a 2 week “wash out period” where patients will receive no treatment. The length of this washout period was determined based on the mean elimination half-life of saproterin and previous clinical trials. The mean elimination half-life of saproterin ranges from 3.9 to 17 hours. In previous trials, washout periods have consisted of 7 and 30 days. No efficacy assessments have been performed to differentiate or support one washout period over the other (BioMarin Pharmaceuticals, Inc. Data on file). Based on the elimination range above, we determined that a two week washout period is sufficient. Patients should maintain their steady diet during this time.

Part 3: length – 4 weeks

The cross-over will occur between treatment groups: A B and B A.

Initial part 3 visit: after 2 week washout period - Food diaries collected for the previous 2 weeks (3 per week). Average weekly Phe calculated, Phe tolerance calculated. - Food frequency tool completed and reviewed. - Baseline Phe level performed by dried blood spot card in office - Urine pregnancy testing in females of reproductive age - Executive functioning and quality of life assessment tools (BRIEF, PKU-QOL) given - Provided with medication kits (high or standard-dose, 4 week supply) - Review of any potential adverse events

Final part 3 visit: after 4 weeks of treatment - Food diaries collected for the previous 4 weeks (3 per week) - Executive functioning and quality of life assessment tools (BRIEF, PKU-QOL) given - Empty medication bottles/ kits collected from each patient to assess compliance - Review of any potential adverse events

During all parts, patients will maintain a stable, Phe restricted diet including formula (same diet as in part 1 of the study). All Phe levels will be blinded and researchers will not have access to them until the conclusion of the study. At the conclusion of the study, after all data have been analyzed, a complete list of blood Phe levels obtained during the study (both on high and standard-dose treatment) will be sent to each patient's primary

metabolic physician. Physicians can then discuss long term Saproterin treatment with their patients based on their Phe levels.

The study will conclude after all parts are complete and groups A and B have both received a trial of high and standard-dose Saproterin. All medication will be discontinued at this time. The primary outcome evaluated will be Saproterin responsiveness based on blood Phe concentration and Phe tolerance. The secondary outcome evaluated will be changes in adaptive behavior, executive functioning and/or quality of life assessments. Improvement in these scores may be an indication for long term Saproterin treatment in these patients.

Safety Management Plan:

BioMarin Standard Language for Adverse Event Reporting: IST Program, Saproterin Adverse Events According to the ICH definition, an adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use

of a medicinal (investigational) product, whether or not considered related to the investigational product.

This definition includes intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions. Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

Adverse event information will be collected in an ongoing fashion through patient reporting AEs to their physician, health care provider or to the investigator. Seriousness and relatedness will be assessed by the investigator, with appropriate reporting. A designated primary contact person based at the treatment center will be responsible for the collection and reporting of AEs for patients participating in the program.

Serious Adverse Events A serious adverse event (SAE) is defined as any AE that: Results in death Is life threatening, that is, places the subject at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization. Admission of a subject to the hospital as an in-patient as a result of an AE, even if the subject is released on the same day, qualifies as hospitalization

 Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the subject's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance. Is a congenital anomaly or birth defect, an AE that occurs in the child or fetus of a subject exposed to the product prior to conception or during pregnancy Important medical event that does not meet any of the above criteria, but may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed above. More than one of the above criteria may apply to any specific event.

Pregnancy Pregnancy in a subject being treated with the product should be reported immediately (within 24 hours of becoming aware of the pregnancy) to BioMarin Pharmacovigilance by using the FDA 3500A (MedWatch Form). Every effort should be made to follow the patient through resolution of the pregnancy (termination or delivery) and report the resolution of the FDA 3500A (MedWatch Form) to BioMarin Pharmacovigilance.

SAE and Pregnancy Reporting

[In Investigator IND studies, SAPROTERIN ™ serious, related, unlabelled, (unexpected) adverse events will be reported to the FDA as required by 21 CFR 312.32 by the Investigator/Sponsor. These reports may be filed utilizing the Form FDA 3500A (MedWatch form). The Investigator/Sponsor will provide BioMarin Pharmacovigilance, with a copy of this report. All serious adverse events (SAEs) and pregnancy reports whether or not considered drug-related should be reported to BioMarin Pharmacovigilance (contact information below) within 24 hours of receipt by the investigator/sponsor by using the FDA 3500A (MedWatch Form). For Comparator Drugs / Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer]. Not pertinent to this study.

In IND-exempt studies, All serious adverse events (SAEs) and pregnancy reports whether or not considered drugrelated should be reported to BioMarin Pharmacovigilance within 24 hours of receipt by the investigator/sponsor by using the FDA 3500A (MedWatch Form). For Comparator Drugs / Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer. Clinicians should not wait to collect additional information that fully documents the event before notifying BioMarin's Pharmacovigilance Department of an SAE or pregnancy. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore it is important that clinicians submit additional information requested by BioMarin Pharmacovigilance as soon as it becomes available. Reporting of SAEs to the IRB will be done in compliance with the

standard operating procedures and policies of the IRB and with applicable regulatory requirements.

Contact information for BioMarin's Pharmacovigilance Department is as follows: BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 Phone: (415) 506-6179 Fax: (415) 532-3144 Email: drugsafety@bmrn.com

Product Complaints Reporting A product complaint ("Complaint") is any direct, written, electronic, or oral communication of dissatisfaction that alleges deficiencies related to the identity, quality, durability, labeling, purity, stability, appearance, effectiveness, safety, and/or design of a drug product after it is released for distribution. Complaints that simultaneously fall under Adverse Event definitions under this Protocol need only be reported via the Adverse Event reporting procedure set forth in this Protocol. Investigator or designee (Reporter) shall capture the following Complaint information as relates to Saproterin used under this Protocol: • Date complaint received • Product Name and Lot Number • Indicate if the product is available for return to BioMarin for investigation • Quantity Affected • Detailed Description of complaint • Study Protocol Number • Investigator Name • Site Contact • Site Number • Subject Number • Name and contact information of the person who is reporting the complaint as well as name and contact information of the complainant. The reporter will be contacted by BioMarin Product Complaint Quality Assurance Department. Investigator or designee (Reporter) will use his or her best efforts to report Complaints to BioMarin within five days of learning of the Complaint. Investigator or designee will submit the complaint information by email at

productcomplaint@bmrn.com or by fax at 415-523-1457. AE language approved 28 May 15, PC language approved 28 May 2015

Statistical Methods:

We will use student's T-test to compare the mean Phe values for diet treatment alone to both the low and high-dose treatment values. The goal is to determine if there is a significant difference between the groups. Similarly, we will compare calculated phe tolerance from each part of the study.

Power calculations were performed and the following was determined:

If the P value (type 1 error) is set at 0.05 and the power (type II error) is set at 80% and the minimal detectable difference between means is set at 200, then 15 patients would be required to find significance. Our enrollment goal is up to 25 patients to allow for dropouts and to increase the probability of having 15 patients homozygous for the common mutation.

Improvement in adaptive behavior/executive functioning and quality of life will be based on our chosen assessment tools. Scores from the surveys will be compared from each group (diet, high-dose and standard-dose).

Timeline for Project:

Regulatory Prep: ~5 months Enrollment: ~ 2 months Phase 1: 4 weeks Washout: 2 weeks Phase 2: 4 weeks Phase 3: 4 weeks: Analysis: 6 weeks Paper preparation: 8 weeks

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