

Project title: **Therapeutic Trial of EPI-743 in Patients with Disorders of Energy Utilization or Oxidation-Reduction**

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Protocol History: 2012 – SRC Review  
2013 – Annual Review  
2014 – Annual Review  
2015 – Triennial Review with SRC  
2016 – Annual Review  
2017 – Annual Review

Study type:

- Archived biological specimens/medical information
- Natural history; definition of phenotype, genotype/phenotype correlation
- Prospective gene identification, NOT providing information to participants
- Prospective linkage/gene identification; information provided to participants
- Social science; assessments of knowledge, attitudes and behavior
- Genetic counseling
- Drugs or devices
- Gene transfer
- Other interventions

Key Words:

- Disease: Undiagnosed Diseases, Oxidation/Reduction, Mitochondrial Disorders
- Population: 20 Children age 2-11 years; Male and Female
- Drugs: EPI-743 (IND #117,000; Holder: William A. Gahl)

Results routinely communicated to subject?  Yes (after CLIA certification)

Research participants to be seen at:  NIH only

### **1. Precis**

The clinical manifestations of disorders of energy metabolism and defects in oxidation/reduction are similar because the basic defect involves the inability to transfer electrons. The same is true for many mitochondrial diseases. Affected patients exhibit a wide variety of signs and symptoms, but the most frequent and earliest dysfunctions occur in the muscle and brain, where energy requirements are high. The diagnosis of this type of defect is problematic because of the nonspecific and protean clinical manifestations of these disorders. Treatment is equally challenging, since the exact locus of the primary defect generally remains enigmatic. As a consequence, physicians rely upon generic cocktails of vitamin co-factors or endogenous intermediates intended to enhance mitochondrial electron transport, diminish the damage of reactive oxygen species, and promote energy production. The field is such a morass that, in general, it calls for trial-and-error treatment based upon empiric data. Edison Pharmaceuticals, Inc, has developed an *in vitro* assay that utilizes patient fibroblasts to model the innate susceptibility to oxidative stress caused by the disorders of energy metabolism and oxidation/reduction. The assay system also determines if the cells respond with increased viability to an IND drug called EPI-743. We propose a clinical trial that enrolls 20 children who meet three criteria. First, they must have a disorder that, based upon studies performed in a clinical protocol such as 76-HG-0238 (“Diagnosis and Treatment of Patients with Inborn Errors of Metabolism and Other Genetic Disorders”), is consistent with a defect in energy metabolism or oxidation/reduction. Second, their cultured fibroblasts must exhibit a defect in the ability to withstand oxidant stress. Third, their fibroblasts must respond to EPI-743 *in vitro* by showing improved viability under conditions of oxidative stress. This protocol is a double-blind, placebo-controlled crossover study with 6-month periods of treatment and a two-month washout period. Patients are admitted to the NIH Clinical Center for 2-5 days every 3 months. The primary outcome measure is quality of life based upon the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) for ages 2-11 years; parts I-III are evaluated separately from part IV. Secondary outcome measures are tailored to each patient’s laboratory, imaging, and clinical abnormalities. Results while receiving EPI-743 will be compared to results while receiving placebo; both repeated measures analyses and Student’s t test will be employed. To date, 10 patients have been enrolled, and three are already in the second arm of the protocol. This study now includes an extension arm, through which all patients are offered open label EPI-743 after completing the crossover trial.

### **2. Objectives and Specific Aims**

This study has two objectives. First, it is intended to identify patients with rare or undiagnosed diseases whose fibroblasts in culture are susceptible to oxidant stress and are protected by EPI-743. Second, the protocol will determine the safety, tolerability, and efficacy of EPI-743 in 20 children whose *in vitro* studies suggest that they could benefit from this drug.

### **3. Brief Rationale and Background**

Individuals with extremely rare or undiagnosed diseases often find it difficult to receive appropriate diagnoses and treatments (1,2). A subset of patients with undiagnosed diseases has clinical findings that suggest defective energy utilization or an abnormal reduction/oxidation (redox) state within cells. These abnormalities are generally attributable to mitochondrial disorders (3), but they could also involve cytoplasmic defects. The diseases themselves are sometimes fatal in the first decade of life. Patients often present with significant neurological and/or muscular findings that can include growth failure, short stature, psychomotor retardation, abnormal respiration, nystagmus, ophthalmoparesis, ataxia, dystonia, optic atrophy, retinitis pigmentosa, hypotonia, myopathy, seizures, or parkinsonism. Laboratory findings can include elevated lactate and pyruvate levels in plasma and/or cerebrospinal fluid, abnormal mitochondrial morphology on muscle, nerve, or liver biopsy, and the presence of mutations on sequencing of mitochondrial genes or nuclear genes encoding mitochondrial proteins. In addition, muscle biopsy can reveal abnormal electron transport chain enzymology, changes in mtDNA copy number, or muscle-specific coenzyme Q deficiency. The diagnosis, clinical investigation, and therapy of this group of disorders are widely considered inadequate and fraught with false positives, false negatives, and huge levels of uncertainty. Rational therapies include the empiric use of supplements of coenzyme Q<sub>10</sub>, idebenone, dichloroacetate, B vitamins, L-carnitine, or a ketogenic diet (4-7). However, a beneficial response is unusual and unpredictable.

To address the problem of diagnosing extremely rare and enigmatic diseases, the NIH Undiagnosed Diseases Program (UDP) was established in 2008 (1,2). The NIH UDP has received over 10000 inquiries, reviewed over 3500 medical records, and admitted over 800 patients to the NIH Clinical Center. Approximately 25% of patients have achieved diagnoses, some of which are amenable to conventional treatments. Most UDP patients, however, never reach a diagnosis or therapy, and many of them exhibit clinical findings suggesting a defect in energy balance or in oxidation/reduction status.

To address the unmet need of treatment for such individuals, Edison Pharmaceuticals Inc., has developed a small molecule therapy (EPI-743) as well as an *in vitro* assay to ascertain which patients are likely to respond to that drug. The assay involves measuring cell viability under conditions of oxidative stress; fibroblasts from patients with abnormal mitochondria or improper redox states die at lower levels of exogenous injurant. Certain patient cell strains can be rescued from injurant-induced lethality by EPI-743. This quinone oxidation product of a vitamin E isomer, alpha-tocotrienol quinone, is a potent rescue agent for cultured cells derived from patients with inherited mitochondrial diseases. EPI-743 is a functional and structural equivalent to coenzyme Q<sub>10</sub>. Edison Pharmaceuticals Inc. currently sponsors IND #107401 for EPI-743 for the treatment of inherited mitochondrial respiratory chain diseases. The drug has not been approved by the FDA.

The cellular efficacy of EPI-743 was established in oxidatively stressed primary fibroblasts cultured from a patient with mutations in *SURF1* resulting in Leigh syndrome (8), a metabolic disease causing subacute necrotizing encephalomyelopathy (9). In this assay, the oxidative stress was induced by depletion of glutathione in the Leigh syndrome primary cell culture, resulting in loss of cell viability. The same level of oxidative stress did not

alter the viability of control fibroblasts from healthy individuals. Pre-treatment of Leigh syndrome cells with EPI-743 resulted in rescue of cellular viability, with an EC<sub>50</sub> of 21 nM. In contrast, the redox silent/locked analogue of EPI-743 (bis-pivoyl ester of EPI-743 dihydroquinone) did not rescue Leigh syndrome fibroblasts, supporting a redox-dependent mechanism of action for EPI-743. Lack of cellular efficacy of the redox silent analogue of EPI-743 was not due to cellular toxicity, since growth inhibition by the compounds was comparable in control patient fibroblasts.

EPI-743 showed similar potency (EC<sub>50</sub> = 18nM) in rescuing the fibroblasts of a patient with a severe CoQ<sub>10</sub> synthetic defect. This form of CoQ<sub>10</sub> deficiency is biochemically similar to Leigh syndrome (SURF1) because both conditions affect the function of the mitochondrial electron transport chain (ETC).

While the exact mechanism of action for EPI-743 has not yet been determined, it is believed that the redox property of the quinone in EPI-743 is integral to its pharmacology. EPI-743 biochemically alters the reduced glutathione pool by the catalytic redox transfer of NADPH reducing hydride equivalents between flavin-dependent oxidoreductases. Key enzymes involved in this EPI-743-mediated transfer are NQO1, which transfers reducing equivalents from NADPH to EPI-743, and in turn glutathione reductase and thioredoxin reductase, which accept the reducing equivalents from EPI-743. The net effect of EPI-743 is to act as an NADPH dependent cofactor to facilitate the repletion of cellular glutathione stores, which appear to be lower in mitochondrial disorders (10). In addition, the ratio of reduced to oxidized glutathione (GSH/GSSG) within tissues appears to reflect the level of oxidative stress (11).

In human studies, several patients with genetically confirmed Leigh syndrome with SURF1 mutation have been treated with EPI-743, and have shown cessation of disease progression and evidence of improvement in global motor function, cognitive function and quality of life as assessed retrospectively by the Newcastle Pediatric Mitochondrial Disease Scale Sections I-IV. Patients with Leber Hereditary Optic Neuropathy have also benefitted from EPI-743 treatment (12).

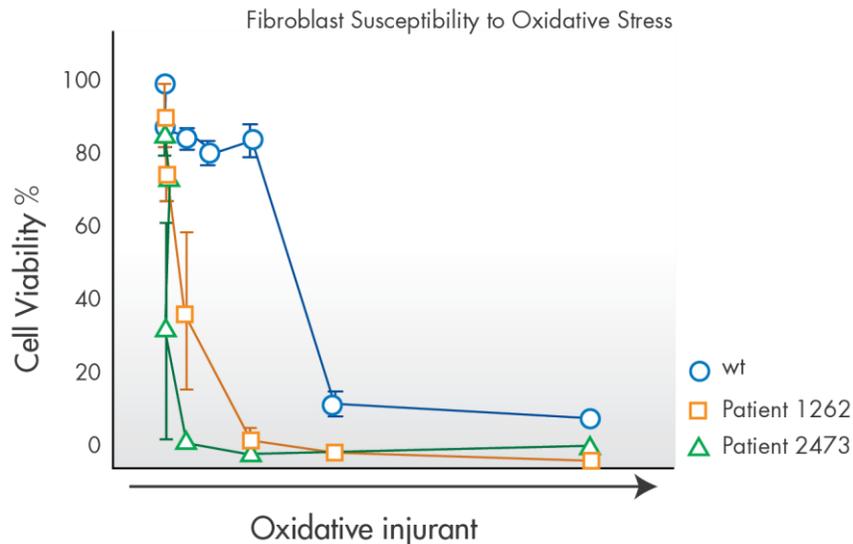
An open-label study confirmed the beneficial effects of EPI-743 in 13 children and one adult with mitochondrial disease (13), including 4 with polymerase- $\beta$  deficiency, 4 with Leigh syndrome, 3 with MELAS, 2 with mitochondrial depletion, and 1 with Friedreich's ataxia. Two patients died of their disease. Ten of the 12 survivors had improved quality of life scores after 13 weeks.

Recent publications have reviewed the role of EPI-743 as an antioxidant (14), correlated brain blood flow (as gauged by Tc99m-HMPAO uptake) with response to EPI-743 in patient with mitochondrial diseases (15), and described glutathione redox status in response to EPI-743 in patients with mitochondrial encephalopathies (16).

We propose to identify patients, both within and outside our UDP cohort, whose fibroblasts in culture both exhibit an abnormal oxidative stress response and are rescued by EPI-743. We will treat these patients with EPI-743, and record quality of life scores using the

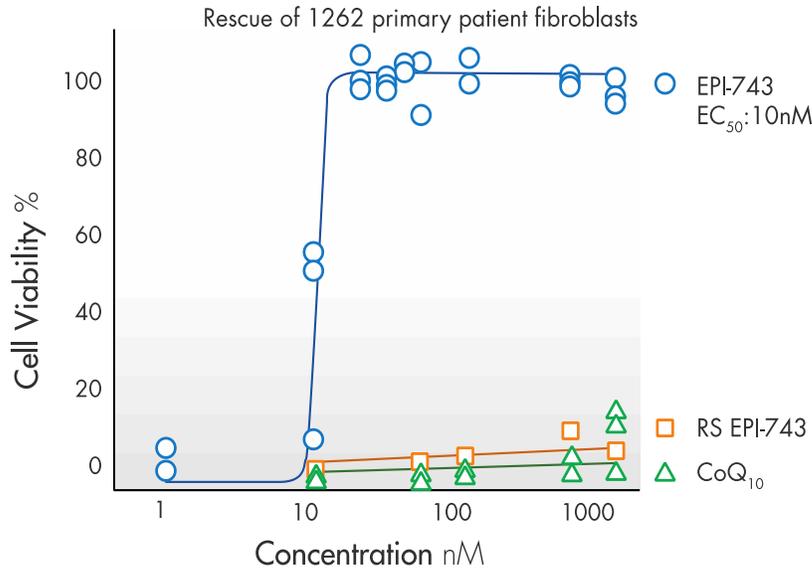
Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) for ages 2-11 years (Appendix A). We will also measure objective outcomes such as growth parameters, plasma lactate, pyruvate, and amino acids, leucocyte CoQ, plasma carnitine levels, ammonia, CPK, serum liver enzymes, urine organic and amino acids, and CSF lactate and amino acids. Clinical changes will be documented by ophthalmology, neurology and audiology evaluations, neuropsychological testing, EKG and echocardiograms, and video-taping of movements. For patients with muscle involvement, the Gross Motor Function Measure (GMFM)-66 will be employed (Appendix B). Possible outcomes include increased strength, decreased fatigue, less acidosis, improved laboratory values, fewer hospitalizations, reduced frequency of seizures, less ataxia or dystonia, and improved hearing and/or vision. For patients with progressive disease/disability, a plateauing of signs and symptoms may be considered beneficial. (See Section 8.1.)

We provide two examples of patients whose fibroblasts have met the *in vitro* criteria for eligibility into this protocol. In the assay for increased susceptibility to oxidant stress, fibroblasts were exposed to increasing concentrations of an oxidizing agent, and cell viability was assessed by measuring uptake of calcein-AM. The fibroblasts of two UDP patients, #1262 and #2473, exhibited 50% cell death at concentrations of oxidating stressor ( $\square$ M) that were significantly lower than those for age-matched control cells (Fig. 1). All cells were between passages 3 and 12.



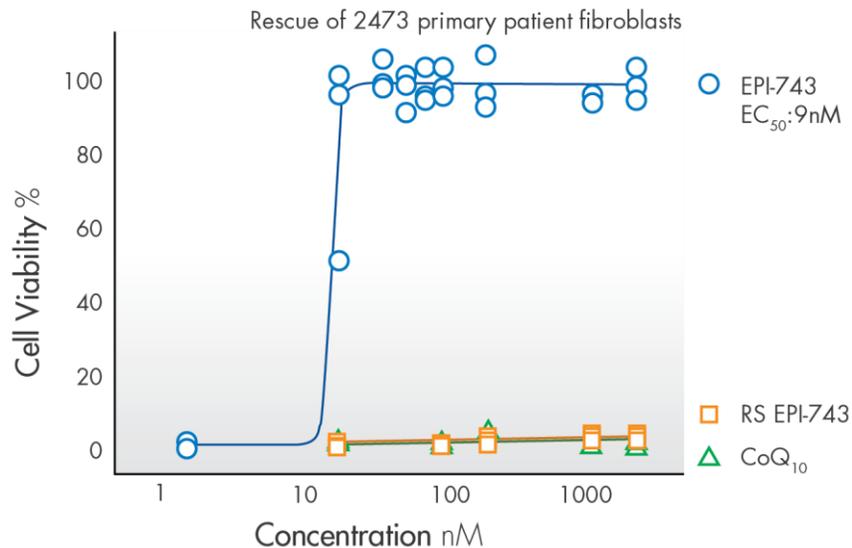
**Fig. 1. Oxidative stress response of wild type (wt) and patient fibroblasts**

Patient #1262 is a 46-year old man with progressive spastic paraparesis with gait problems but normal cognitive function. He is heterozygous for a pathogenic *SPG7* mutation, a known cause of autosomal dominant spastic paraplegia and gait disease. His cultured fibroblasts had their cell viability rescued by 10 nM EPI-743, while there was no effect of coenzyme Q<sub>10</sub> or an inactivated EPI-743 molecule, i.e., RS EPI-743 (Fig. 2)



**Fig. 2. Response of patient #1262 fibroblasts to EPI-743.**

Patient #2473 is a 10 year-old girl with congenital myopathy, contractures, mild microcephaly, other dysmorphisms, and failure to thrive. She has a tube placed for feedings and has a tracheostomy with home ventilation. She has undergone two muscle biopsies showing increased lipid but providing no diagnosis, and has had a rod placed in her spine for scoliosis. A brain MRI is normal. Despite losing some milestones, her cognition is average. An extensive metabolic and genetic evaluation has not yielded a diagnosis. Again, EPI-743 rescued the viability of her fibroblasts (under conditions of oxidant stress) with an EC<sub>50</sub> of 9 nM (Fig. 3).



**Fig. 3. Response of fibroblasts of patient #2473 to EPI-743.**

To date, we have identified 33 patients age 2-11 years whose fibroblasts in culture are responsive to EPI-743; all have clinical signs of impaired redox status or energy production. Of these, 12 have been found not to meet eligibility criteria. Nineteen patients have enrolled, but three came off study; one subsequently died. Twelve have completed both arms of the trial two remain in treatment arm #1, while two have moved to arm #2. One new patient is expected to be enrolled in the first quarter of calendar year 2017. Screening is ongoing.

Nine patients are enrolled on the extension study of active drug; and five are scheduled to return. Two families declined participation in the extension.

There have been 22 Serious Adverse Events, all reported to the IRB and DSMB in moderate detail. These SAEs are listed below in cursory form; patient EPI-03, an 8 year old boy with POLG1 deficiency, died of his disease.

**SAE #1 – March, 2014**

**Grade 3, attribution unlikely**

EPI-02: Infection requiring IV antibiotics

Hospitalized for fever with subsequent pseudo-bowel obstruction (3/15-3/31/2014). The patient was transfused with RBCs during this hospitalization with a hemoglobin of 7.8 g/dL, down from a previous baseline of 9.8 g/dL.

**SAE #2 –April, 2014**

**Grade 3, attribution unlikely**

EPI-02: Infection requiring IV antibiotics

Hospitalized for ear infection with subsequent electrolyte abnormalities managed medically (4/11-4/24/2014).

**SAE #3 – May, 2014**

**Grade 4, attribution unlikely (\*grade changed to reflect the CTCAE)**

EPI-03: Seizure, Life-threatening; prolonged repetitive seizures

Hospital admission for status epilepticus on May 3, 2014. He required intubation for airway management and also multiple phenobarbital and pentobarbital comas. At day 9 of hospitalization he was transferred to Duke Medical Center. Due to inability to travel for 3 month visit, he was discontinued on the trial as of June 19, 2014. He died in hospice care in November, 2014.

**SAE #4 – May-June, 2014**

**Grade 3, attribution unlikely**

EPI-02: Infection requiring IV antibiotics

Hospitalized for low grade fever with subsequent finding of bacteremia likely secondary to central line used for total peripheral nutrition treatment (5/23-6/8/2014). The patient was also transfused with RBCs during this hospitalization with a hemoglobin of 7.9 g/dL.

**SAE #5 – September 24, 2014**

**Grade 3, attribution unlikely**

EPI-02: Infection requiring IV antibiotics

Subject had been off study drug for 55 days during study-drug wash period of 2 months. Subject hospitalized on 9/24/2014 for fever and lethargy secondary to suspected viral or bacterial illness.

**SAE #6 – December 2014 – January 2015**

**Grade 3, attribution unlikely**

EPI-02; upper respiratory infection requiring hospitalization; fever with line infection

Subject developed upper respiratory symptoms and was hospitalized December 3, 2014. She became febrile on December 14. Cholecystectomy performed. Antibiotics and antifungals were given, and the line grew out yeast. Supportive therapy yielded slow recovery. There was no disruption of Epi-743 drug/placebo doses. This hospitalization was consistent with the underlying disease course.

**SAE#7-July 2015**

**Grade 2, attribution unlikely**

Epi-10: Dehydration requiring IV fluids

Our PNP was notified at 10 AM on July 8, 2015 that EPI-10 was admitted to the Children's Hospital of Michigan on the evening of July 7, 2015. She spoke with the Senior Renal Fellow who reported that EPI-10 came through the ED with a 2-day history of fever, vomiting & diarrhea. EPI-10 was afebrile but with a heart rate of 148-155 in the ED. EKG showed sinus tachycardia, no other abnormalities. EPI-10 was given three 10 ml/kg normal saline boluses and remained tachycardic so she was admitted for a fourth bolus and started on 1.5x maintenance continuous IV fluids. The white blood cell count was 8.9 with a normal differential; electrolytes were normal except for a potassium of 2.6 mEq/L. (EPI-10 receives electrolyte supplements because of her renal tubular acidosis). On July 8, the local dietician learned that the family was treating this girl's constipation with Mango juice, 2–3 ounces with every feed. The local team feels that the Mango juice is at least in part contributing to diarrhea, since there is nothing to support a severe viral gastroenteritis. The local Investigational Pharmacist was in touch with the NIH pharmacist, as well as our PNP, and was sent a copy of the protocol and the signed consent. EPI-10 has not missed any doses of study drug.

**SAE #8 –November 2015**

**Grade 3, attribution unlikely**

EPI-11: Intractable vomiting, progression Leigh syndrome

Hospitalized for intractable vomiting and underwent brain MRI that identified progression of Leigh syndrome into brainstem. (November 19-December 21, 2015) This hospitalization, length of hospitalization, and course are consistent with underlying progression of disease and the patient was withdrawn from the study.

**SAE #9 – December 2015**

**Grade 3, attribution unlikely**

EPI-02: Suprapubic catheter leak requiring surgical intervention

Hospital admission (December 30, 2015 – January 2, 2016) on the Extension Open-label

portion of this study, experienced loose sutures and leaking around her suprapubic catheter without a fever or signs of illness and was admitted the evening of December 30<sup>th</sup> with plans to go to the Operating room today for replacement of her suprapubic catheter. She is expected to be discharged as soon as fully recovered from Anesthesia .

**SAE #10 – February, 2016**

**Grade 3, attribution unlikely**

EPI-02: Infection requiring IV antibiotics

Hospitalized on the Extension Open-label portion of this study, experienced vomiting and back pain with a fever of 104.2 and was admitted for IV antibiotics presumed to be a urinary tract infection/pyelonephritis given her suprapubic catheter as the risk factor.

**SAE #11– February 2016 filed with DSMB family did not tell us right away**

**Grade 3, attribution unlikely**

EPI-15: Respiratory illness with dehydration requiring IV fluids

Hospitalized November 9-11, 2015 however did not notify us until next safety visit to NIH. This hospitalization is consistent with underlying disease course.

**SAE #12 – February 2016**

**Grade 4, attribution possibly related**

EPI-01, Neutropenia without Fever

EPI-01 On February 5, 2016, EPI-01's mother notified the NIH Senior Nurse Practitioner that her son underwent routine monthly lab work. The sedimentation rate was 45, exactly what it was at the beginning of the Extension study Jan 4, 2016. However, his neutrophil count was 0; it had been 340 on Jan 4th. Mother reported that EPI-01 had cold symptoms for 4 days, but no fever or cough. He had a runny nose and was sleeping from 5PM-6AM every day, which his Mom considers lethargic for him. He was seen for routine follow up of his hypothyroidism with his endocrinologist Feb 5 and was to get his monthly CK checked per the EPI-743 protocol. The endocrinologist repeated the CBC with differential & ESR along with regular endocrine labs and CK. When the absolute neutrophil count of 0 was called to the endocrinologist, he referred EPI-01 to his PCP. The PCP sent him to the Emergency room of Rady Children's hospital, San Diego where Dr. Jim Harley (858-966-8001; Fax 858-966-6769) the ED Attending evaluated him. A curb-side Hematology evaluation reported that EPI-01 was not febrile, nor did he have any signs of infection, and he was acting normally; no further lab work was performed but an outpatient Hematology appointment was planned. Over the 14-month course of the crossover study, EPI-01 had no ESR elevations or low neutrophil counts. He was on the open-label drug since January 4, 2016 and did not miss any doses.

**SAE #13 – February, 2016**

**Grade 3, attribution Unlikely**

EPI-09: Dehydration requiring IV fluids

Epi-09 who is in on the Extension study, was admitted to his local hospital February 25-26, 2016 for a vomiting, cough, hypokalemia with a potassium of 3.2 and no fever. He was treated with 24 hours of IV fluids and discharged with a potassium 4.4. EPI-09 did not miss any doses of study drug over the 24 hour hospitalization.

**SAE #14 – February, 2016**

**Grade 3, attribution Unlikely**

EPI-10 who is in on the Extension study, was admitted to her local hospital February 25-26, 2016 for a vomiting and cough, without fever. She was treated with 24 hours of IV fluids and discharged. EPI-10 did not miss any doses of study drug over the 24 hour hospitalization.

**SAE #15 – March, 2016**

**Grade 4, attribution possibly related**

Epi-01: Neutropenia without Fever

EPI-01 is a nearly 9 year-old boy on the EPI-743 extension study, having completed both arms of the crossover study on May 15, 2015. EPI-01 has an ongoing Grade 4 SAE for decreased neutropenia without fever that has been reported to, and discussed with, the DSMB on several occasions. EPI-01 was identified on February 5, 2016 with an ANC of 0; it had been 340 on Jan 4, 2016 at the initiation of the Extension portion of this study. Dr Janet Yoon, Hematology, at Rady Children's hospital evaluated EPI-01 and identified a manual ANC of 64 on February 16, 2016 and EPI-01 was taken off EPI-743 until his ANC rose above 300 on March 9, 2016 at which time we restarted EPI-743 with consensus from Dr Yoon and the DSMB. His repeat CBC on March 28, 2016 revealed WBC of 3.8 with an ANC of 210. Dr Yoon feels the EPI-743 should be stopped if the ANC drops below 200 and is planning on repeating the CBC in 1 week.

**SAE #16 – April 2016**

**Grade 4, attribution possibly related**

Epi-01: Neutropenia without Fever

EPI-01 is a nearly 9 year-old boy on the EPI-743 extension study, having completed both arms of the crossover study on May 15, 2015. This is follow-up of an ongoing Grade 4 SAE for neutropenia without fever that has been previously reported as SAE #15. To review, EPI-01 was identified on February 5, 2016 with an ANC of 0; it had been 340 on Jan 4, 2016 at the initiation of the Extension portion of this study. Dr. Janet Yoon, hematologist at Rady Children's Hospital in San Diego, evaluated EPI-01 and identified a manual ANC of 64 on February 16, 2016. EPI-01 was taken off EPI-743 until his ANC rose above 300 on March 9, 2016, at which time he restarted EPI-743 with consensus from Dr. Yoon and the DSMB. His repeat CBC on March 28, 2016 revealed a WBC of 3.8 with an ANC of 210. At that time the plan was to repeat his CBC in one week and discontinue Epi-743 if his ANC was less than 200.

On April 7, 2016 Epi-01 had a repeat CBC that revealed an ANC of 41 by manual count, and the EPI-743 was discontinued based upon previous plans formulated by the NHGRI team, the DSMB, and Dr. Yoon, and upon ongoing discussions with Dr. Yoon. Repeat neutrophil auto-antibodies are pending and will take several weeks to return results.

Our Senior Nurse Practitioner also discussed these results with the parents, who feel that Epi-743 makes a huge difference in the quality of life of their son and the entire family. They are very disappointed to stop Epi-743 again, although they state that they

understand the team's decision. They would like an opportunity to retry the study drug again if possible. Dr. Yoon has proposed repeating CBC's and neutrophil auto-antibodies monthly until such time as the auto-antibodies become negative and the ANC is greater than 500, then restarting Epi-743 and obtaining a repeat CBC and auto-antibodies 2 weeks after that. If the auto-antibodies revert to positive with exposure to Epi-743, Epi-01 would have to be removed from the Extension portion of this study.

**SAE #17 – April-May, 2016**

**Grade 4, attribution Unlikely**

Epi-02 Line infection with Fever

Epi-02 started the open-label Extension portion of this study December 3, 2015. On April 26, 2016 our Senior PNP was notified that Epi-02 was hospitalized on April 8, 2016 for a planned suprapubic catheter change. Apparently her central line was cut or damaged during the OR procedure and broke the next day on the floor. With the history of hypoglycemia and TPN dependence, no central access was a potentially life-threatening event. A bedside PICC insertion was successfully placed and Epi-02 was taken to the Operating room 2 days later for placement of a new double lumen Broviac. Subsequently, Epi-02 grew out *Candida Krusei*, a resistant yeast in her urine susceptible to Amphotericin B. However, Infectious Diseases felt that bladder washes rather than IV Amphotericin should be tried. Eight days later Epi-02 had complete bowel shutdown with vomiting every 45 minutes for 5 days per her Mom. An NG tube was placed and suction from the NGT and her J-tube was used and her ileus has returned to baseline. Now she has developed pancreatitis with a peak Lipase of 607. One IV dose of Amphotericin B was given on April 25, 2016. An ERCP is planned for later this week. Discharged May 23, 2016.

**SAE#18 – April 2016**

**Grade 2, attribution Unlikely**

Epi-12: Prolonged seizure requiring hospitalization

EPI-12 is a nearly 8 year-old boy on the EPI-743 Extension study since April 12, 2016 having completed both arms of the crossover study. His diagnosis is MED-12 related XLID and he has seizures at baseline. His mother called to report that he had experienced a generalized seizure of indeterminate length overnight. Since he is a noisy sleeper, mom did not immediately recognize his noises as seizure related. The boy is currently an inpatient in his local hospital undergoing a 24 hour EEG. He has missed one dose of Epi-743 so far.

**SAE#19 – July 2016**

**Grade 3, attribution Unlikely**

Epi-02 Line infection with Fever

Epi-02 started the open-label Extension portion of this study December 3, 2015. On July 25, 2016 our Senior PNP was notified that Epi-02 was hospitalized Sunday July 24, 2016 for a fever of 102.5 without a source. As of this AM there is no evidence of a UTI and a urine culture is pending. Blood cultures from her Broviac are negative. She has not missed any doses of Epi-743 to date.

**SAE#20 – August 2016**

**Grade 4, attribution Unlikely**

Epi-02 Line infection with Fever

EPI-02 is a 13 year old girl who started the open-label Extension portion of this study December 3, 2015. On August 29, 2016 our Senior PNP was notified that EPI-02 was hospitalized for a fever of 105 and rigors. CBC, CMP, amylase & lipase are within normal limits. Blood cultures from her broviac and suprapubic catheters are negative to date. She is currently receiving vancomycin and ceftadixime. She has not missed any doses of Epi-743 to date. EPI-02 has been the subject of previous SAEs based upon her hospitalizations for line-related infections, pseudo-obstruction, and possible pancreatitis, all related to her primary disease.

**SAE#21 – October 2016**

**Grade 3, attribution Unlikely**

Epi-15 Prolonged partial prothrombin time

Epi-15 started the blinded portion of this study August 5, 2015 and was at NIH for her 14 month visit on October 5, 2016 (end of the blinded study) when an elevated PTT was noted to be elevated at 68, a repeat was higher at 72.6. PT was 15 & 15.6 with normal CBC, ESR & LFT's. A Hematology consult was obtained and she was found to have positive Lupus anticoagulant antibodies. She subsequently developed vomiting and 1 episode of liquid diarrhea without normal oral intake so she required IV fluid rehydration over-night. She had no fever and no blood or mucous in her diarrhea. She has missed 6 doses of Epi-743 to date.

**SAE#22 – November 2016**

**Grade 2, attribution Unlikely**

EPI-02 is a 13-year old girl who started the open-label Extension portion of this study December 3, 2015. On November 16, 2016 our Senior PNP was notified that EPI-02 was hospitalized for surgical replacement of a cracked central line. She did not have any signs of infection or metabolic instability and is expected to be discharged in 24-48 hours. She has not missed any doses of Epi-743 to date.

One non-serious but severe Adverse Event was experienced by patient EPI-06 in May of 2015. This 4 year-old girl had a Grade 4 elevation of her serum creatine phosphokinase (CK) in month 3 of arm 2 of this study. We do not know if that arm provided placebo or EPI-743. The CK value fell while she remained on drug, then rose again, prompting discontinuation of drug. After the CK normalized, drug was restarted and the CK again rose to Grade 4 levels. The drug was stopped, and the patient terminated from the study, but the CK has remained elevated for several weeks. At one point, this Adverse Event was considered probably related to the drug, whether placebo or EPI-743. Based upon the recommendations of the NHGRI DSMB, monthly monitoring of CK values has been incorporated into this protocol, and a letter has been sent to patients notifying them of this event. In addition, elevated CK is noted as a risk in Section 9.1 and in the consent.

One non-serious but possibly related Adverse Event was experienced by Epi-01 who had non-febrile neutropenia with an ANC less than 500 mm<sup>3</sup> and recurrent neutrophil

autoantibodies when on open-label Epi-743 in the Extension portion of the study. This was reported to the FDA via Medwatch and monthly CBC with differentials on all participants have been requested at this time.

#### 4. Study design

This is a double-blind, placebo-controlled, cross-over trial involving 20 children whose clinical findings and *in vitro* (i.e., cultured fibroblast) studies suggest that they could benefit from EPI-743. These patients will be admitted to the NIH Clinical Center for 2-5 days and will receive either EPI-743 or placebo, t.i.d., for 6 months. The dosage will be 15mg/kg three times a day up to a maximum of 200 mg three times a day. Patients will return after 1, 3, 6, 8, 9, 11, and 14 months; safety labs studies and phone evaluations will be spaced between these visits. After the 6-month admission, there will be a two-month washout period and then the drug will be changed to either placebo or EPI-743. PK data show a terminal half-life for EPI-743 of 24 hours at low plasma concentrations. A two-month washout should be sufficient, since washout is typically assumed to be complete at 5 times the terminal half-life. Safety will be assessed by history, physical examination, laboratory testing and consultations. Efficacy will be determined based upon comparison of the 6 months on EPI-743 with the 6 months on placebo. The primary outcome parameter will be the quality of life score on the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) for ages 2-11 years; parts I-III will be assessed separately from part IV. Secondary outcomes will consist of a set of laboratory and clinical measures customized for each patient, such as the GFMF-66 and specific laboratory tests. After completing the crossover portion of this trial, all patients will be offered open-label EPI-743.

#### 5. Procedures

After screening is performed to assess for entry criteria, baseline data relevant to the patient's individual disorder will be collected. Patients will remain on any supplements they have been receiving. Accepted patients will be randomized by the NIH Pharmaceutical Development Service to receive either EPI-743 (15mg/kg three times a day) or placebo, with meals to a maximum dose of 200 mg three times daily with meals. Admissions to the NIH Clinical Center will occur according to the schedule of Table 1 and last for 2-5 days. The one-month and 9-month NIH admissions, along with local laboratory testing at 2 weeks, 2 months and 2 weeks after each treatment period, will be for safety reasons. Crossover will take place after the first 6-month treatment period plus a two-month washout period. Testing will be individualized based upon clinical findings, but certain studies will be performed on every patient (Table 1).

**Table 1. Timing of NIH Clinical Center Investigations for All Patients**

	Base- line	1 m o	2 m o	3 m o	4 m o	5 m o	6 mo	2-mo was h o	8 mo	9 m o	10 m o	11 m o	12 m o	13 m o	14 m o	
History, Physical	X	X		X			X		X	X		X				X
Study																

bloods <sup>1</sup>	X			X			X		X			X			X
Safety bloods <sup>2,3</sup>		X								X					
CBC with differential	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CK	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EKG	X	X		X			X		X	X		X			X
Urin <sup>4</sup>	X			X			X		X			X			X
NPMS	X			X			X		X			X			X
CRF	X	X		X			X		X	X		X			X
Drug <sup>5</sup>	Star t 1						Sto p		Star t 2						
Other <sup>6</sup>	X						X		X						X

<sup>1</sup>CBC with differential, ESR, acute care, mineral and hepatic panels, lipids, PT/INR, aPTT, CK, T4/TSH, amylase, lactate, pyruvate, amino acids, carnitine, ammonia, urine organic acids, urine amino acids, urinalysis, GSH

<sup>2</sup>CBC with differential, ESR, acute care, mineral, and hepatic panels, PT/INR, aPTT, CK

<sup>3</sup>Safety bloods for CK testing will be obtained locally at 2, 4, 5, 7, 10, 12, and 13 months, so that CK values are, in total, obtained monthly.

<sup>4</sup>Organic acids, urine amino acids, urinalysis

<sup>5</sup>EPI-743 or Placebo treatment

<sup>6</sup>For some patients, testing will be customized based upon clinical manifestations. In those cases certain of the following studies will be performed at baseline and at 6, 8, and 14 months: GMFM-66, brain MRI/MRS, CSF lactate, amino acids, folate, neurotransmitters, neuropsychological testing, video, ophthalmology, audiology, ECG, Echo, and EEG

Additional laboratory studies will be obtained locally for safety reasons at week 2, month 2, and 2 weeks after the end of each treatment period (see below).

### 5.1 Medical information

Upon admission to the NIH Clinical Center, a history and physical examination will be performed in addition to several laboratory tests, procedures, and consultations. Patient data will be entered into the Clinical Trials Database (CTDB), an electronic data capture system provided by NICHD. Computer files will use numbers assigned to patients and will have secure passwords. This database is secure and password protected. Data entered directly in CTDB will be considered digital source documentation. CTDB is a Web-based application that supports protocol design, clinical data collection and reporting. Clinical Trials Database (CTDB) is a secure password-protected encrypted database, where patient information is stored by patient study ID number until required for analysis. Descriptions of CTDB and its security information are included in Appendices A and B, attached.

#### Baseline and follow-up outcome measures (0, 3, 6, 8, 11, 14 months)

Laboratory tests include a routine urinalysis with dipstick and testing for organic acids. A two-hour urine protein will be obtained if the urinalysis is positive for protein. Blood will be drawn for CBC and differential, platelets, erythrocyte sedimentation rate, acute care, mineral and hepatic panels, cholesterol, triglycerides, amylase, lipase lactate, pyruvate, ammonia, prothrombin time and INR, partial thromboplastin time, plasma and urine amino acids, carnitine (at 0,3, 6, 8, 11 and 14 months), creatine phosphokinase (CK), and glutathione. When indicated, leucocyte coenzyme Q will be obtained. Endocrine studies will include thyroxine and TSH. Blood (15 ml) will also be drawn for research purposes, such as measurement of creatine (17) and FGF-1. Unused plasma and serum will be banked

for use at a later date for questions related to this and future studies. Coagulation studies (PT, PTT, INR) will be repeated 2 days after the start of treatment, since steady state should have been reached by this time. (The initial half-life of EPI-743 approximates 4 hours.)

“Study” blood volumes will be 68 mL for the baseline and 8-month admissions and 60 mL for the 3, 6, 11, and 14-month admissions. Additional blood will be drawn for medically indicated tests. Total blood volumes, monitored by the protocol coordinator and by phone calls to determine local blood draws, will be consistent with Clinical Center guidelines. Specifically, no more than 9.5 mL/kg or 550 mL will be drawn for research purposes over any 8-week period, and no more than 5 ml/kg will be drawn at one time. When blood volumes are limiting because of the patient’s weight, the least relevant studies will be eliminated. For example, some patients may not need to have lactate, pyruvate, ammonia, amino acids, or carnitine drawn.

Procedures will include electrocardiograms at every NIH admission. In addition, studies that are medically indicated for the patient’s disorder will be performed. These may include an audiogram, ABR, measurement of visual acuity, visual field testing using the Humphrey 32.3 chart, sedated electroretinogram and/or optical coherence tomography, MRI/MRS of the brain, spine or muscle, electromyogram/nerve conduction velocity, EEG, videotaping, or neuropsychological testing. These standard studies, if indicated to characterize or diagnose the patient’s basic illness, will be repeated at the beginning and end of each treatment period to assess the effects of the treatment. Another outcome measure will be total days hospitalized for disease-related illnesses.

Consultations may include neurology, neuropsychology, audiology, cardiology, rehabilitation medicine, ophthalmology, and others as indicated by the disease process. If a lumbar puncture is performed, the CSF will be sent for lactate, pyruvate, 5-methylene tetrahydrofolate, neurotransmitters, and pterins.

#### Safety studies (0.5, 1, 2, 6.5, 8.5, 9, 10, 14.5 months)

These safety-related blood draws will be obtained locally and the results sent to the NIH. They include CBC with differential, ESR, acute care, mineral and hepatic panels, PT/INR, aPTT, and creatine phosphokinase (CK), for a total volume of ~14 mL. (These same blood tests are included in the “study” bloods drawn at 0, 3, 6, 8, 11, and 14 months.) In addition, a CBC with differential and CK will be drawn locally at 4, 5, 12, and 13 months to comply with the DSMB and FDA’s requirement to follow these tests monthly. Additional lab studies will be performed if there are abnormal results. Specifically, if the CK is elevated during this study or was elevated in the past, a CK will be performed more frequently, based upon the investigator’s medical judgment.

All patients enrolled in this protocol will also be enrolled in protocol 76-HG-0238, “Diagnosis and Treatment of Patients with Inborn Errors of Metabolism and Other Genetic Disorders.” Under that study, a variety of blood tests (requiring 52 mL plus up to 100 ml for research), consultations, and procedures (photos, radiographs, skin biopsy) may be performed. When studies for the two protocols coincide, they will not be duplicated, and the total amount of blood drawn for both protocols will not exceed NIH limits.

Medical summaries and laboratory test results will be stored in a locked file cabinet and treated as confidential clinical data. A Clinical Report Form (Appendix C) will be completed for each patient at each admission. The CRF includes the delineation of a specific set of laboratory and clinical criteria that will comprise the secondary outcome measures for each patient. The Clinical Report Form can be computerized and the data maintained as files. Computer files will use numbers assigned to patients, with the key kept in a locked file cabinet. The principal investigator and associate investigators will have access to the locked file.

**Extension Study:** Individuals who complete the two arms of this study or who were terminated from this study due to an event that was not determined to be drug related will be invited to receive open label EPI-743 until either the protocol stops seeing patients, the drug is approved for human use, or the company stops supplying drug. Individuals who have reached the age of 12 years old will be allowed to participate in the extension study. The Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) for ages 12-18 years will be employed to assess quality of life scores in these patients.

There will be at least a two-week wash-out between stopping the blinded study and beginning the extension study to establish a baseline for the extension study.

Baseline studies will include history and physical exam, CBC, ESR, platelets, acute care, CK, mineral and hepatic panels, PT/INR, aPTT, research Glutathione levels, EKG and echocardiogram. Neuropsychiatric testing, Newcastle primary outcome scores, and in some cases patient-specific secondary outcome measures will also be obtained.

Patients who have participated in the Extension study for at least 6 months without any drug-related adverse events will be moved to annual follow up at NIH.

Follow-up will include the following safety studies:

- Every month: CBC with differential and CK, locally.
- Every 3 months: CBC with differential, ESR, acute care, mineral and hepatic panels, PT/INR, aPTT, EKG completed locally. A follow up phone call will be completed every 3 months.
- Every 6 or 12 months: History and physical exam, CBC with differential, ESR, acute care, mineral and hepatic panels, PT/INR, aPTT, research Glutathione levels, EKG and Echocardiogram at NIH Clinical Center for follow-up.

The NHGRI DSMB will follow adverse events and safety issues as it is currently doing.

Measures of efficacy:

- 6-month Extension patients: Neuropsychiatric testing, Newcastle primary outcome scores, patient-specific secondary outcome measures on some patients at the Investigator's discretion.

- 12-month Extension patients: Newcastle primary outcome scores, patient-specific secondary outcome measures on some patients at the Investigator's discretion.

Patients who choose not to receive EPI-743 through this extension will be asked to engage in this follow-up as well.

#### 5.2 Diagnostic studies

Medically indicated diagnostic tests will be performed. In addition, a skin biopsy will already have been obtained from each patient under protocol 76-HG-0238. Fibroblasts will be grown from those biopsies, and the cells will be studied by Edison Pharmaceuticals Inc., using the injurant oxidant system as described in Section 3.

#### 5.3 Biological specimens.

Blood and urine will be obtained for medical and research purposes during each admission. These samples will be stored indefinitely and may be shared with collaborators; the samples will be sent with clinical information but without names or identifiable information.

#### 5.4 Approved drugs.

There are no approved drugs being used for research purposes through this protocol.

#### 5.5 Unapproved drugs.

EPI-743, or 2-[(3R,6E,10E)-3-hydroxy-3,7,11,15-tetramethyl-6,10,14-hexadecatrienyl]-3,5,6-trimethyl-2,5-cyclohexadiene-1,4-dione, will be provided by Edison Pharmaceuticals, Inc. A common chemical name of EPI-743 is alpha-tocotrienol quinone. EPI-743 is the quinone oxidation product of alpha-tocotrienol, one of the eight naturally occurring forms of vitamin E. EPI-743 is a viscous yellow oil. It will be administered as a mixture with sesame oil NF/USP at 100 mg/mL, with meals. Dose formulations will be prepared by the NIH Clinical Center pharmacy.

Experience with EPI-743 in patients is as follows:

- 1.) For patients with Leigh syndrome treated with EPI-743 at 100 mg t.i.d. (both under the US expanded access protocol and in the phase 2 study conducted in Rome), the mg/kg dose ranged from 3.3 to 14.8 mg/kg. No drug-related adverse events or safety laboratory abnormalities were observed during the course of treatment at these doses.
- 2.) Based on the PK data obtained from the studies with EPI-743 at the dose of 100 mg t.i.d., we estimate that a dose level of 15 mg/kg, with a maximum dose of 100 mg t.i.d., will expose patients to an AVERAGE daily AUC of approximately 9,000 ng.h/mL, and a MAXIMUM daily AUC of approximately 46,000 ng.h/mL. Based on the NOAEL in 28-day rat toxicity studies (AUC 55,000 ng.h/mL), these exposures correspond to safety margins of 6- and 1.2-fold, respectively.
- 3.) Because the dose-limiting effect of EPI-743 in non-clinical safety studies is inhibition of coagulation, the protocol includes appropriate monitoring of

coagulation parameters during the study, as well as a provision to lower the dose in those patients where INR increases to Grade 2 or greater severity. (See Section 9.1.)

Recent data, described in Section 9.1, indicate that EPI-743 is safe and well tolerated at a dosage of 15mg/kg three times a day, with a maximum dose of 200 mg three times a day, and this is the dosage to be used in this protocol.

Each bottle of EPI-743 contains approximately 20 g of Active Pharmaceutical Ingredient (API) and is labeled with the following: the product name, product lot number, protocol number, recommended storage conditions, expiration date, "Caution: New Drug-Limited by Federal Law to Investigational Use," and Edison Pharmaceuticals' company name and address. Labeling will comply with the requirements of 21 CFR 312.6.

Placebo will be supplied by Edison Pharmaceuticals and will contain sesame oil.

All study drugs will be stored and dispensed in accordance with the Food and Drug Administration (FDA) and local regulations concerning the storage and administration of investigational drugs. Drug use will be tracked by asking the patients to return their unused supplies; this will be recorded on the CRF.

#### 5.6 Results given to participants.

Parents, guardians, and referring physicians will be given the results of medically relevant tests. Patients and families will not be told when they are receiving EPI-743 or placebo, nor will they be told the results of studies that constitute outcome measures. Upon request, the investigators will provide results of the NPMDS to the families at the end of the study.

#### 5.7 Questionnaires.

Quality of life questionnaires will be administered in the form of the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) for ages 2-11 years (Appendix A). For patients on the extension study who are more than 11 years old, the NPMDS for ages 12-18 will be employed. The results will serve as the primary outcome parameter. The GMFM survey will serve as a secondary outcome measure and will be completed as appropriate.

#### 5.8 Genetic counseling.

The Principal Investigator and his staff will provide counseling regarding the patient's disorder. In cases where specific issues require in-depth counseling, one of the genetic counselors in the Office of the Clinical Director will perform this service.

#### 5.9 Criteria for withdrawal.

Patients may withdraw from the study at any time and for any reason, such as because they are no longer able or willing to travel to the NIH, or have a mild adverse event associated with the study.

An individual patient can be removed from the study if he/she:  
develops a severe adverse [events https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE\\_5.0/](https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_5.0/) or cannot tolerate EPI-743;

- exhibits clear evidence of noncompliance;

- fails to make an admission;
- shows significant progression of underlying disease;
- uses an excluded therapy.

If an individual is removed from this study, the data will be analyzed on an intent-to-treat basis. Since this study involves an IND, records cannot be destroyed even if requested by a subject.

## 6. Description of Study Population

### 6.1 Estimated number of participants.

This study will enroll 20 children, largely from the NIH UDP. Patients will already be enrolled in protocol 76-HG-0238, "Diagnosis and Treatment of Patients with Inborn Errors of Metabolism and Other Genetic Disorders." The subjects will have either rare or unknown disorders characterized by:

1. Molecular genetic, biochemical, and/or enzymatic findings consistent with a cellular oxidation/reduction or energy production defect;
2. *In vitro* evidence of such a defect, based upon the Edison assay performed using the patient's fibroblasts in culture; and
3. *In vitro* evidence of rescue by EPI-743 of the energy or redox defect.

We expect that several of the subjects enrolled in this protocol will have mitochondrial disorders.

Patients will be enrolled on a rolling basis.

### 6.2 Inclusion/exclusion criteria.

Inclusion criteria involve enrollment in protocol 76-HG-0238, "Diagnosis and Treatment of Patients with Inborn Errors of Metabolism and Other Genetic Disorders". In addition, patients must:

- Be 2-11 years of age upon entering the crossover part of the study;
- Manifest clinical findings of a neuromuscular disease with a component of impaired energy or oxidation/reduction. Typical symptoms would include hypotonia, dystonia, or seizures.
- Have a disorder that is untreatable or poorly treatable.
- Have cultured fibroblasts that exhibit reduced viability under conditions of oxidative stress, compared to age matched control fibroblasts.
- Have cultured fibroblasts that achieve at least 80% viability rescue with EPI-743 at 1micromolar upon exposure to oxidative stress and that have a half maximal effective concentration of EPI-743 of less than or equal to 50 nanomolar.
- Be willing to abstain from initiating the use of dietary supplements and non-prescribed medications, foods or beverages or bars fortified with coenzyme Q<sub>10</sub>, vitamin E, super fortified "functional" foods or beverages, and idebenone.
- Be able to travel to the Clinical Center for at least 8 visits during the crossover part of the study.

Exclusion criteria include:

- Age < 2 years or >11 years
- Diagnosis of mitochondrial diseases benefiting from treatment and at risk from being moved to placebo
- Allergy to EPI-743 or sesame oil
- Hepatic insufficiency with liver function tests greater than 3-times the upper limit of normal
- Renal insufficiency requiring dialysis
- Significant malabsorption of fats precluding drug absorption
- Allergy to vitamin E
- Significant coagulation abnormalities as evidenced by abnormal PT/PTT tests
- Severe end-organ hypo-perfusion syndrome secondary to cardiac failure resulting in lactic acidosis
- Ventilator-dependence
- Chronic pancreatitis
- Clinical history of bleeding requiring ongoing medical management
- Abnormal red cell parameters requiring significant ongoing medical management besides iron supplementation
- A platelet disorder
- Neutrophils less than 500 mm<sup>3</sup>

#### 6.3 Location of study.

This is an inpatient study involving patients admitted to the NIH Clinical Center.

#### 6.4 Recruitment strategies.

Patients are recruited largely through the NIH UDP, but other referrals will be permitted via enrollment in protocol 76-HG-0238.

#### 6.5 Existing samples/data.

None.

#### 6.6 Financial compensation

No financial compensation will be provided. Travel and lodging may be paid for.

### **7. Statistical Considerations (Appendix D)**

In this cross-over study, patients will serve as their own controls. Outcome measures during the EPI-743 treatment period will be compared with values during placebo treatment.

The primary outcome measure will be the quality of life score on the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) for ages 2-11 years (Appendix A). This will be completed every 3 months during the treatment periods. Parts I-III will be analyzed separately from part IV. Change over the period of therapy (6 months) will be compared with change over the period of placebo (6 months). That is, the midway score for each period will not be considered, since some NPMDS items assess status over the past 6 months. For analysis of the entire 20 patients, mean changes on and off EPI-743 will be compared using

Student's t-test. Subsets of the NPMDS scale that do not assess the previous 6 months will include scores every 3 months, and can be analyzed using repeated measures.

The NPMDS data will be analyzed via a two-treatment crossover design in which half the patients will be randomly assigned to receive treatment A (EPI-743) in period 1 and B (placebo) in period 2, while the other half will receive B in period 1 and A in period 2. There will be a 2-month washout between the two time periods. It is assumed that there will be no carry-over after the washout period. NPMDS will be measured at 4 time points: (X1, X2) = immediately before and immediately after period 1, then, after the washout, (X3, X4) = immediately before and immediately after period 2. The changes from baseline in each period are then given by  $Y1 = X2 - X1$  and  $Y2 = X4 - X3$ . SAS code will be used for this analysis. If there is carry-over, the crossover design must be abandoned in favor of a student's two-sample t-test of the differences between arms in period 1 only. The presence of significant period-by-treatment interaction will falsify the no carry-over assumption, and this will be tested. If the paired t-test of X3 against X1 (both treatment groups pooled) is not significant, this will encourage belief in no carry-over.

Secondary outcome measures, besides the number of days of disease-related hospitalization in the past 3 months, will be customized to each patient and recorded on the CRFs. They will include biochemical parameters reflecting a change in laboratory findings specifically selected at baseline. Examples are serum lactate and pyruvate, blood creatine, glutathione, FGF1, and CO<sub>2</sub>. For patients with muscle involvement, the GMFM-66 will be employed (Appendix B). Since all these data will be obtained every 3 months during the treatment periods, a repeated measures analysis will be used to compare rates of change on EPI-743 and on placebo for each individual. In addition, qualitative measures of change (e.g., video evidence of improved gait or movement) will be evaluated.

We will not address futility or success in terms of the protocol in its entirety, since improvement in even a single individual would be gratifying. Hence, there will be no interim analysis of composite data for efficacy. (Safety is addressed in Appendix D.) Both futility and success will be evaluated for each patient based upon a comparison of the outcome parameters at the 3 time points on treatment with the 3 time points on placebo. Individuals in whom treatment was deemed successful will continue to receive EPI-743.

If patients are removed from the study and no longer receive treatment, their data will be retained and analyzed on an "intent to treat" basis.

## **8. Potential Benefits**

### **8.1 Direct benefits to participants.**

Individual patients could benefit from EPI-743 by having some of their myopathic and neurological signs and symptoms stabilize or even improve. Patients enrolled in this study will also receive optimal care and follow-up of their illness.

### **8.2 Collateral benefit to participants.**

The results of clinical testing performed at the Clinical Center will provide baseline and follow-up medical data for each patient's record.

### 8.3 Benefits to society.

This protocol should help determine the safety and efficacy of EPI-743 for various disorders of energy and oxidation/reduction.

## 9. Likelihood and Seriousness of Harms and Means to Maximize Safety

### 9.1 Therapeutic interventions

The greatest risks of this protocol are associated with the administration of EPI-743. For example, non-clinical pharmacology and toxicology studies have not excluded the possibility of human Ether-a-go-go-Related Gene (hERG) inhibition because of the very low water solubility of EPI-743. *hERG*, also called *KCNH2*, codes for a potassium ion channel. Therefore, ECG monitoring prior to and during the initial dose stages will be performed. *In vivo* toxicology information is limited to short term studies (up to 28 days) at this time. A decrease in hematocrit and hemoglobin was observed 14 days after single dose at 1000 mg/kg and 2000 mg/kg in rats. In dogs and rats, coagulation parameters were inhibited after 7 daily doses of 300 mg/kg and higher, with death occurring with signs of internal hemorrhage. In a 28-day study in rats and dogs, coagulation times (PT and aPTT) were elevated by 15-80% at doses of 300mg/kg (rats) and 100 mg/kg (dogs). A structural similarity to other agents suggests that EPI-743 might act as a weak vitamin K antagonist. Therefore, standard clinical hematology tests will be performed periodically throughout the trial and prothrombin time/INR and PTT will be monitored throughout the trial. EPI-743 also caused increases in plasma cholesterol levels in rats and dogs, so cholesterol levels will be monitored during the clinical trial. The No Observable Adverse Effect Level (NOAEL) of EPI-743 after daily oral administration for 28 days to rats and dogs is 100 mg/kg, corresponding to AUC levels of approximately 60,000 and 180,000 ng.h/mL, respectively. Based on the pharmacokinetic results from the open label studies in patients treated with EPI-743 thus far, the AUC resulting from a dose of 100 mg p.o., t.i.d. is estimated to be below 3,000 ng.h/mL.

EPI-743 was not mutagenic in standard *in vitro* tests. EPI-743 did not cause any neurological changes as indicated by the functional observation tests conducted as part of the 28-day toxicity studies in rats, at doses up to 300 mg/kg. EPI-743 did not cause ECG changes in dogs during the 28-day toxicity studies at doses up to 100 mg/kg. EPI-743 did not cause changes in respiratory parameters in rats after single oral doses of up to 300 mg/kg.

The potential for enzyme induction by EPI-743 was assessed *in vitro*. Exposure of human hepatocytes to 0.1, 1.0 and 10.0  $\mu$ M EPI-743 did not result in significant induction of enzyme activities associated with CYP1A2, CYP2B6 or CYP3A4 isoforms *in vitro*. EPI-743 is not a significantly transported substrate or inhibitor of human P-glycoprotein *in vitro*.

As of September 2014, over 300 adults and children have been dosed with EPI-743 for a total of over 300,000 dosing days. Patients ranged in age from 22 days to 56 years. There

have been no dose-limiting toxicities reported; one patient had a possibly drug-related serious adverse event (clinically diagnosed pneumonia in a child with mitochondrial disease). In the current study (12-HG-0161), one patient had a non-serious Grade 4 elevation of serum CK considered probably related to the drug, although we do not know if the drug was EPI-743 or placebo.

As of November 2014, the dose level of 15 mg/kg has been evaluated in several trials in addition to the NIH study including:

1) Rett syndrome placebo-controlled trial: EPI-743 was administered at a dose of 15 mg/kg TID (dose ranging from 145 to 405 mg TID) for 6 months. There were no laboratory abnormalities related to drug treatment, no drug-related SAEs and no dose-limiting toxicities. 2) Leigh syndrome trial: A total of 31 children have been enrolled in an ongoing placebo-controlled trial (EPI743-12-002) at a dose level of 15mg/kg TID up to 200 mg TID. While treatment assignments are still blinded, all subjects have received at least 6 months of EPI-743 therapy based on the delayed start format of the trial. On review of blinded data, there have been no drug-related SAEs or dose-limiting toxicities and coagulation data show no abnormalities in PT/INR.

Blood chemistries, histories, and physical examinations obtained on every admission will help to detect any potential, unforeseen, or chronic toxicity of EPI-743. In addition, specific questioning about side effects will be performed on every admission by the nurse coordinator or an associate investigator.

Patients who develop grade 3 or 4 toxicity that is probably or certainly related to EPI-743 will be withdrawn from the study. Patients who develop grade 3 or 4 toxicity that is possibly related to drug, and patients with grade 1 or 2 toxicity per CTCAE criteria version 4.3 that is thought to be related to treatment with EPI-743, will have their EPI-743 dosage reduced to 100 mg TID and monitored in 2 weeks for correction of AE of concern, and thereafter per protocol. Should the INR toxicity not resolve to at least grade 1 in severity within two weeks of the initial dose reduction, administration of EPI-743 may either be further reduced or discontinued.

Once a patient's dose is reduced due to elevated INR thought to be related to study drug administration, the dose should not be re-escalated.

To further ensure safety, an interval analysis will be performed if and when 5 Serious Adverse Events have occurred, or after 10 patients have completed their courses of treatment, whichever comes first.

If a patient is terminated from the study for any reason, every attempt will be made to obtain a final evaluation that will include a history and physical examination, AE evaluation and documentation, and determination of the reasons for early termination, and follow-up laboratory testing and EKG.

## 9.2 Diagnostic interventions

The primary procedure-associated risk of this study consists of phlebotomy requirements, which are approximately 68 mL per admission and will never exceed 5 mL/kg at any one time or 9.5 mL/kg in any 8-week period, including blood drawn for ancillary tests and for other protocols. Blood volumes will include that collected locally, which will be monitored by the interim phone calls and by history upon scheduled admissions to the NIH Clinical Center. Discomforts include venipunctures (usually 2 per admission).

In selected patients, some procedures that are medically indicated to be performed once will be repeated to provide outcome measures. These may include an MRI of the brain, spine or muscle, EEG, videotaping, or neuropsychological testing.

#### 9.3 Radiation.

Only medically indicated radiation exposure will be incurred.

#### 9.4 Sedation.

Sedation will be given only for medically indicated studies.

#### 9.5 Psychological harms.

This protocol may give false hope; the principal investigator and NHGRI genetic counselors will attempt to avoid this.

#### 9.6 Risks to family relationships.

This therapeutic clinical trial does not involve DNA diagnostics *per se*, so there is no risk of divulging family relationships.

#### 9.7 Discrimination.

To the fullest extent possible, the investigators will not disclose to third parties any information about the participants without their expressed consent.

### **10. Privacy and Confidentiality of Medical Information/Biological Specimens**

#### 10.1 Will participant identifiers be attached to data?

Data files will have only numbers, not names. However, an identifier will be attached to the data, all of which will be maintained in records in a locked file cabinet within a secured laboratory or office.

#### 10.2 Clinical/demographic information.

Age, sex, diagnosis, and laboratory results will be part of the Clinical Report Form kept in a locked file within a secured office.

#### 10.3 How might information make specific individuals identifiable?

It is doubtful that any demographic information could identify an individual.

#### 10.4 Access to the key for patient identification.

The code to patient identities, as well as other patient data, will be kept in a locked file. Access to the code will be restricted to the Principal Investigator and Associate Investigators.

10.5 Will pedigrees be published?

We have no plans to publish pedigrees from this protocol. If we do, elements of a pedigree that could uniquely identify it will be disguised by, for example, not specifying the genders of certain members.

10.6 Will results be provided to participants?

Clinical data will be given directly to the patients at a debriefing session just prior to discharge. The results of outcome measures such as the NPMDS score, however, will be withheld so as not to unblind patients and their families.

10.7 Will identifiable information be released to third parties?

The investigators will not disclose to third parties any information about the participants without their expressed consent.

10.8 Sharing of data/samples with other researchers.

Data and samples may be shared with collaborators, but will not be transferred to another site with any identifiers. The fibroblasts sent to Edison Pharmaceuticals for stressor and EPI-743 response will be kept pending FDA action, and then destroyed.

10.9 Additional features to protect confidentiality.

None.

**11. Assessment of Significance of Study (Balance of Risks and Benefits)**

Patients eligible for this protocol have no proven therapy for their disorders. While the prospects of slowing the progression of disease are unknown, adverse events related to EPI-743 treatment are expected to be minimal. The risk/benefit ratio favors attempting EPI-743 treatment.

**12. Collection, Monitoring, Analysis and Reporting of Adverse Events**

Adverse events, protocol deviations, unanticipated problems (UP), serious adverse events, are defined as described in NIH HRPP SOP 16 (“Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations.”). All adverse events occurring during this study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and Acting CD as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and Acting CD as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event. Deaths will be reported to the Acting Clinical Director within 7 days after the PI first learns of the event.

The PI will immediately report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The PI will record nonserious AEs

and report them to the Sponsor according to the timetable for reporting specified in the protocol (21 CFR 312.64(b)).

Some minor protocol deviations are expected, and will not be reported to the IRB unless they occur at a rate greater than anticipated. These deviations include drawing blood later than planned, occasional missed individual lab test or EKG, rare missed vital sign documentation, missed NIH visits related to local hospitalizations or severe local weather impacting safe patient travel, failing to have the patient fast for a blood draw, creating a hematoma on blood drawing, etc.

#### 12.1 Exclusion of adverse event reporting.

There are a large number of expected events associated with the disorders affecting patients enrolled in this protocol.

Anticipated adverse events that known to be associated with EPI-743 and that are Grade 1 or Grade 2 will not be reported to the IRB unless they occur at a rate above 5%. Examples of expected adverse events include but are not limited to those events detailed in the Investigator's Brochure for [EPI-743](#) and in the protocol's risk section. The severity of organ and laboratory abnormalities is defined in the CTCAE ([http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE\\_4.03\\_2010-06-14\\_QuickReference\\_8.5x11.pdf](http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf)). If the rate or severity of these events exceeds the rate or severity anticipated in the protocol or investigator's brochure, the events will be classified and reported as though they are Unanticipated Problems.

In addition, certain non-serious Protocol Deviations are expected to occur approximately once every 3-6 months per patient, and these will not be reported to the IRB except at the time of the annual report. These include certain missed laboratory tests (such as an ESR, often missed locally), late laboratory tests (such as blood lactate levels or urine test results), and late telephone calls to check on the patients; any of these could be delayed by 1-2 weeks. Other reporting exclusions would be follow-up visits that are late by up to 3 weeks.

#### 12.2 Monitoring of adverse events related to this protocol.

Adverse events (AEs) will be collected from the time of study drug initiation to the 14.5-month followup. In addition, SAEs will be collected from the time informed consent is obtained to the 14-month end of study visit + 30 days for SAEs that are ongoing at the 14-month study visit. All AEs, whether observed by the Investigator, reported by the patient, from clinically significant laboratory findings, or other means, will be recorded in the patient's medical records and on the CRF. An Adverse Event Collection Form comprises Appendix E.

Adverse events will be monitored by phone calls or direct interviewing every week for the first month of each arm of the study, monthly after that, and 2 weeks after the last study drug dose. Adverse events occurring at the NIH Clinical Center will be recorded in the medical records and in study documents. Nurses on the unit (1NW) will monitor excessive blood drawing by tabulating totals. In addition, patients will have easy access to the treatment team to report adverse events by phone or in person.

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations"). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems, Unanticipated Adverse Device Effects and serious protocol deviations, will be reported to the IRB and CD (Clinical Director) as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

### 12.3 Data Safety and Monitoring Board.

Data from this study will be reported to the standing NHGRI DSMB. Reports will be made every 6 months. Early reporting will relate to safety, and the report to the DSMB will tabulate symptoms and abnormal laboratory findings.

Stop/Pause rules are given in Appendix D. A pause means that no new patients will be enrolled until the DSMB lifts the pause; enrolled patients will continue to receive drug. The DSMB will decide whether to stop/pause the study. Specifically, if 2 different patients experience the same Grade 3 adverse event, or if any patient develops a Grade 4 adverse event, the DSMB will be notified immediately and will decide if the study should be stopped. In addition, since 5 serious adverse events (SAEs) have occurred, the DSMB will be notified in real time of each subsequent SAE, and will make a determination regarding whether to continue the study or to impose a pause. A pause will occur automatically when 5 additional patients have SAE occurrences.

In addition, the DSMB will decide if individual patients who experience Grade 3 or higher adverse events that are probably or certainly related to EPI-743 will be withdrawn from the study.

For the extension study, the DSMB will continue to assume its current role with respect to adverse event evaluation and safety issues.

The FDA will be informed of any Stop in the protocol, and if the protocol is terminated.

### **13. Alternatives to Participation**

Patients with mitochondrial defects, other disorders of energy metabolism, or oxidation/reduction abnormalities frequently receive a cocktail of drugs designed to stabilize the electron transport chain. This alternative, often already attempted in our patients, remains a possibility.

#### **14. Consent Process**

14.1 The Principal Investigator or an Associate Investigator will obtain permission from both parents, as well as assent. Ample opportunity will be provided for the family to discuss the protocol with the research team and ask questions.

14.2 Parental permission and assent will be obtained in person at the NIH Clinical Center. If only one parent is physically available to provide consent, then consent may be obtained from the other parent via telephone, mail, or fax consistent with NIH Clinical Center policies.

On occasion, when verbal consent has been obtained but the patient has gone home to await the results of a test of eligibility, the parental permission form will be signed at home and mailed to the NIH.

14.3 The parental permission and assent forms are attached. Care is taken to inform each subject that EPI-743 may provide no benefit whatsoever, and could even be harmful.

14.4 Protections for vulnerable participants.  
An assent form is attached for children. In cases of legal guardianship, the rule of law will apply and the decision of the appointed surrogate will be honored. In questionable cases, an ethics consult will be obtained, and consent monitoring will be invoked. We will also assess for signs of agreement or objection conveyed through body language.

14.5 Special circumstances.  
None.

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