**In Utero Enzyme Replacement Therapy for Lysosomal Storage Diseases**

Presented by Jennifer L. Cohen, MD, Assistant Professor of Pediatrics, Division of Medical Genetics, Duke University

Dr. Cohen gave an overview of lysosomal storage diseases (LSD) which are individually rare, but collectively affect 1 in 5,000 live births. The clinical spectrum for LSDs ranges in severity and age of onset and there are subcategories of diseases. Dr. Cohen briefly discussed four main available and approved treatment modalities for disease treatment beyond symptomatic/supportive management including Enzyme Replacement Therapy (ERT). The goal of treatments is to reduce the build-up of the toxic substrate.

Dr. Cohen explained the pathophysiology of Pompe disease, disease classifications, and incidence. Infantile onset (IOPD), the focus of today’s presentation, has an onset less than 12 months old with cardiomyopathy. Late Onset (LOPD) affects individuals with onset less than 12 months without cardiomyopathy and all individuals with onset greater than 12 months old. Following implementation of U.S. newborn screening (NBS), the overall incidence of Pompe (IOPD and LOPD) is 1 in 16,000 to 1 in 22,000 live births. Thirty-six states currently conduct NBS for Pompe disease. Although Pompe disease is a part of the Recommended Uniform Screening Panel (RUSP), some states are slower to implement screening, due to legislative, infrastructure, and other issues. Strides are being made to fully implement screening.

CRIM

Dr. Cohen discussed cross-reactive immunologic material (CRIM), especially important in regard to IOPD. CRIM-positive indicates that there is some internally produced enzyme (occurs in most Pompe patients). CRIM-negative patients make little or no enzyme. This is the most severe form of IOPD, requiring immunosuppressive regimens to prevent anti-ERT antibody production. Blood tests can help predict CRIM status.

ERT for Pompe disease

Dr. Cohen discussed Pompe disease ERT, early treatment, alternatives, and limitations to current treatment. The two FDA-approved treatments are Myozyme/Lumizyme (standard of care for ERT) and Nexviazyme which was FDA-approved in August 2021 for LOPD and internationally approved for IOPD. FDA approval for IOPD patients is anticipated. Without ERT, IOPD usually results in death by age 1-2 years. Dr. Cohen reviewed the literature over the past several years. Studies have demonstrated that ERT begun before 6 months of age improves survival and morbidity. CRIM-positive NBS patients that are treated at median age 16 days had improved survival and improved ventilator-free survival. Timing of treatment is optimized through NBS with earlier diagnosis and treatment. A Duke University research group (Li, et al., 2021) demonstrated that early treated CRIM-negative patients (following NBS and at less than 4 weeks old) had improved overall and ventilator-free survival, improved left ventricular mass index (LVMI), and improved creatine kinase (CK) levels. CK is a muscle enzyme. Elevated CK levels indicate muscle injury or disease. Extensive research and experience show that days matter in diagnosis and treatment of IOPD. There are significantly better clinical outcomes when treatment is initiated within the first few days of life.

Alternatives to ERT

Alternatives to ERT are the emerging therapies and clinical trials for Pompe disease. Trials are conducted as observational and interventional studies for IOPD and LOPD. Interventional studies include next generation ERT, gene therapies, and IUERT for 8 different infantile onset LSDs. In terms of next generation ERT, the FDA is reviewing Biologics License Application (BLA) for cipaglucosidase alfa and the New Drug Application for misglustat for ATT-GAA. Gene therapy is through direct gene transfer or indirect gene transfer (which involves stem cell transplant). Multiple active gene therapy trials for Pompe disease can be found at the clinicaltrials.gov website. Challenges to gene therapy are being studied through trials and preclinical work.

Limitations of ERT in IOPD and a History of Fetal Interventions

Dr. Cohen outlined the limitations of ERT in IOPD: disease sequelae (consequences) present at birth; inadequate reversal of skeletal muscle damage postnatally; incomplete prevention of IgG anti-ERT antibodies; and residual disease despite early diagnosis with NBS. As context for discussion of IUERT, she gave a brief history of fetal surgery and fetal therapy beginning with amniocentesis in 1881 and a century later, in utero animal model interventions and the first successful human fetal surgery. She also described several other significant events. Dr. Cohen outlined molecular fetal therapies leading to 2020-present with the IUERT active Phase 1 clinical trial for eight LSDs. She noted that for the future, we hope gene therapy will be clinically available for hemophilia and LSDs.

IUERT: Advantages, Proof of Principle, Feasibility

*Advantages* of IUERT include tolerance induction in the fetus since the immature immune system may be tolerable to foreign antigens. This is due to special maternal-fetal immunology. Other advantages are prevention of disease sequelae and the ability for medication to penetrate brain cells before the blood-brain barrier forms. Postnatally, the blood-brain barrier cannot be crossed. The ability for medication to cross the barrier is especially important to LSDs with a neurological component. Single-gene disorders, which can be diagnosed prenatally, are potential candidates for IUERT and include LSDs. For prenatal diagnosis, there is rapid genetic diagnosis with molecular testing using amniocentesis or chorionic villus sampling (CVS); a family history of affected sibling(s), and parental carrier status determined with preconception carrier testing. Prenatal diagnosis through biochemical testing for LSD enzyme levels or molecular testing allows for early management. Dr. Cohen commented that if in utero efficacious treatment becomes clinically available, prenatal screening practices may change to include more of these conditions. In terms of delivery of IUERT, approved postnatal therapeutics are delivered intravenously. IUERT is administered systemically by accessing the umbilical veins with ultrasound guidance. This method is a routine procedure in fetal blood transfusions and the same is being used for in utero hematopoietic stem cell transplant trial for Alpha Thalassemia.

*Proof of principle* was conducted by colleagues at the University of California/San Francisco (UCSF) through research on another LSD, mucopolysaccharidosis VII (MPS VII). Dr. Cohen reported on their preclinical work involving in-utero ERT in the MPSVII mouse model. Their findings showed improved survival to birth, reduced storage material in the liver and spleen, improved bone growth, improved neurologic outcomes (grip strength, decreased brain cell inflammation), and tolerance of recombinant enzyme.

*Feasibility*

Dr. Cohen reported on the Phase I IUERT clinical trial. Primary objectives are to determine the safety to both fetus and mother and the feasibility of identifying patients who desire in utero therapy for each prenatally diagnosed LSD. Secondary objectives, efficacy-based, are to determine whether IUERT reduces biomarker accumulation, whether in utero exposure to recombinant enzyme induces tolerance, and long-term outcomes. Safety endpoints for the mother are allergic reaction, infection, and pregnancy complications. Safety endpoints for the fetus are survival to birth, survival to hospital discharge, allergic reaction, and anti-drug antibodies. Dr. Cohen noted that when the trial was launched, umbilical vein injection safety statistics indicated 0 fetal loss in 123 procedures at UCSF. Dr. Cohen discussed efficacy endpoints which vary according to the individual LSD but can include serum enzyme levels, amniotic fluid and urine biomarkers, anti-drug antibodies/tolerance to recombinant enzyme, and improvement of disease measures.

IUERT Phase I Clinical Trial – First Case

Dr. Cohen displayed a schematic of the IUERT trial steps: diagnostic confirmation with CVS or amniocentesis; IUERT infusions for 18-35 weeks, postnatal care with standard ERT, and yearly follow-up for five years. When the IUERT trial was launched, the patient community was engaged with outreach to groups such as AMDA, Duke University’s Pompe disease clinical program, Pompe Awareness, and National MPS Society. Published research by colleagues at UCSF (Schwab et al., 2022) on the preliminary interest of patients with LSD and their parents showed that a majority were likely to enroll in a Phase 1 clinical trial for fetal IUERT. A majority was also likely to choose an FDA-approved fetal ERT.

Dr. Cohen presented a case history of the first patient treated with IUERT. The patient was an IOPD CRIM-negative fetus. Dr. Cohen recapped the prenatal presentation of IOPD with onset less than 12 months AND cardiomyopathy. Symptoms include in utero hypertrophic cardiomyopathy and postnatally, cardiomyopathy, failure to thrive, respiratory distress and hypotonia. Treatment limitations of ERT are disease sequelae at birth, inadequate reversal of skeletal muscle damage, incomplete prevention of IgG anti-ERT antibodies, and residual disease despite early diagnosis with NBS.

The rationale for the IUERT clinical trial is underpinned by the knowledge that earlier postnatal treatment with ERT can improve outcomes, prenatal diagnosis is feasible, there has been successful preclinical work, and there is closely correlated genotype-phenotype association for both IOPD and CRIM status that allows for identification of affected fetuses who will be concordant with siblings, meaning having a similar clinical course for IOPD and CRIM status. This dictates the severity of disease and the need for immune tolerance induction (ITI) postnatally.

The case Dr. Cohen presented was of a pregnant female who presented at The Ottawa Hospital in Canada with a fourth pregnancy affected by CRIM-negative IOPD. Diagnosis was made using CVS and showed the same familial pathogenic GAA variant as in previous affected children. Prior collaboration between physicians at The Ottawa Hospital and Duke University in caring for the family led to discussion of options for this fourth pregnancy. The first affected baby, Sibling #1, was diagnosed based on clinical presentation. The baby received ITI and ERT at 6.6 months per protocol but died at age 29 months due to progressive disease. For the second affected baby, Sibling #2, the election was made to give palliative care. Sibling #2 died at age 8 months. For the current patient, Sibling #3, the option of IUERT interested the family. Discussions about care began early in the second trimester of pregnancy.

Dr. Cohen discussed the international collaboration and timeline for this first case. In the fall of 2020, the Phase I clinical trial of IUERT was launched at UCSF. In February 2021, video consultation was conducted with the family and medical teams at Ottawa, Duke, and UCSF. Due to COVID-19, the family was unable to receive treatment in California as part of the trial. Consequently, physicians at The Ottawa Hospital sought and received ethics approval for the provision of clinical care in a single patient at the hospital using the trial’s Institutional Review Board (IRB) protocol. On March 24, 2021, the first IUERT was administered to Sibling #3. Between March-June 2021, at 24-34 weeks gestational age, the baby received ERT, repeated every two weeks for a total of six infusions. In June 2021, at 37 weeks gestational age, Sibling #3 was born through an uncomplicated induced delivery.

Dr. Cohen discussed data analysis methods and control comparisons with siblings and CRIM-negative IOPD patients identified through NBS. Fetal echocardiograms showed no cardiac hypertrophy after IUERT in Sibling #3 compared with Sibling #2 who had been monitored prenatally. There was normal and sustained postnatal cardiac LVMI after IUERT in Sibling #3, compared with siblings and CRIM-negative NBS. Age-appropriate early motor skills were also seen in the IUERT patient (Sibling #3), compared with hypotonia and delayed motor skill milestones in the NBS controls. The IUERT patient walked independently at 11.5 months of age and showed consistent age-appropriate motor skills through one year. The IUERT patient’s CK levels were normal at birth and have remained normal. Glucose tetrasaccharide (Glc₄) levels in amniotic fluid matched levels seen in unaffected control patients. Glc₄ levels in the IUERT patient’s urine were stable and normal. The absence of deposits and glycogen-filled lysosomes in the placenta of the IUERT patient indicated normal placental pathology. The use of ITI postnatally in the IUERT patient eventually resulted in negative antibodies compared with Sibling #1 who had anti-ERT antibodies and required a second course of ITI.

Dr. Cohen summarized outcomes of this first human IUERT case. There were no adverse safety events, a full-term uncomplicated delivery, normal cardiac status, age-appropriate motor skills and assessments, normal and stable biomarker levels, and normal placental pathology. The patient is tolerating enzyme infusions postnatally and clinically doing well at the age of 16 months. She underscored that in 2021, the first child treated in the fetal period was successfully delivered, demonstrating safety for mother and fetus. Feasibility of IUERT with improved early clinical outcomes has also been demonstrated. There may be improvement in longer-term outcomes as well. The ultimate goal is treatment in the prenatal period.

Dr. Cohen thanked the patient and her family who made this all a reality. She also gratefully acknowledged her colleagues at The Ottawa Hospital, University of California/San Francisco, Duke University School of Medicine, and University of Washington. She noted that the IUERT Clinical Trial is now open for enrollment (ClinicalTrials.gov identifier NCT04532047). There is funding to support travel and medical costs. For more information, contact Dr. Cohen at [Jennifer.Cohen@Duke.edu](mailto:Jennifer.Cohen@Duke.edu) or [billie.lianoglou@ucsf.edu](mailto:billie.lianoglou@ucsf.edu) or [tippi.mackenzie@ucsf.edu](mailto:tippi.mackenzie@ucsf.edu).

**Question and Answer**

Q1: How early can IUERT be started?

A: 18 weeks gestation (the earliest it can be started according to the trial)

Q2: How accurate are preconceptions and prenatal testing for Pompe disease, especially given the number of rare mutations?

A: That is a good question. You have to know about what methodology the lab is using for testing panels. Are they looking for founder mutations or doing gene sequencing? It depends on which commercial lab. They might not capture 100% but may pick up more common mutations.

Q3: What dose was used?

A: 20 mgs per kg every two weeks which was the label dosing and using estimated fetal weight. Later the dose was boosted to 40 mgs per kg weekly based on recent published literature from multiple groups showing improved efficacy with this higher and more frequent dose.

Q4: Were there any changes in milestones with dosage change?

A: Dosage change did not occur until age 9.5 months, late to attribute responsibility for good outcomes but will certainly help the patient going forward.

Q5: Was there rounding of dosage?

A: With such small doses, not even a full vial, rounding was not done.

Q6: Is it accurate that with the IUERT, the ITI was started before the first dose was given after birth to prevent immune response after birth?

A: Yes, the ITI was given before the first dose postnatally, following standard of care. It also helped to bring down the small antibody response. Of note, Sibling #1 received ERT naïve ITI and mounted the same response and sustained it even longer. This is not totally unexpected that we did not get to zero in utero and obviously something under study.

Q7: The information about the blood-brain barrier in utero is intriguing. When does the blood-brain barrier form?

A: It forms postnatally. ERT administered postnatally does not cross this barrier. Based on the preclinical mouse model, the thought is that IUERT can reach the brain because there is no barrier. This will be looked at as a secondary outcome in the human trials.

Q8: So, if ERT does not cross the blood-brain barrier postnatally, you would start to see accumulation of glycogen in the brain.

A: Yes, that is accurate. The hope is that some damage from accumulation can be prevented in utero as a bridge to potentially other therapies that are coming on line, such as gene therapy.

On behalf of the AMDA, Tiffany House thanked Dr. Cohen for the incredibly interesting presentation and giving hope that Pompe disease may become a chronic disease and not fatal. She also thanked everyone for attending the webinar and the session was adjourned.