CLINICAL STUDY PROTOCOL

PROTOCOL TITLE: **Pilot study to assess clinical and pivotal biomarkers in the urine to predict the progression of nephropathy in Fabry disease.**

PROTOCOL NO: 23-LDRTC-01

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Principal Investigator Signature:_____**Date:**_____

INTRODUCTION

Nephropathy is one of the major contributors to morbidity and mortality in Fabry disease (FD). The standard non-invasive clinical laboratory tests for kidney disease (creatinine, glomerular filtration rate (GFR), and cystatin C) are not sensitive enough and may delay the therapeutic decision-making process. Additionally, the disease-specific biomarkers globotriaosylceramide (Gb-3) and globotriaosylsphingosine (Lyso-Gb-3) are not considered clinical entities associated with kidney damage. Therefore, the precise detection of the presence and the progression of kidney involvement is crucial for not only the development of effective therapies but also the prevention of late and irreversible complications in FD.

In FD, the typical clinical signs of kidney involvement may be absent, but biopsies may already show chronic glomerular and interstitial damage with fibrosis early in the disease course. The renal histology is characterized with intracellular Gb-3 deposits, even with normal GFR and minimal proteinuria. A characteristic finding is that the vacuoles correspond to extracted Gb-3 deposits in podocytes and epithelial cells. Heterozygote females develop the same kidney involvement as hemizygous males with FD (1).

The progressive accumulation of Gb-3 and its deacylated form lyso-Gb-3 creates an inflammatory environment (Figure 1). Furthermore, the accumulated Gb-3 and lyso-Gb-3 have been released into the cellular milieu from the ruptured lysosomes, leading to cell damage (2). Naturally sequestered inside live cells, these molecules are passively released extracellularly upon cell death and loss of membrane integrity. The elevated levels of both molecules in plasma and urine correlate with the impact of Fabry disease and may correspond to the short-term response to therapy with the reversal of cellular apoptosis.

Cells can die through several different mechanisms. Two of the significant types of cell death are necrosis and apoptosis. Sphingolipids are associated with apoptosis, where cells die while maintaining the integrity of their plasma membrane but are ingested by the tissue macrophages, in turn releasing pro- or anti-inflammatory signals such as TGF-beta or IL-10 (3). In contrast, necrotic cell death occurs in response to an insult such as infection and is associated with rapid loss of cell membrane integrity. Pyroptosis is a form of necrotic cell death due to a pro-inflammatory response (4). This pathway is uniquely dependent on Caspase-1, which is not involved in apoptotic cell death. Caspase-1 activation in macrophages results in the processing of Il-1β with subsequent activation of the transcription factor NF-κB.

The transcription factor NF-κB regulates multiple aspects of immune functions and serves as a pivotal mediator of inflammatory responses (5). NF-κB controls the expression of pro-

inflammatory cytokines (e.g., IL-1, IL-6, IL-8, and TNFα), chemokines (e.g., MIP-1, MCP1, RANTES, and Eotaxin), and vascular adhesion molecules (e.g., ICAM1, VCAM1, and E-selectin). NF-κB is also a central mediator of TGF-β1, the pathway central to the progression of fibrosis in multiple organs.

The responses induced by cell injury can cause significant damage while trying to heal the injured tissue. This is one of the reasons why it's important to elucidate these molecules in the urine not only to understand the order of pathogenic events associated with renal injury but also potentially block the cellular triggers of inflammation. Thus, this proposed study of pivotal urinary biomarkers may offer further insight on renal injury beyond current diagnostic tests.

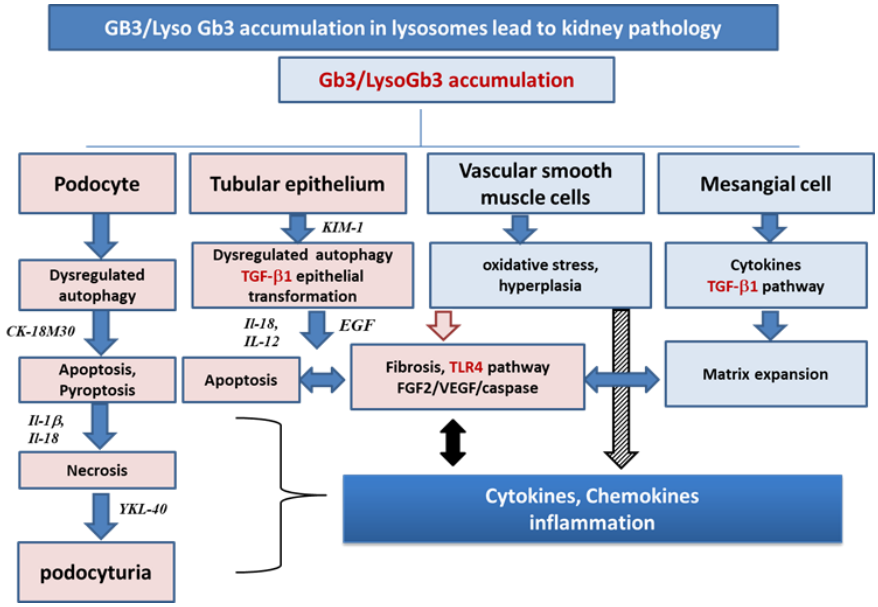

Figure 1. Fabry nephropathy. The pathogenesis leads to the activation of inflammatory cascades and fibrosis. The model was adopted from publication (6).

BRIEF RATIONALE AND BACKGROUND

Progressive nephropathy, glomerulosclerosis, tubular atrophy, and interstitial fibrosis are associated with inflammatory cellular infiltration. The Gb-3 accumulation in lysosomes leads to early cellular dysfunction followed by cell death, which activates inflammatory pathways and stimulates tissue fibrosis in Fabry disease. Progressive nephropathy, including glomerulosclerosis, tubular atrophy, and interstitial fibrosis, are associated with inflammatory cellular infiltration in the kidney. While Gb-3 accumulation is primarily located in endothelial and epithelial glomerular cells, histological studies demonstrate that the buildup of Gb-3 in podocytes plays an essential role in glomerular damage (7). Moreover, the study of urine-derived FD kidney epithelial cells and podocytes *in vitro* demonstrates that the cause of this damage lies in deregulated autophagy

pathways (7, 8). Deposition of Gb-3 in glomerular cells leads to focal segmental glomerular sclerosis. In addition, Gb-3 deposition in podocytes leads to activation of necroptosis (Figure 1). Podocyte injury, detachment, and podocyturia might be the initial pathogenic events in FD nephropathy (Figure 1). Gb3 accumulation in podocytes has been shown to resist ERT clearance partially. Moreover, the Gb3 accumulation in lysosomes continuously activates inflammatory pathways and stimulates pathogenetic processes, while the cellular clearance of Gb-3 by ERT limits histological damage.

It is well known that the NF-kB, TGF-β1, and Nothch1 signaling pathways play an essential role in nephropathy (Table 1 and 2). Moreover, Gb-3 deposition is associated with the increased release of TGF-β1 and epithelial-to-mesenchymal cell transition, leading to the over-expression of profibrotic molecules (Figure 1). TLR4 is involved in the initiation of this process leading to renal fibrosis via inflammasome activation in renal epithelial cells and, at the same time, dysregulated immune response (Tables 1 and 2).

Table 1:Urinary inflammatory biomarkers investigated in this study:

	NF-kB pathway	**TGF-β1 pathway**	**TLR4 pathway**
Cytokines	IL-6, IL-1β, TNF-α , Il-10	TGF-β1, Il-4, INF-gamma	Il-18, Il-12
Chemokines	MCP1, IL-8, RANTES, *BAFF*, APRIL		plasminogen activator inhibitor (PAI-1)

Common clinical biomarkers related to FD nephropathy (alpha-1-microglobulin, microalbumin, creatinine) will be compared with the pivotal biomarkers associated with the inflammatory and dysregulated immune pathways.

Table 2: Suggested pivotal biomarkers and pathways involved in Fabry nephropathy:

	Relationship to pathway or involvement		**Biomarkers**
Inflammatory biomarkers	Cytokines	NF-kB pathway	IL-1β, IL-6, TNF-α, Il-10
		TGF-β1 pathway	TGF-β1, Il-4, INF-gamma
		TLR4 pathway	Il-18, Il-12
	Chemokines	NF-kB pathway	MCP1, IL-8, RANTES, *BAFF*, APRIL
		TLR4 pathway	PAI-1
Biomarkers of kidney function and proteinuria	clinical laboratory urine biomarkers		alpha-1-microglobulin, albumin, cystatin C, GFR
	We will measure in our laboratory		B2M, bikunin, NGAL, osteopontin, clusterin, creatinin.
Acute kidney injury	*Marker* of *renal* tubular injury		KIM-1
	Inflammation-associated glycoprotein		YKL-40
	Growth factor, fibrosis		EGF
	Apoptotic marker		CK-18 M30
	Pyroptotic markers		*IL-1β, IL-18* (included in the list of cytokines)

This is a study to assess the markers related to autophagy, apoptosis, pyroptosis, and inflammatory markers related to NFkB, TNF-alpha, and TGF-β1 pathways in the urine. Urinary biomarkers will then be compared to the standard measures of kidney function and proteinuria: GFR, cystatin-C, B2M, bikunin, NGAL. Gb3 and Lyso-Gb3, urine microalbumin, and urine protein-to-creatinine (UPCR) ratio. We will also analyze the role of therapy, especially for the inflammatory responses in patients on stable enzyme replacement therapy (ERT) with that of patients naïve to therapy.

OBJECTIVE AND SPECIFIC AIMS

Primary objective*:* Identify biomarkers in urine for the detection and progression of kidney involvement in naïve male and female patients with FD, and compare with patients on ERT to assess the effects of ERT.

Specific Aims*:*

1) *To identify urinary inflammatory biomarkers associated with nephropathy in FD.*

2) *Analyze the association between urinary inflammatory markers, clinical urine biomarkers, circulating lyso-Gb3, and kidney involvement in naïve FD patients.*

3) *Analyze the association between urinary inflammatory markers, clinical urine biomarkers, circulating lyso-Gb3, and kidney involvement in FD patients on stable ERT.*

4) *Assess the role of the time of initiation and duration of therapy (ERT), respectively, inactivation and reversibility of inflammation cascade, and how these relate to the degree of kidney involvement in patients with FD.*

SUBJECT SELECTION

Number of Subjects

A total of 50 subjects, male or female, between the ages of 18 to 80 years of age, include 25 healthy individuals and 25 patients with Fabry disease.

Study population

FD patients n=25 (5 without clinical evidence of nephropathy and 15 patients with clinical evidence of nephropathy), NAÏVE FD patients (n=5), and healthy controls n=25 (controls, urine samples are not actual patients, urine samples will be purchased from a commercial company).

The study will involve 50 age-and gender-matched subjects and controls divided into the following cohorts:

1. FD subjects without clinical evidence of nephropathy (normal eGFR and no microalbuminuria) naïve or on ERT **(n=5**),
2. FD subjects with clinical evidence of nephropathy **(n=15)**
   Subcohorts:
   a. *FD subjects on ERT with CKD stage 1 (estimation n=5)*
   b. *FD subjects on ERT with CKD stage 2 (estimation n=5)*
   c. *FD subjects on ERT with CKD type 3 a or b (estimation n=5)*
3. FD patients naïve to therapy (**n=5**). Can we be included in Cohort group 1 or 2. Additional analysis will be stratified NAÏVE vs. ERT.
4. Non-FD age-matched controls with no known kidney disease n=25.

	FD	**Healthy control**
Total FD	**25**	**25**
without nephropathy	5	
with nephropathy	15	
NAIVE	5	

Table 3. Clinical staging of FD patients:

Clinical parameter(s)	**FD patients and kidney involvement**				**Age-matched controls**
	Stage 1	**Stage 2**	**Stage 3a**	**Stage 3b**	
Kidney involvement					
Kidney damage	Normal/	Mild reduction	Moderate reduction	Moderate reduction	normal
GFR ml/m1.73 m^2	>90	60-89	45-59	30-44	90 min
Cystatin-C	0.5-0.9	1.0-1.2	1.3-1.5	1.4-1.9	0.5-0.9
Urine B2M	-	0.428-0.603	0.603-0.950	0.950-1.034	-
FD biochemical markers					
α–Gal A	Deficiency	Deficiency	Deficiency	Deficiency	Normal
GLA mutations	Yes	yes	yes	yes	No
Gb-3	Yes	yes	yes	yes	No
Lyso-Gb-3	Yes	yes	yes	yes	No

Gender of Subjects

Subjects of both genders will be included in the research, and it is expected that this inclusion will be representative of the FD patients population.

Age of Subjects

Subjects will be between the age of 18 to 80 years of age.

Racial and Ethnic origin

There are no enrollment restrictions based on race or ethnic origin, and it is expected that racial and ethnic representations in this study will represent the FD population.

INCLUSION/EXCLUSION CRITERIA

Inclusion criteria:

a. Male and Female subject is greater than 18 but not older than 80 years.
b. Subject willing to sign the informed consent and/or assent.
c. Confirmed diagnosis of Fabry disease based on deficient α–Gal A enzymatic activity and molecular analysis demonstrating pathogenic variants in the *GLA* gene.

Exclusion criteria:

a. Any other known genetic condition associated with CKD,
b. Evidence of hepatitis B or C infections or other chronic infectious diseases,
c. Pregnancy or breastfeeding.
d. Any other chronic condition, as per PI's discretion, that makes the subject ineligible.

Description of criteria for withdrawal from the study

A subject may withdraw from the study at any time. The study is entirely voluntary.

RECRUITMENT:

Upon identifying patients with FD disease by the Principal Investigator, the research coordinator will recruit the study subjects. The research coordinator will recruit healthy controls in the clinic or purchase from a commercial company.

SETTING/LOCATION

Research subjects will be recruited at the **Lysosomal and Rare Disorders Research and Treatment Center, Inc (LDRTC)** located at 3702 Pender Drive, Suite 170, Fairfax, VA 22030, and/or 121 Congressional Lane, Ste 320, Rockville, MD, 20852.
Subjects recruited at these facilities will be receiving advice from Ozlem Goker-Alpan, MD, and be under her clinical care. All experimental/laboratory procedures will be performed by or under the supervision of Margarita Ivanova, Ph.D. All informed consent processes will take place at the indicated research sites.

Laboratory tests will be performed at **Lysosomal & Rare Disorders Research & Treatment Center, Inc,** whereas some assays such as Lyso-Gb3 will be performed outside collaborating laboratories such as Labcorp Diagnostic Laboratories and/or Centogene, Rostock, Germany.

STUDY PROCEDURES

Study type

Descriptive and observational study of molecular components detected in biological specimens of urine.

Study design and plan

This prospective study aims to establish novel biomarkers for non-invasive detection of kidney involvement in FD. FD patients will be assessed using the definition of Kidney Disease Outcomes Quality Initiative (KDOQI) 2002. The patients with CKD stages 3 or less with a progressive decline in kidney function, as evidenced by a decreasing GFR and a slope of less than -1.5, will be further assessed.

The glomerular filtration rate will be calculated from serum creatinine (CKD-EPI). The UPCR will be used to measure proteinuria. Additionally, the patients will be stratified as having low renal involvement (LRI) if the UPCR is less than or equal to 0.5 g/g. Anti-drug antibody status and other adverse events, such as the occurrence and the progression of cardiomyopathy as evidenced by LVMI, LVPWD, and neurological events such as strokes, will be recorded through the study. The categorization of kidney involvement via renal biomarkers will be defined (Table 4).

Table 4. Clinical staging of FD patients:

	FD patients and kidney involvement				**Age-matched controls**
Clinical parameter(s)	**Stage 1**	**Stage 2**	**Stage 3a**	**Stage 3b**	
Kidney involvement					
Kidney damage	Normal/	Mild reduction	Moderate reduction	Moderate reduction	Normal
GFR ml/m1.73 m^2	>90	60-89	45-59	30-44	90 min
Cystatin-C	0.5-0.9	1.0-1.2	1.3-1.5	1.4-1.9	0.5-0.9
Urine B2M	-	0.428-0.603	0.603-0.950	0.950-1.034	-
FD biochemical markers					
α–Gal A	Deficiency	Deficiency	Deficiency	Deficiency	Normal
GLA mutations	Yes	yes	yes	yes	No
Gb-3	Yes	yes	yes	yes	No
Lyso-Gb-3	Yes	yes	yes	yes	No

The study will take place over 24 months. The clinical part of the active study to collect clinical data and the samples will be over 12 months. The urine samples will be collected three times (0, 6, and 12 months) (Table 5). A physical examination will be performed during the visits, and samples will be collected. We will review medical records as a part of the clinical evaluation. After a urine specimen collection, samples will be aliquoted and stored at -80^0 C until biomarkers are screened. We expect to recruit the subjects within 6 months of the study initiation; thus, the last visit for the last subject is expected to be 19 months after the study starts.

Table 5. Visit and assessment schedule:

	baseline		
visits	**1**	**2**	**3**
	month 1/ enrollment	**month 6**	**month 12**
Vital signs	X	X	X
Height and weight	X		X
Physical examination	X		X
Concomitant medications	X	X	X
Adverse event reporting	X	X	X
Informed consent form	X		
Eligibility (inclusion/exclusion)	X		
Urine pregnancy test (part of eligibility)	X		
HBV/HCV testing (if clinical results are not available)			
Medical history	X	X	X
Retrospective chart review	X		
Urine sample collection and urinalysis	X	X	X

STUDY METHOD AND PROCEDURES

Informed consent will be obtained prior to the execution of any research procedures. The subjects will be followed for a 12 month period during which urine will be collected three times every six months. Subsequent samples will be obtained based on the Principal Investigator's order. Biological samples are only collected for research purposes and will not be sold or distributed for other purposes.
After the study is completed, biological samples may be retained for possible future exploratory analysis by the Principal Investigator and/or by the Co-Investigator.

CONSENT/ASSENT:

Enrollment of a subject or a family member in this study will be completed once all inclusion and no exclusion criteria have been met and the subject has signed the informed consent form (ICF). The clinical research coordinator will approach subjects identified by the Principal Investigator.

The Principal Investigator will discuss the research study with the subject explaining research procedures, potential risks, and benefits. The Principal Investigator will give the subject an

opportunity to ask questions, and ample time will be given to the subject to review the ICF in detail. The clinical research coordinator will repeat this process using verbiage found in the consent to explain the study.

The alternative method for consenting:

The ICF could be emailed or mailed to the patient with a pre-paid addressed return envelope provided for convenience. The patient should be contacted at a later time to confirm receipt of the ICF and be given an opportunity to ask questions. The potential subject will have ample time to review the ICF in detail. Once all questions have been answered to the subject's satisfaction, research personnel should provide guidance as to what page requires the subject's printed name, signature, date, and witness signature (if applicable). A copy of the original signed ICF will be provided to the patient.

DEMOGRAPHICS AND MEDICAL HISTORY

A review of demographic parameters, including age, gender, race, and ethnicity, will be performed at screening.

Past and present medical and surgical history will be recorded according to the Schedule of Assessments. Any ongoing condition and signs and symptoms observed prior to the initiation of the study will be recorded as past medical history.

Disease history specific to FD will be recorded and includes the date of diagnosis, primary symptoms at time of diagnosis, and disease progression history. A separate chart review will be conducted as indicated in the schedule of events to assess Fabry disease-related kidney, cardiac, and nervous system involvement, history of strokes and presence and nature of pain, and the history of acute pain crises, history of the treatment of FD including Enzyme Replacement therapy, and other treatments such as ACE and ARBs for proteinuria, presence of anti-drug antibodies and history of immune hypersensitivity reactions will be specifically noted.

CONCOMITANT MEDICATIONS AND PROCEDURES

Subjects will be asked to report any new or changes in previously reported prescription and non-prescription medications, including dosage, frequency, and administration dates. Information will be entered in the source records. Information regarding any medical or surgical procedures performed since the last visit will also be collected.

URINALYSIS

In addition to the exploratory laboratory tests, a clinical urinalysis will be performed.
Urinalysis will include the following: Specific gravity, appearance, color pH, glucose, protein

(total), ketones, bilirubin, urobilinogen, hemoglobin, leucocyte esterase, reducing substances, and nitrite.

OTHER LABORATORY TEST

Virus Serology

Subjects are required to have negative serology test results for hepatitis B surface antigen and hepatitis C antibody at screening to participate in the study.

Use of Biological Samples for Future Research

Researchers may use leftover samples from already collected and deidentified biological samples obtained from consented subjects during the study for other future investigations

Vital Signs

Vital signs will include temperature, blood pressure, respiration rate, and heart rate. Vital signs will be measured as specified in the Schedules of Assessments (1) and as needed per PI.

Physical Exam

A physical examination will be performed by a study physician or designee, as specified in the Schedule of Assessments (Table 2). The physical examination includes skin, head, ears, eyes, nose, throat, heart, lungs, abdomen, and neurologic systems. An additional examination may be performed by the Investigator.

Weight and Height

Weight and height will be measured using proper scales. No bulky clothes or shoes should be worn.

BENEFITS:

There are no immediate benefits to subjects for participation; however, knowledge obtained from this study may lead to improved understanding, diagnosis, and therapeutic interventions in the future.

SUBJECT COMPENSATION:

Costs: There are no costs to subjects. Subjects will not be charged for the costs of procedures or sample analysis.

Payment: No subject compensation will be provided.

HUMAN SUBJECT PROTECTIONS

Risks:

There is some risk to subjects participating in this study:

There are risks of personal information being inadvertently released. The research team will try its best to protect research participants' health and personal information. To minimize this risk, only researchers at **Lysosomal & Rare Disorders Research & Treatment Center, Inc** will have access to biological specimens collected in this protocol, and all specimens will be deidentified. In the case of any sample being shared with other researchers, they will only receive specimens labeled with a generic study identification number that cannot be linked back to the research subject. No other identifying information will be shared with them.

Alternatives:

The subject has the right to decide not to participate in the research study.

Confidentiality:

All patient data will remain confidential in accordance with the Health Insurance and Portability and Accountability Act (HIPAA). No patient identifiers will be used to label biological specimens. All biological specimens will be deidentified using a generic identification number.

Adverse Event Reporting:

All adverse events will be reported to the IRB according to the Internal Conference of Harmonization (ICH) Good Clinical Practice (GCP) adverse reporting guidelines section 6 and **Lysosomal & Rare Disorders Research & Treatment Center, Inc** standard operating procedures section 6 regarding reporting of adverse events and unanticipated problems.

Adverse events (AE) Definitions: An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, a new disease, worsening in severity or frequency of a concomitant disease, temporally associated with the use of a medicinal product, whether or not the event is considered causally related to the use of the product.
Although abnormal laboratory values are typically not considered AEs, the following considerations may result in an abnormal laboratory value being considered an AE:

- A laboratory test result that meets the criteria for an SAE
- A laboratory test result that requires the patient to receive specific corrective therapy
- A laboratory abnormality that leads to discontinuation of therapy
- A laboratory abnormality that the health care provider considers to be clinically significant

Serious Adverse Events: A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- Results in death. Note that death is an outcome of an event. The event(s) causing death should be recorded.
- In the view of the Health care provider, it places the patient at immediate risk of death (a life-threatening event); however, this does not include an event that, had it occurred in a more severe form, might have caused death
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- An SAE may also be any other medically important event that, in the opinion of the Health care provider, may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. (Examples of such medical events include

A Special Situation Report (SSR) includes any of the following events:

- Suspected transmission of an infectious agent: Suspected (in the sense of confirmed or potential) transmission of an infectious agent by a medicinal product.
- Lack of efficacy
- Accidental/Occupational exposure
- Use outside the terms of the marketing authorization, also known as "off-label"
- Use of falsified medicinal product
- Drug-drug interactions and drug-food interactions
- Inadvertent or accidental exposure with or without an AE
- Unintended benefit

  An SSR should be reported even if there is no associated AE.

SAEs, AEs, ADRs, SSRs and PQIs in the healthcare record/source or other applicable source data that are part of the study objectives or endpoints

Events/issues which are part of the study objectives or endpoints will be systematically identified and collected from healthcare records or other applicable source records and summarized as part of any interim analysis and in the final study report. Such events do not need to be notified as individual reports to Takeda Pharmacovigilance.

Events/Issues which are not part of the study objectives and endpoints will not be abstracted or collected from healthcare records or other applicable source records.

Reporting of Adverse Drug Reactions to Regulatory Agencies and IRB/EC

LDRTC is responsible for reporting serious and non-serious adverse drug reactions suspected of being related to to regulatory authorities. The Investigator is responsible for reporting adverse drug reactions to the IRB/EC, if required by national law or regulation, within the timelines required by such law or regulation. The Investigator shall maintain records of all such submissions.

Study-specific committees

No steering committee, data safety monitoring committee, or clinical endpoint committee will be used in this study.

STUDY OVERSIGHT

Premature Termination of Study:

Ozlem Goker-Alpan, MD, may prematurely terminate the study. Reasons for termination may include but is not limited to the following:

- Series of adverse events or incidences associated with subject participation.
- Low subject enrollment.

Clinical Research Quality Assurance:

To ensure that GCP and good clinical research are conducted per regulatory requirements and guidelines, the study will be made available for monitoring, auditing, review, and inspection by regulatory authorities such as IRB and FDA. This will be accomplished by providing direct access to study-related documents.

Data management

The acquired data will be entered into Excel for further analysis. Data will be analyzed using GraphPad Prism software (Graphpad Inc., La Jolla, CA) using ANOVA analysis and Student's t-test for statistical analysis. $p<0.05$ is considered significant.

Samples and data storage

Once subjects have signed the informed consent form, they will be assigned a personal generic research study identification number. This number will be matched with the patient's name and demographic information in a file stored on a password-protected computer. Only members of the research team at **Lysosomal & Rare Disorders Research & Treatment Center, Inc** will have

access to this file. Urine samples shipped for further analysis and viable cells stored long-term in liquid nitrogen freezers will be done without any identifiers attached. Research data from urine analysis will be stored in a password-protected separate file shared online between researchers at **Lysosomal & Rare Disorders Research & Treatment Center, Inc.**

IRB REVIEW/ ETHICS/ INFORMED CONSENT

The protocol, informed consent, and all other relevant documents will be submitted to the IRB for review, and approval will be obtained before the study is initiated per the required guidelines. This study will be conducted with responsible clinical research practices in accordance with the ethical principles that have their origins in the Declaration of Helsinki and that are congruent with GCP guidelines established at the ICH and other applicable regulatory requirements. The study will be conducted in accordance with the regulations of the United States Food and Drug Administration (FDA) as described in 21 CFR 50 and 56, applicable laws, and IRB requirements.

The Principal Investigator will submit any changes to the protocol to the IRB for review and approval before implementing such changes. However, a protocol change will be implemented immediately if the change is intended to eliminate or minimize harm to subjects. Such change will be notified to the reviewing IRB and the FDA within 10 working days.

The Principal Investigator is responsible for providing each subject with full and adequate verbal and written information using the IRB-approved informed consent form as described above. Informed consent will be obtained prior to performing any study-related procedures. A copy of the signed consent form will be given to the subject.

Clinical research quality assurance

To ensure GCP and good clinical research conduct per regulatory requirements and guidelines, the study will be made available for monitoring, auditing, review, and inspection to regulatory authorities such as IRB and FDA. This will be accomplished by providing direct access to study-related documents.

Monitoring will be done in accordance with the LDRTC SOPs. The Site will maintain Source documents. Source documents are defined as original documents, data, and records. The Investigator guarantees access to source documents by the sponsor and by the IRB. All aspects of the study and its documentation will be subject to review if required, including but not limited to the patient's medical records, informed consent documentation, documentation of the patient or the participant's caregiver authorization to use personal health information, and patients' database and associated source documents.

Protocol Deviations

The Investigator should not deviate from the protocol, except where necessary, to eliminate an immediate hazard to study patients. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the Investigator should consult with the sponsor or designee (and IRB, as required) to determine the appropriate course of action. There will be no exemptions (a prospectively approved deviation) from the inclusion or exclusion criteria.

The Site should document all protocol deviations in the patient's source documents. In the event of a significant deviation, the Site should notify the sponsor or its designee (and IRB/IEC, as required). Significant deviations include but are not limited to those that involve fraud or misconduct, increase the health risk to the patient, or confound the interpretation of the primary study assessment. A Protocol Deviation Form should be completed by the Site and signed by the sponsor or designee for any significant deviation from the protocol.

DATA HANDLING AND RECORDKEEPING

The Site will collect patient information from patient medical charts.
The Site will follow its internal procedures for the Development of Study Specifications for the Development of the password-protected Excel, where patient data will be extracted. The Site will follow their internal procedures for the testing and live production of the study-specific database. The Site will document their entry method, QC process, and if entry/readability of file is limited to certain staff. The Site will keep Master Study File where they will document data entry processes. The Site will follow their internal procedures for the management of user access to the system and database. The Site will have data transfer and quality control procedures in place and documented to minimize data transcription errors if applicable. All Site staff involved in the handling of study data will have documented training on the relevant procedures.
The Site will ensure that protected health information is handled in compliance with national privacy/data protection laws and regulations. The Site will ensure that all personal health information has been de-identified. Study data and patient records will be archived per the Site's policies and will be made available to Regulatory Agency upon request. AEs, medical history, and concurrent medical conditions will be coded using the most recent Medical Dictionary for Regulatory Activities.

CONFIDENTIALITY

All subject data and personal information will remain confidential in accordance to the Health Portability and Accountability Act and the Privacy Rule. However, all subject study-related records upon request may be disclosed to regulatory agencies such as the United States FDA and overseeing IRB for inspection. If shared with them, the Privacy Rule no longer covers subject information, but these entities are also committed to keeping subject information confidential. Subject information will be kept confidential and will not be made publicly available. If any results

are published as a result of this research study subject identity will remain confidential, as only aggregate data will be reported.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov.
This Web site will not include information that can identify the subjects. At most, the Web site will include a summary of the results. Subjects can search this Web site at any time.

INTENDED USE OF DATA

The data collected and analyzed will be used to understand the renal function in Fabry disease better. Data may be used and stored until all research-related activities are completed.

REFERENCES

1. Fogo AB, Bostad L, Svarstad E, Cook WJ, Moll S, Barbey F, et al. Scoring system for renal pathology in Fabry disease: report of the International Study Group of Fabry Nephropathy (ISGFN). Nephrol Dial Transplant. 2010;25(7):2168-77.
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