Evaluation Of Safety and Efficacy of Metreleptin Treatment for Patients with Multiple Symmetric Lipomatosis With Additional Phase for Long-Term Treatment

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Protocol

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This protocol will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

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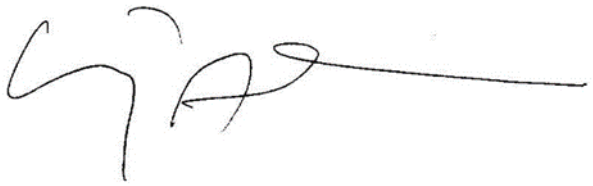

Elif A. Oral, M.D.
08/30/2024

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List of Abbreviations

AE	Adverse event
ATDC	Adipose tissue dendritic cells
ATM	Human WAT macrophages
BAT	Brown adipose tissue
BP	Blood pressure
BUN	Blood urea nitrogen
CK	Creatine kinase
COL1A1	Collagen, type I, alpha 1
COL6A1	Collagen, type VI, alpha 1
CRP	C-reactive protein
DEXA	Dual-energy X-ray absorptiometry
EC	Endothelial cells
ECG	Electrocardiogram
GGT	Gamma-glutamyl transferase
HbA1c	Glycated hemoglobin
HDL	High-density lipoprotein
HR	Heart rate
iPSCs	Pluripotent stem cells
LDL	Low-density lipoprotein
LDH	Lactate dehydrogenase
MFN2	Mitofusin 2 gene
MRI	Magnetic resonance imaging
MSL	Multiple Symmetrical Lipomatosis
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
SAE	Serious adverse event
SAT	Subcutaneous WAT
SVF	Stromal vascular fraction
SOA	The Schedule of Assessments
TG	Triglyceride
VAT	Visceral WAT
VLDL	Very low-density lipoprotein
WAT	White adipose tissue

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1.0 BACKGROUND AND RATIONALE

1.1 Multiple Symmetric Lipomatosis and Adipose Tissue Physiology

Adipose tissue (AT) depots differ in embryological origins, gene expression profiles and hormonal regulation pattern according to anatomical location (visceral or subcutaneous) and major cell type constituent (white, brown, or beige/brite adipocyte) (Kahn, Wang et al. 2019). Understanding the metabolic regulation of different adipose depots and their interaction with systemic metabolism can help identify therapeutic targets for several human pathogenic conditions.

Multiple symmetric lipomatosis (MSL) is a rare disease characterized by symmetrical deposition of adipose tissue (AT) in the neck and upper body, associated with severe lipoatrophy in the distal segments of arms and legs (Herbst 2012). The association of MSL with mitofusin 2 (MFN2) was first described in three patients from one pedigree showing compound heterozygous *MFN2* pathogenic variants p.G108R and p.R707W (Calvo, Funalot et al. 2009). Subsequently, case series of patients with MSL, partial lipodystrophy and distal axonal neuropathy have been reported to be associated with biallelic variants in *MFN2* (homozygous recessive p.R707W pathogenic variant) (Calvo, Funalot et al. 2009, Sawyer, Cheuk-Him Ng et al. 2015, Rocha, Bulger et al. 2017). In addition, these patients had insulin resistance, non-alcoholic fatty liver disease, elevated lactate, extremely low leptin, and low adiponectin (Rocha, Bulger et al. 2017).

MFN2 gene encodes a GTPase protein localized at the mitochondrial outer membrane involved in mitochondrial dynamics such as homotypic interaction (Carr, Polke et al. 2015) and previously associated with Charcot-Marie-Tooth disease type 2, a hereditary axonal polyneuropathy (de Brito and Scorrano 2008, Dorn 2020). R707 is in the carboxy-terminal domain of *MFN2*, critical for homotypic and heterotypic interactions of MFN2 and regulates mitochondrial membrane fusion (Honda, Aihara et al. 2005, Cohen and Tareste 2018). Histological examination of the AT from affected human subjects have shown unilocular adipocytes and negative UCP1 immunostaining (Rocha, Bulger et al. 2017). Gene expression profile revealed atypical features with characteristics of both white and beige/brown AT, and transcriptional study of the AT revealed a gene expression pattern consistent with mitochondrial dysfunction (Rocha, Bulger et al. 2017, Capel, Vatier et al. 2018). Increase in body fat can reduce the *MNF2* mRNA in humans, and specific deletion of MFN2 in AT results in an obese phenotype (Mancini, Pirruccio et al. 2019). Besides the broad knowledge of the mitofusin's role in Charcot-Marie-Tooth disease, there is still a lack of information on its role in adipose tissue in general and specifically the pathophysiology in MSL (Dorn 2020).

Additionally, fibroblast growth factor 21 (FGF21) levels were found to be elevated in patients with MSL due to *MFN2* mutations, suggesting that thermogenesis could play a role in the pathogenesis of this disease (Enzi, Busetto et al. 2015, Capel, Vatier et al. 2018). Obesity and lipodystrophy represent opposite states of AT accumulation, but both can have metabolic disorders such as insulin resistance and metabolic syndrome (Fox, Massaro et al. 2007, Kahn, Wang et al. 2019, Foss-Freitas, Akinci et al. 2020). The association of increased AT mass with obesity may depend not only on the balance between energy intake and utilization but also on the balance between white and brown AT (Nedergaard, Bengtsson et al. 2011). On the other hand, pathologic AT deficiency, observed in lipodystrophy (Foss-Freitas, Akinci et al. 2020), is also associated with metabolic disorders, illustrating the complex interaction between body fat and metabolic homeostasis (Cypess and Kahn 2010, Fisher, Kleiner et al. 2012). Furthermore, AT depots differ in embryological origins, gene expression profiles and hormonal regulation pattern according to anatomical location (visceral or subcutaneous) and major cell type constituent (white, brown, or beige/brite adipocyte) (Kahn, Wang et al. 2019).

1.2 A brief summary of patients with MFN2 associated lipodystrophy/MSL

At the University of Michigan, we are currently following three pedigrees, each with two affected siblings with *MFN2* associated MSL. Homozygous *MFN2* R707W (c.2119C>T) pathogenic variant was detected in all patients. Patient 3 (Now an 18-year-old men at diagnosis) and patient 4 (14-years-old boy at diagnosis) are siblings from another pedigree. The older sibling in this pedigree has evidence for axonal neuropathy and foot deformities. Both parents are heterozygous for R707W. Besides these two patients, the family also has two other boys, one is confirmed to be normal, and the other is heterozygous for R707W. Patient 5 is a 56-year-old female, reporting face and upper body fat tissue expansion starting when she was 15 years old; she also presented with distal axonal neuropathy. Her sister has clinical manifestations compatible with MSL (patient 6). Patient 7 is a 61 years-old female presenting increase in fat tissue in face and upper body since she was 17 years old and evolving with acromegalic features and macroglossia, was also, diagnosed with Charcot Marie-Tooth neuropathy at age 44. All patients share the phenotypic features of the disease characterized by the accumulation of fat in the upper part of the body and lipoatrophic upper and lower limbs. Table 1 summarizes the metabolic profile of the patients eligible for treatment with Metreleptin, followed at the University of Michigan. Although variable, all patients have some degree of insulin resistance, but none of them has crossed the clinical threshold of diabetes so far. Triglyceride levels are elevated ranging from around 200 mg/dL to 1300 mg/dL. Remarkably, all patients have very low leptin levels and an enlarged liver with mildly impaired liver function. Obstructive sleep apnea is diagnosed in two patients due to the accumulation of AT in the neck. Affected patients have variable degrees of scoliosis, likely a secondary effect of adipose tissue overgrowth during adolescence.

Table 1: Metabolic profile of patients followed at the University of Michigan and eligible for treatment with Metreleptin. (data from September 2021)

	P3	P4	P5	P7
Sex	M	M	F	F
Current age (years)	21	18	56	61
Diagnosis age (years)	17	14	53	45
Age of onset (years)	14	14	15	17
Height (cm)	167	151	151	167
Weight (kg)	74	53.8	62.2	69.5
BMI (kg/m^2)	26.5	23.5	27.2	24.7
Glucose state	Normal	Normal	Normal	Normal
Insulin resistance	Yes	Yes	NA	NA
Triglyceride (mg/dL)	200-1300 range	<200	177	238
eGFR	84	NA	106	111
Liver enzymes (U/L)	100-200 range	Normal	Mildly elevated	Normal
Enlarged liver	Yes	Mildly enlarged	NA	NA
Fatty liver	Yes	No	NA	NA
Leptin level (ng/mL)	undetectable	undetectable	0.7 ng/mL	undetectable

Adiponectin level (mcg/mL)	<2	<2	<2	2
Medical comorbidities	Neuropathy (Charcot-Marie-Tooth disease type 2), hypertension, mild asthma, thrombosis of inferior vena cava, OSAS	OSAS	Fasciculations, PCOS	Acromegalic features and macroglossia. Neuropathy (Charcot-Marie-Tooth disease).

NA: Not available, not assessed on site, eGFR: estimated glomerular filtration rate.

1.3 Leptin levels are low in MSL

Despite prominent upper body adipose overgrowth in MSL, levels of adipocyte hormones leptin and adiponectin are strikingly low, adding the name of the disease to the list of disorders with severe leptin deficiency in humans. Rocha et al. showed that leptin secretion from adipose explants taken from the neck and abdominal subcutaneous adipose tissue of one patient and surgical biopsies of overgrown interscapular subcutaneous adipose tissue in two patients was undetectable, and leptin mRNA expression was suppressed. Capel et al. further confirmed that unlike normal expression of several mature adipocyte markers, leptin and adiponectin expression was markedly decreased in adipose tissue from subjects with MSL.

The mechanism of suppressed leptin expression in MSL is not clear. One hypothesis would be low levels of leptin expression in the pathologically expanding cell lineage as reported for brown adipocytes. On the other hand, leptin gene expression could be altered by complex cellular mechanisms sensing mitochondrial function and cellular energy state.

From a therapeutic point of view, one observation has been that although liposuction may only offer temporary benefit and MSL returns, it may promote short term improvement in glucose control. On the other hand, levels of thermogenic factors FGF21 and CITED1 were found to be elevated (although UCP1 was very low), and 18F-FDG-PET-scan revealed increased fat metabolic activity in patients with MSL, proposing the idea that FGF21 promotes thermogenesis, glucose uptake, and metabolite oxidation which may embody a protective mechanism against metabolic complications in these patients. Although the presence of moderate metabolic abnormalities in most patients (despite very low leptin) supports this idea, the amount of evidence is limited, and some patients may exhibit severe hypertriglyceridemia and clinically significant hepatic steatosis.

1.4 Myalept: a treatment for lipodystrophy and leptin deficiency

Metreleptin was approved in the US as an adjunct to diet as replacement therapy to treat the complications of leptin deficiency in patients with congenital or acquired generalized lipodystrophy (USPI).

The pivotal efficacy data supporting the approval was from an open-label, single-arm study evaluating metreleptin treatment in patients with congenital or acquired generalized lipodystrophy and diabetes mellitus, hypertriglyceridemia, and/or increased fasting insulin. Of the 48 patients enrolled, 32 (67%) had congenital generalized lipodystrophy and 16 (33%) had acquired generalized lipodystrophy. Overall, 36 (75%) patients were female, 22 (46%) were Caucasian, 10 (21%) Hispanic, and 9 (19%) Black. The median age at baseline was 15 years (range, 1-68 years), with 35 (73%)

patients being less than 18 years of age. The median fasting leptin concentration at baseline was 0.7 ng/mL in males (range, 0.3 to 3.3 ng/mL) and 1.0 ng/mL in females (range, 0.3 to 3.3 ng/mL). Substantial and clinically meaningful improvements in HbA1c, fasting plasma glucose (FPG), and triglyceride (TG) were observed over Year 1 with metreleptin treatment, with greater effects observed in those patients with elevated baseline parameters. Long term durability of response to metreleptin treatment was also demonstrated. Similar to adult patients, treatment with metreleptin in pediatric patients led to marked reductions in key efficacy parameters. The pivotal study was not placebo-controlled, and the lack of a placebo control limits interpretation of relatedness of AEs to metreleptin, which is particularly relevant in this patient population that often has multiple serious co-morbidities as part of their underlying disease. These co-morbidities may be expected to progress over the course of a study spanning several years. This is further confounded by the relatively small patient numbers and the relative lack of published data on the natural history of the condition. Overall, metreleptin has a favorable safety profile with the majority of patients remaining on treatment, some for over 11 years. The most frequent adverse reactions are reported in the USPI are:

Table 2: Adverse Reactions of 5% or Greater Incidence in Patients with Generalized Lipodystrophy Receiving MYALEPT in an Open-Label, Single-Arm Study.

	All Subjects N=48 (%)
Headache	6 (13)
Hypoglycemia	6 (13)
Decreased weight	6 (13)
Abdominal pain	5 (10)
Arthralgia	4 (8)
Dizziness	4 (8)
Ear infection	4 (8)
Fatigue	4 (8)
Nausea	4 (8)
Ovarian cyst	4 (8)
Upper respiratory tract infection	4 (8)
Anemia	3 (6)
Back pain	3 (6)
Diarrhea	3 (6)
Paresthesia	3 (6)
Proteinuria	3 (6)
Pyrexia	3 (6)

Frequent AEs that were considered by the investigator to be related to metreleptin treatment included hypoglycemia, fatigue, nausea, and decreased weight, although there was no difference in incidence of fatigue or nausea between metreleptin-treated and placebo-treated obese subjects (without lipodystrophy) in the integrated Amgen obesity safety studies.

In the pivotal study, 11% of patients reported hypoglycemia. The majority of hypoglycemia events were mild or moderate, and most occurred in the setting of insulin use (alone or with oral agents). In the integrated Amgen obesity studies, hypoglycemia was more frequent in metreleptin-treated versus placebo-treated subjects (3.6% vs. 1.4%). The most notable safety observation in the generalized LD patient population was the observation of 2 cases of peripheral T-cell lymphoma not otherwise specified (PTCLNOS) and 1 case of ALK+ anaplastic large cell lymphoma (ALCL) in the NIH studies. All 3 events occurred in patients with a specific type of LD (acquired generalized lipodystrophy) and the 2 events of PTCL-NOS occurred in patients who had significant hematologic

abnormalities. The ALK+ ALCL occurred in the setting of a chromosomal translocation, and metreleptin had no evidence of mutagenicity in toxicology studies. Thus, based on the available evidence, the development of peripheral T-cell lymphoma is likely associated with the underlying acquired LD disease but a potential contribution of metreleptin to progression cannot be entirely excluded.

Patients with LD are predisposed to acute pancreatitis due to severe and sometimes extreme hypertriglyceridemia. During metreleptin treatment, 7% of patients had an episode of pancreatitis. All had a history of pancreatitis and most had a history of hypertriglyceridemia. Four patients had abrupt withdrawal or suspected non-compliance with metreleptin. Thus, despite metreleptin effects to substantially reduce TG, some patients likely remain predisposed to developing pancreatitis due to prior history of pancreatitis and/or continuing hypertriglyceridemia (e.g., if they are not compliant with or discontinue metreleptin therapy). (In the NIH and FHA101 studies, 4 patients experienced events of hypersensitivity (1 with anaphylaxis attributed to food, and 3 with events of urticaria). All patients continued metreleptin treatment without further events, suggesting that the events were not related to metreleptin. However, in the integrated Amgen obesity studies, events of hypersensitivity occurred more commonly with metreleptin versus placebo treatment (3.3% vs. 1.4%) although the incidence was low.

Neutralizing activity has been observed in patients treated with metreleptin in clinical studies (obesity and lipodystrophy development programs). The development of neutralizing activity could neutralize the activity of metreleptin, resulting in loss of efficacy, as well as neutralize the activity of endogenous leptin, potentially affecting normal physiological processes regulated by leptin (including immune function). In lipodystrophy patients who are leptin deficient at baseline, the consequences of neutralizing endogenous leptin are not clear. There was evidence of loss of efficacy in the lipodystrophy patients who developed neutralizing activity to metreleptin. Sepsis was reported in 2 LD patients who also had other factors that could predispose them to infection but has not been consistently associated with neutralizing activity.

Leptin has a known role in regulating immune function that is complex. This has relevance given the potentially dysregulated immune systems of LD patients (especially those with acquired LD and/or autoimmunity). How mitofusin 2 affects immune function is not known.

In this study, we will treat four patients from our cohort with Mitofusin 2 defects. We can broaden the studies if we have a positive signal in these cases.

1.5 Rationale for proposed studies

Taken together, our data suggest that the mutated MFN2 impairs mitochondrial fusion ability, increasing fission and leading to mitochondrial fragmentation and mitophagy in white AT. Therefore, we hypothesize that *MFN2* mutation has different consequences in white and brown AT, leading to an uncontrolled increase of dysfunctional brown adipocytes (hypertrophic depots) and adipocyte loss in white AT depots. In addition, the low or undetectable levels of leptin in these patients could play a role in the development of the lipomatous tissue. We would like to observe the clinical effects of Metreleptin treatment in patients with MSL and leptin deficiency. We will specifically seek if correction of hypo leptinemia will cause a reduction in the hypertrophic depots in addition to the expected clinical effects of improving metabolic abnormalities such as IGT, hypertriglyceridemia and NAFLD/NASH.

We have access to highly motivated patients who have been clinically diagnosed with MSL and genetically confirmed the R707W pathogenic variation. Access to these unique cohorts (with all patients seeking their regular care with us) is unprecedented and presents a tremendous opportunity to advance our understanding of this disease.

With this study, we will have the opportunity to evaluate the effects of Metreleptin on body

composition and morphometry. We hypothesize that Metreleptin will cause a reduction in the hypertrophic depots. In the long-term treatment phase, we propose that continued treatment will be safe and will help maintain long-term reduction of lipomatosis phenotype and be associated with improved metabolic parameters.

Since MSL does not have any specific treatment and is quite disfiguring, identifying potential leads for intervention is of clinical importance. Given that the disorder is associated with severe leptin deficiency, the fat cell hypertrophy can perhaps be corrected with leptin replacement strategy. We will have the opportunity to document the potential clinical and molecular effects of treatment with Metreleptin in a small number of individuals with MSL.

2.0 PATIENT-SPECIFIC PROTOCOL OBJECTIVE AND ENDPOINTS

2.1 Protocol Objectives

The study aims to evaluate the safety and effectiveness of Metreleptin in individuals with clinical diagnosis of MSL. This study will document the metabolic, morphometric, and molecular changes associated with Metreleptin treatment.

2.2 Protocol Endpoints

Evaluate the effects of Metreleptin on body composition, truncal adiposity and AT gene signatures while patients are being treated with commercial grade Myalept to target their metabolic defects.

Outcome measures: Truncal adiposity by DEXA, total adiposity (Primary) - performed at baseline and visit 3 (week 24)

2.3 Exploratory Endpoints

Triglyceride levels, liver fat percentage, insulin sensitivity by HOMA_IR, adipose tissue sensitivity by Adipo-IR (exploratory)

Adipose tissue gene expression and morphometry (detailed below) (exploratory)

Patient self-evaluation of distress scale (RAND SF-36 and DDS adapted to lipomatosis)

Measure of hyperphagia

2.4 Additional data elements collected from medical records which will be collected in the course of clinical care before and after treatment

Changes in signs and symptoms of obstructive sleep apnea e.g., CPAP requirements

Full neurological assessment.

Dose decreases in or discontinuation of medication used to treat metabolic comorbidities

Linear growth, bone age and pubertal status in case P4

3.0 INVESTIGATIONAL PLAN

3.1 Overview Design and Plan of the Protocol

This is a pilot protocol to evaluate and document the clinical effects and safety of Metreleptin in patients with MSL. The protocol includes four visits. The endpoints will be evaluated by the change in parameters from baseline and six months after starting the treatment with metreleptin (Visit 3). When the study was designed, we had intended to discontinue therapy at 6 months unless a substantial benefit can be demonstrated by a reduction of at least 30 percent in liver fat and triglyceride levels from baseline and significant improvement in physical appearance. Though we have not completed our 6-month assessments, the physical appearance improvement is so striking and so important to the participants that we feel obligated to also add a long-term treatment phase. Clinically, we believe that the improvement is most striking in the younger male cases but also very noteworthy on the older two female participants.

In the original protocol, two additional safety visits at six and 12 weeks after drug initiation was planned for safety measurements and drug compliance assessment. These were hoped to be done in person but could also be conducted remotely due to COVID-19 pandemic or schedule difficulties of participants. When opting for the remote visit, patients will be instructed to get a blood draw in a local lab for measurement of safety labs. The baseline and 6-month assessments have to be performed on site. Additionally, two more phone visits will be performed one week and two weeks after baseline. Detailed description of procedures is presented in Table 4.

We are now adding a long-term extension phase that will include two in person visits/year and one 12 week assessments in between two in person visits.

3.2 Investigation of a unique cohort of patients

As previously described in section 1.2, we follow three pedigrees at the University of Michigan, each with affected individuals with MFN2 R707W mutation.

3.3 Eligibility Criteria

The study will enroll the patients identified in table 1. . The patient under 18 will only be enrolled after we have preliminary evidence of benefit in the 2 adult patients, or the patient has reached the age of 18.

Eligibility criteria are as follows:

- Have the clinical diagnosis of MSL and being followed at University of Michigan (cohort to be studied in this proof-of-concept study is already available at Michigan)
- Leptin levels <4 ng/mL
- Willing and able to tolerate the study procedures.
- Willing and able to tolerate blood sampling.
- Having no condition that may impede successful data collection or interfere with testing parameters.
- If female of childbearing potential:
    - Not breastfeeding.
    - Negative pregnancy test (human chorionic gonadotropin, beta subunit) at baseline.
- Can read, understand and sign approved informed consent form, communicate with study physician, and study team, and understand and comply with protocol requirements.

3.4 Exclusion Criteria

- Presence of advanced liver disease (abnormal synthetic function, PT, or albumin) in medical records
- Evidence of other etiologies of viral hepatitis in medical records
- Presence of active hematologic, bone marrow or other abnormalities that may increase risk of bleeding in medical records.
- Presence of HIV infection in medical records.
- Presence of ESRD, active cancer, or >class 2 congestive heart failure based on medical history and physical examination.
- Active chronic infection (e.g., known chronic osteomyelitis or TB). May have transient infections but must be free of active infection for two weeks prior to study visits.
- Unable to ambulate or tolerate trips to the University of Michigan Clinical Research Unit.
- Clinically relevant CAD: history of stent or CABG with cardiologist confirmed angina.
- Presence of autoimmune disease.
- Hypersensitivity to metreleptin.
- General obesity not associated with congenital leptin deficiency.
- Any other condition that, in our opinion, may impede successful data collection.

3.5 Lifestyle Guidance

Lifestyle guidance for healthy metabolic health will be provided per the 2020 Diabetes Care guidelines of ADA.

4.0 Study Drug:

The study drug is Metreleptin (Myalept) as manufactured by Amryt Pharmaceuticals

4.1 Study Drug Preparation

Study drug for injection is supplied in a carton containing 30 vials for reconstitution. Each vial contains 11.3 mg of the study drug as a sterile, white, solid, lyophilized cake or powder to deliver 5 mg/mL of the study drug when reconstituted with 2.2 mL of bacteriostatic water. After reconstitution, the mixture should be clear and colorless. When the study treatment is reconstituted with bacteriostatic water, the vial can be used for up to 72 hrs if refrigerated appropriately. In the event of bacteriostatic water shortage reconstitution can be made with sterile water (2.2 mL). If reconstituted with sterile water, the vial needs to be discarded after a single use.

The daily dose will be self-administered by the patients or a designated person (e.g., caregiver) after being properly trained by qualified study personnel. The first occurrence of self-administration must be observed by the study personnel.

4.2 Labeling

Metreleptin labels will include the statement “Caution: New Drug – Limited by Federal law to investigational use”. The label will also include information on the storage conditions, lot number and expiry date.

4.3 Dose and Treatment Regimens

a) Starting and Maximum Doses

The starting doses of the study drug to be administered will be as follows 2.5 mg (0.5mL) for males and 5 mg (1mL) for females daily. The maximum daily dose should not exceed 10 mg (2mL).

b) Dose Adjustments

The dose adjustments of the study treatment are shown in Table 3. Dose increases should be made monthly according to the schedule below until the highest tolerated dose is implemented, to ensure consistency across the study. Safety reasons for stopping the dose increase include hypoglycemia and excessive weight loss.

Table 3: Study Drug Dose Increase

		INCREASE DOSE TO:			
Gender	Starting Daily Dose (Injection Volume)	Month 1	6 weeks	12 weeks	
Males	2.5 mg (0.5 mL)	5 mg (1 mL)	7.5 mg (1.5 mL)	10 mg (2 mL)	
Females	5 mg (1 mL)	7.5 mg (1.5 mL)	10 mg (2 mL)	No Dose Change	

Abbreviations: HbA1c = glycated hemoglobin.

* Dose increases should be made in addition to increases based on weight changes.

Dose reductions will only be considered if, in the Investigator's opinion, there is a safety risk to the subject such as massive weight loss.

4.4 Storage

The study drug in a form of lyophilized powder should be stored in the refrigerator at 36°F to 46°F (2°C to 8°C) in a carton and protected from light until prepared for use; the study drug should not be frozen. When the study drug is reconstituted with WFI, it should be administered immediately and cannot be stored. Unused reconstituted solution should be discarded.

4.5 Compliance

The administration of the study treatment (dose and duration) should be recorded by the investigator monthly. Subjects will keep dose diaries to be provided by the investigative team to document timing and administered dose as another source of compliance.

4.6 Study Drug Handling and Accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received, and any discrepancies are reported and resolved before use of the study treatment. All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the storage conditions with access limited to the Investigator and authorized study center staff. Only

subjects enrolled in the study may receive study treatment and only authorized study center staff may supply or administer study treatment. After proper training patients will be responsible for in home handling and study drug administration. Drug will be delivered to the patients at each visit.

The drug will be dispensed with a prescription from research pharmacy and delivered to the clinical site where subjects are seen. The kits handed to the patient will be recorded by the investigative team and returned vials as well as dosing diaries will be tracked by the investigative team.

The Investigator is responsible for study treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). The Investigator, a member of the study center staff, or a hospital pharmacist must maintain an adequate record of the receipt and distribution and return of all study treatment using the Drug Accountability Form. These forms must be available for inspection at any time.

4.7 Concomitant and Other Treatments

Unless excluded, medication considered necessary for the subject's safety and well-being may be given at the discretion of the Investigator and should be recorded in the appropriate sections of the eCRF. Special attention should be paid to recording changes to the subject's anti-diabetic, lipid-lowering or immunomodulatory treatments as these modifications may affect efficacy determination.

Metreleptin may affect the formation of cytochrome P450 (CYP450) enzymes and may be clinically relevant for coadministered CYP450 substrates with narrow therapeutic index, where the dose is individually adjusted. None of these drugs are at play for these subjects (Table 4). However, if any subjects who are being treated with drugs that use Cyp450 as their metabolic clearance, we will carefully evaluate the therapeutic effect and drug concentration when appropriate and the dosage will be adjusted as needed.

Table 4: List of concomitant medication in use by each patient.

P3	P4	P5	P7
Cyanocobalamin	Cyanocobalamin	Cholecalciferol	Alprazolan
Fluticasone	Melatonin	Omega 3	Hydrocodone-acetominophen
Trazodone	Anatrozole	Biotin	Pregabalin
Omega 3	Cholecalciferol	Fluoxetine	Multivitamin
Amlodipine	Vitamin E	Cetirizine-pseudoephedrine	
Azilsartan	Omeprazole		
Busprione	Rizatriptan		
Fluoxetine	Sumatriptan		
Loratadine	Loratadine		
Vitamine E	Lisdexamfetamine		
Esomeprazole			
Fenofibrate			
Olmesartan			
Tretionin			
Cholecalciferol			

5.0 PROTOCOL VISITS

5.1 Duration of Patient Participation

The total protocol duration will be four visits over a period of 7 months. There will be a baseline visit and then the first two visits (Visit 1 and Visit 2) will be 6 weeks apart from baseline and Visit 3 will be three months (12 weeks) after visit 2. There will be a follow-up visit 4 weeks after the end of the treatment period (in person or remote). We had initially planned treatment discontinuation after 6 months of therapy; however, given the substantial improvement we have noticed, we will offer the option to continue treatment for this month.

Due to the unique characteristics of fat distribution in this phenotype and a potential risk of unknown adverse event, for safety reasons, we will perform two telephone visits one week and two weeks after start of Metreleptin treatment.

The patients have the right to withdraw from the protocol at any time, for any reason, and without repercussion.

5.2 Protocol Discontinuation

The execution of this protocol may be prematurely terminated if, in the opinion of the Principal Investigator, there is sufficiently reasonable cause. The Principal Investigator will provide written notification documenting the reason for protocol termination to the IRBMed and also to the FDA, if applicable, and the manufacturer.

5.3 Open-Label Treatment Extension

If patients experience substantial reduction of disfiguring lipomatosis and/or experience reduction in triglycerides or liver fat, we will offer a long-term extension phase after the 4-week follow up. Participants will have the option to continue the treatment during the first month defined as the follow up month or to stop treatment. Participants can also have the option of restarting after 1 month discontinuation if they wish to evaluate the impact upon discontinuation to their physical appearance and disfigurement.

The total duration of the long-term treatment extension study will be 5 years or until approval of this indication by the FDA.

If a patient decides to continue with treatment, they will have a baseline visit to obtain informed consent for the long-term treatment, and to document medical history, medication changes, and physical findings. The remaining visits will be clinical visits aimed at documenting medical history, medication changes, and physical findings. All of the procedures that will be performed at these visits are detailed in Table 6.

PROTOCOL ASSESSMENTS

6.1 Overview of Schedule of Assessments

The Schedule of Assessments (SOA) to be conducted during the protocol is depicted in Table 4.

Laboratory and testing assessments are also described in Table 4. Patients will be required to

fast overnight on the day preceding all visits. In addition, they will be allowed to take their usual medications with a sip of water on the morning of each clinic visit. Therefore, assessments may occur over multiple days during the scheduled protocol visits. If patients have been studied in the META (Metabolic and Molecular Evaluation of Individuals with Multiple Symmetric Lipomatosis, HUM00211960) study, the baseline data can be obtained from META study if done within 6 weeks of scheduled study day.

Table 5: Schedule of Assessments

Visits	**Baseline**	**Phone visit 1 (Week 1)±3 days**[17]	**Phone visit 2 (Week 2)** ±3 days[17]	**Visit 1 (Week 6) ±1 week**	**Visit 2 (Week 12) ±1 week**	**Visit 3 (Week 24) ±1 week**	**Follow-up**[18] **(week 28) ±1 week**
Informed consent/assent[1]	X						
Inclusion/Exclusion criteria	X						
Medical history[2]	X	X	X	X	X	X	X
List of medications	X	X	X	X	X	X	
Physical examination[3]	X			X	X	X	X
Weight	X			X	X	X	
Waist circumference[5]	X			X	X	X	
Hip circumference	X			X	X	X	
Height	X			X	X	X	X
Patient photos[6]	X			X	X	X	X
Vital signs[7]	X			X	X	X	X
ECG (12-lead)[8]	X			X	X	X	
Laboratory tests[9*]	X			X	X	X	
Safety labs[10]	X			X	X	X	X
Future research samples[11]	X					X	
DEXA fat distribution	X					X	
Liver MRI/ MRI for Body Composition[*]	X					X	
Indirect calorimetry[12]	X					X	
Adipose Tissue Biopsy[13]	X					X	
Skin Biopsy[*]	X					X	
Adverse Events	X	X	X	X	X	X	X
Body temperature		X	X				
Pulse rate		X	X				
First study drug administration/training	X						
Self administered questionnaires[14]	X					X	
Evaluation of sleep apnea syndrome symptoms and full neurological assessment[15].	X					X	

Linear growth, pubertal status and bone age in pediatric patient P4 (when treated)[16]	X					X	
Antidrug and Neutralizing antibodies	X				X	X	X

Table 6: Schedule of Assessments: Long-Term Treatment (will be continued for up to 5 Years)

Visits	Baseline (Week 28)	Month 3 Visit (Week 40)±1 week Virtual/Phone	Month 6 Visit (Week 52) ±1 week	Month 9 (Week 64) ±1 week Visit Virtual/ phone	Month 12 Visit (Week 76) ±1 week
Informed consent/assent[1]	X				
Inclusion/Exclusion criteria	X				
Medical history[2]	X	X	X	X	X
List of medications	X	X	X	X	X
Physical examination and photos[3]	X		X		X
Weight	X		X		X
Waist circumference[5]	X		X		X
Hip circumference[5]	X		X		X
Height	X		X		X
Vital signs[7]	X		X		X
Laboratory tests[9*]	X		X		X
Safety labs[10]	X	X	X	X	X
Adverse Events	X	X	X	X	X
Body temperature		X	X	X	
Pulse rate		X	X	X	
Study drug administration/training	X				
Antidrug and Neutralizing antibodies	X				X

Footnotes for Table 6: Schedule of Assessments

1. Before any program procedures are performed, the details of the program will be described to the patient, and the patient will be given a written informed consent document to read. If the patient agrees to participate in the program, consent will be indicated by signing and dating the informed consent document in the presence of program personnel.

2.The medical history will include detailed lipodystrophy history, including prior medication/investigational products and procedures for the treatment of lipodystrophy

3. A complete physical examination will be conducted, as noted in the SOA. Tanner Staging for assessment of pubertal development will be conducted according to the SOA; if the patient has not reached Tanner Stage V. Whenever possible, the same trained health care professional will conduct the exam and Tanner Staging.

4. Weight will be measured at the clinic using the same scale after the patient has emptied her bladder and while fasting. The patient will be asked to wear light clothing or underwear, no shoes, and will be weighed at approximately the same time of day.

5. Waist and hip circumference will be done as single measures.
6. Patient photographs will be taken at each in-clinic study visit. Left lateral and frontal pictures of the patient's head and neck region will be taken at a distance of approximately 18-24 inches. Left lateral, frontal, and posterior full-body pictures will be taken from a distance of approximately 10 feet. Patients will place their arms at their side for the left lateral full-body picture and extend their arms at a 45-degree angle distally with the wrist rolled vertically for the frontal and posterior full-body pictures. Patients will be photographed in tank top/shorts or in their underwear under normal clinic lighting. The patient eyes will be obscured by black marking in finalized pictures. Areas of specialized interest or unique morphology may be photographed in more detail at the investigator's discretion.
7. All BP and HR measurements will be obtained in the sitting position following at least 5 minutes of rest. All measurements will be taken in triplicate, approximately 2 minutes apart. When possible, BP will be taken in the non-dominant arm throughout treatment, using the same methodology (automated or manual) according to Appendix 10.A. Body temperature (°C) and respiration rate (breaths/minute) will be obtained in the sitting position following at least 5 minutes of rest.
8. A single 12-lead ECG will be performed in the supine position following a period of at least 10 minutes of rest.
9. Fasting TGs and lipid panel, AST, and ALT,will be measured at each clinic visit. HbA1c will be drawn at Baseline, 12 week, and 24 week visits. Fasting samples (10-hour minimum) are required at all-time points. Hormones secreted by fat cells and immune system cells will be measured if any funding is available. Protease inhibitors will be added into the tubes if necessary. These measurements will be done clinically as part of the patient's medical management.
10. Safety labs will include kidney function test, blood count and other liver function test not included in item 9.
11. Serum/plasma will be kept at -80 C. Protease inhibitors will be added into the tubes if necessary.
12. Indirect calorimetry will be measured in fasting state and before the oGTT.
13. Adipose tissue biopsy will be performed on affected and unaffected areas of the trunk and dorsocervical area.
14. Self-administered questionnaires will include the RAND SF-36, Disease Distress Scale and hyperphagia scale (see Appendix).
15. Assessment of sleep apnea and C-pap support will be collected on clinical grounds. In addition full neurological assessment by a neurologist will also be conducted during clinical care before treatment begins and after 6 months are concluded.
16. in case P4, if and when he is treated, clinical assessments of linear growth, pubertal assessment and bone age will be collected from his clinical care documents and included in the data collection.
17. Phone visit 1 will be performed one week after starting of Metreleptin treatment and Phone visit 2 one weeks after the previous phone visit. The telephonic visits will be performed for safety management and questions on drug management. This procedure will be repeated after each dose adjustment of Metreleptin.
18. Follow -up visit will occur for all individuals wishing to discontinue treatment. Individuals with benefit and who do not wish to have an interruption in therapy will also have 1 month follow up while on drug and then return to clinic and begin long-term treatment portion.

6.2 Measurements and Sampling

All studies will be performed in the fasted state (10-12 hours). Baseline and 6 month assessments will be conducted on site. Visit 1 and 2 may also be conducted on site if pandemic-related conditions allow.

6.3 Clinical Procedures

a) Informed Consent/Assent

A complete description of the protocol will be presented to the patients and their parents or guardian. The signed and dated informed consent and/or assent will be obtained before any protocol-specific procedures are performed. In the case of minor patients, the assent document must be signed by the patient, and at least two legal guardians (unless documentation of only one parent with parental rights) must sign the informed consent prior to the patient's enrollment in the study. Minor patients that reach the age of majority will consent at the next research visit after their 18th birthday.

b) Demographics and Medical History

Data will be recorded in the source document and CRF. Recent medical history will be obtained. This recent medical history includes a review for changes from baseline as well as a review of the patient's current medication use and to assess whether any changes have occurred since the previous visit.

c) Physical Examination

A complete physical examination will include a review of body fat distribution, peripheral lymph nodes, head, eyes (including conjunctiva), ears, nose, mouth and oropharynx, neck, heart, lungs, abdomen, musculoskeletal including back extremities and neurologic. Tanner Staging will also be assessed at each visit until patient reaches Tanner Stage V. All physical examinations will be conducted in adequate light. Height (cm) will be measured, without shoes, according to the SOA using a wall-mounted stadiometer. Weight will be measured at the clinic using the same scale after the patient has emptied her bladder and while fasting. The patient will be asked to wear light clothing or underwear, no shoes, and will be weighed at approximately the same time of day. Waist and hip circumference will be done as single measures.

d) Medication Review

A review of medications will be conducted during the Baseline Visit and at every protocol visit. In addition, any medications taken by the patient will be recorded in source documents.

e) Vital Signs

Vital signs will be obtained in the sitting position following at least 5 minutes of rest each time they are measured.

Blood Pressure and Heart Rate

Blood pressure (BP; mmHg) and heart rate (HR; bpm) will be performed using the same methodology throughout the protocol (manual or automated) as outlined in Appendix 10.A. All BP and HR measurements are to be obtained in the sitting position following at least 5 minutes of rest. All measurements will be taken in triplicate, approximately 2 minutes apart. When possible, BP will be taken in the non-dominant arm throughout the protocol. Repeat measures and more frequent monitoring can be implemented for significant increases in BP or HR.

Body Temperature and Respiration Rate

Body temperature (°C) and respiration rate (breaths/minute) will be obtained in the sitting

position following at least 5 minutes of rest.

f) 12-Lead Electrocardiogram

Single 12-lead electrocardiograms will be performed following a period of at least 10 minutes of rest in the supine position.

g) Metabolic measurement

Blood will be drawn to determine circulating glucose, free fatty acids (FFA), insulin, glycerol, AST, ALT and C-peptide. Surrogate estimates of insulin resistance (HOMA-IR and Adipo-IR) will be calculated based on fasting insulin, glucose and FFA concentrations. Other samples that can be collected may include adipokines and samples for incretin hormone measurements.

h) Oral glucose Tolerance test

An oral glucose tolerance test will be performed with a 75 g glucose load. After a 12 hour fast, a blood sample will be collected (time = 0). After this blood sample, the patient will be given 75 grams of dextrose to consume. Blood will be drawn to determine circulating glucose, free fatty acids (FFA), insulin, and C-peptide at 30-minute intervals for five hours. Surrogate estimates of insulin resistance (HOMA-IR and Adipo-IR) will be calculated based on fasting insulin, glucose and FFA concentrations. The lipolysis index will be determined from fasting glycerol per fat weight as determined by Dual X-Ray Absorptiometry. Other samples that can be collected may include adipokines and samples for incretin hormone measurements. Indirect calorimetry will be performed to analyze fasting energy expenditure (24). During the OGTT, blood samples will be collected to measure glycerol and FFA, and these measurements will allow comparison of glycerol and FFA suppression from affected individuals compared to non-affected. We do not plan on repeating the OGTT during the long-term treatment portion unless a clinical need arises, such as a substantial increase in the HbA1c.

i) Safety labs

Safety labs will include blood count, creatinine, BUN creatinine kinase, GGT and HbA1c. Other lab tests to be drawn will include fasting serum levels of triglycerides, total HDL and LDL cholesterol, CRP, circulating adipokines, including leptin and adiponectin, and inflammatory markers. A pregnancy test will be performed before any procedure at each visit. The full set will be done provided that sufficient funding is available. Otherwise, the investigator will modify the list and testing will include only safety labs, fasting glucose, lipids, insulin, and CRP. Additional serum and plasma will be stored to run additional testing if future funding becomes available.

j) Body composition

Two independent methods for estimating body composition will be used, as one technique alone does not fully capture the distribution of fat within the body and because previous studies have not documented the time course of fat loss in humans affected with *MFN2* mutations. First, we will use MRI fat quantification (3 Tesla magnet; Philips Healthcare) and a custom MatLab computer program developed in-house, which integrates the amount of fat mass observed in the images (Sparti, DeLany et al. 1997, Ajluni, Meral et al. 2017). We will then estimate whole-body composition, including fat and lean body masses, by DEXA (GE Lunar Prodigy, model PA +41744). Next, hepatic fat quantification will be performed on a 3 Tesla clinical MRI system (Philips Healthcare) using the vendor-provided, torso phased-array surface coil positioned on

the abdomen. In addition to breath-hold, single-shot, turbo-spin-echo sequences for localization, fat will be quantified using two image-based and one spectroscopy-based technique as previously published (Oral, Reilly et al. 2017). Body composition assessments can be planned in the long-term treatment phase based on investigator discretion no more than once a year depending on clinical findings and availability of funding.

k) Adipose tissue biopsy

A critical aspect of this proposal is to obtain tissue samples from patients who have the *MFN2* mutation. Thus, we will perform incisional biopsies from the affected (subcutaneous region) and from the unaffected areas. The procedure will be performed by a plastic surgeon. Briefly, biopsy sites will be sterilized, and Xylocaine (1%) will be used for local anesthesia. After induction of local anesthesia, an incision will be made through the skin, and ~1-3 grams of adipose tissue will be excised for the analyses described below. The incision will be closed with sutures, and the patient will remain under observation in the clinic for one hour.

Skin scrapings will also be saved and studied. Approximately 200 mg of the obtained fat tissue will be fixed with paraformaldehyde for histologic analysis, 100-200 mg will be frozen immediately in liquid nitrogen for isolation of RNA, 100-200 mg will be frozen for single nuclei RNAseq, and 500 mg will be used to isolate stromal vascular and adipocyte fractions for further analyses, including single-cell RNAseq and isolation of RNA and protein or confirmation of regulated genes by qRT-PCR and immunoblotting. The remainder will also be frozen for future experimentation.

The following studies are planned with the adipose tissue biopsy: Adipocyte size will be evaluated by morphometric analysis of H&E-stained adipose tissue sections as previously described (Parlee, Lentz et al. 2014). Adipose tissue inflammation and macrophage recruitment will be examined using standard F4/80 immunostaining techniques (Taniike and Suzuki 1995). We will also investigate markers of fibrosis in adipose tissue biopsies, stain the extracellular matrix with Picrosirius Red, and evaluate expression of collagen genes (*COL1A1, COL6A1*) by qRT-PCR and immunoblotting. Finally, immunohistochemistry will be used to identify the tissue and cellular localization of proteins that are identified as differentially regulated in our mouse and human molecular profiling studies (Berry, Church et al. 2014)

No adipose tissue biopsies are planned in the long-term treatment phase.

l) Evaluation of Sleep Apnea Syndrome

Assessments for sleep apnea will be obtained as part of clinical care before any treatment begins and after 6 months. In the long-term treatment phase, we will follow patients clinically.

7.0 ADVERSE EVENTS AND RISKS

7.1 Adverse events

Patients will be carefully monitored for the development of any AEs throughout the protocol from the time the patient provides informed consent through the Final Protocol Visit. Biopsy-related bleeding and scarring will be closely monitored. Patients will be questioned on skin integrity, erythema, swelling, and structural abnormalities a week following the procedure. Because participants live away from the University of Michigan, phone calls and patient-provided pictures and

alternative visualization methods such as Skype or Face-time technology will be used to provide monitoring of patients. All interactions will be performed using secure methods.

7.2 Monitoring of Adverse Events and Period of Observation

AEs will be recorded in the source documents. SAEs and deaths will be recorded on the SAE CRFs starting from the time the ICF is signed and continuing through the Final Protocol Visit. All AEs will be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

Due to the unique pattern of fat distribution in this population and the potential of unknown adverse events due to an increase in metabolic activity on the atypical hypertrophic adipose tissue, we will ask the patients receiving Metreleptin to monitor body temperature with a calibrated electric thermometer and heart rate daily for the first month and after each drug dosage adjustments for 2 weeks.

7.3 Risks associated with evaluation methods

m) Imaging modalities

While MRI scanning is thought to be safe, the procedure may cause anxiety in some patients since current equipment used at the University of Michigan uses a closed tube.

Dexa is fairly simple, but subjects need to lie flat for about 15 minutes or less. The radiation exposure from DEXA scanning is thought to be negligible (<5 mrem per measurement that is less than 1% of a CXR). Women of Childbearing potential will undergo regular urine pregnancy tests per study schedule.

n) Risks with blood tests

The total amount of blood drawn from the patients will be kept within the IRB restrictions. Total amount of blood to be drawn with the outlined studies is estimated not to exceed 90 cc over the course of 16 weeks. Each blood draw is associated with pain and risks of infection, though these risks are minimal.

o) Risks with study procedures

In routine care, the frequency of scheduled medical visits for any stable prediabetic patient is at least 3 to 4 times a year. For all intents and purposes, the patients who harbor mutations on the *MFN2* gene, even if young, would be considered in the prediabetic category. In this study, patients are asked to have extensive visits annually. In addition, some patients may find the time needed to complete the research studies an inconvenience in their routine lives. Each participant in the study will clearly understand that participation is totally voluntary.

7.4 Mitigation of Risk

The potential risks will be carefully mitigated using strategies included in the description of each risk and via our adverse event monitoring strategies outlined above. Subject privacy will also be protected via team training and all the precautions listed in the appropriate sections above.

8.0 DRUG SAFETY

8.1 General Considerations

The Investigator is responsible for ensuring that all staff involved in the study are familiar with the content of this section. All AEs, including AEs leading to discontinuation of study treatment, AESIs and SAEs will be recorded on the applicable eCRF. Additionally, all SAEs and AESIs requiring immediate reporting (per Section 8.4) will be captured on the applicable paper report form and sent to the Sponsor designee (per Section 8.6).

8.2 Definition of Adverse Events

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the study product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the clinically significant abnormal results of an investigation (e.g., laboratory findings).

8.3 Definition of Adverse Events of Special Interest

AESIs include the following:

- All serious and severe infections (regardless of suspicion of loss of endogenous leptin or metreleptin action)
- Potential Hy's Law events (aspartate aminotransferase [AST] or alanine aminotransferase [ALT] ≥3× upper limit of normal [ULN] and total bilirubin ≥2×ULN without any other explanation other than drug involvement)
- AEs that lead to the study treatment discontinuation
- Clinically significant abnormal lab results that lead to the study treatment discontinuation
- Medication errors, including errors during administration of the study treatment and overdose
- Any event concerning bone metabolism, brain development or sexual maturity
- Hypersensitivity reactions
- Pancreatitis
- Severe hypoglycemia
- Any malignancy
- Hepatic AEs including potential drug-induced liver injury
- New diagnoses of autoimmune disorders (e.g., autoimmune hepatitis, glomerulonephritis, lupus erythematosus, antiphospholipid antibody syndrome, rheumatoid arthritis)
- Autoimmune disease exacerbation

A sample should be collected for measurement of in vitro neutralizing activity in all subjects with the following AESIs:

- Suspected loss of response (worsening of metabolic control)

8.4 Definition of Serious Adverse Events

An SAE is an AE occurring during any study phase (i.e., period between ICF and first administration of study treatment, treatment, withdrawal, follow-up), that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening defined as an event in which the subject was, in the judgment of the Investigator, at risk of death at the time of the event; it does not refer to an event that

hypothetically might have caused death had it been more severe

- Requires in subject hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above. In addition, for the purposes of this protocol, the following AESIs must be handled in the same fashion as SAEs and are reportable to the Sponsor designee (per Section 8.6) within the SAE reporting time frame regardless of whether the AESI meets the seriousness criteria described above in this section:
- Necrotizing pancreatitis
- Hepatic AEs including potential drug-induced liver injury
- Severe hypoglycemia
- Severe hypersensitivity reactions
- New diagnoses of autoimmune disorders (e.g., autoimmune hepatitis, glomerulonephritis, lupus erythematosus, antiphospholipid antibody syndrome, rheumatoid arthritis)
- Autoimmune disease exacerbation. Pre-existing diseases that are present before entry in the study are described in the medical history, and those that manifest with the same severity, frequency, or duration after study treatment exposure, will not be recorded as AEs. However, when there is an increase in the severity, duration, or frequency of a pre-existing disease, the event must be described on the AE eCRF
- Severe infection
- All cancers (excluding non-melanoma skin cancer) by cancer type

8.5 Recording of Adverse Events

i) Time Period for Collection of Adverse Events

All AEs and SAEs will be collected from the signing of the ICF until the follow-up visit and as soon as informed to the investigator.

ii) Follow-up of Unresolved Adverse Events

Any AEs, including SAEs, AEs leading to discontinuation of the study treatment and AESIs that are present at the subject's last assessment as appropriate in the study, will be followed up by the Investigator for as long as medically indicated and the outcome of the AE will be updated in the applicable eCRF, if needed. Sponsor retains the right to request additional information for any subject with ongoing AE/SAE(s) at the end of the study, if judged necessary.

iii) Variables

The following variables will be collected on the applicable report form for each AE:

- AE (verbatim)
- Date when the AE started and stopped or if it is ongoing
- Maximum intensity
- Whether the AE is serious or not
- Investigator causality rating against the study treatment
- Action taken about the study treatment
- Whether the AE led to the subject's withdrawal from the study
- Outcome

In addition, the following variables will be collected on the applicable report form for SAEs:

- Date AE met criteria for SAE
- Date Investigator became aware of SAE
- Reason AE is serious
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to study procedure(s)
- Description of AE

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 8.4. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE unless it meets the criteria presented in Section 8.4. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE when it satisfies the criteria.

Intensity rating scale to be used:

- Mild: Mild symptoms causing no or minimal interference with usual social and functional activities, with intervention not indicated.
- Moderate: Moderate symptoms causing greater than minimal interference with usual social and functional activities, with intervention indicated.
- Severe: Severe symptoms causing inability to perform usual social and functional activities, with

intervention or hospitalization indicated.

iv) Causality Collection

The Investigator will assess causal relationship between the study treatment and each AE, SAE, AE leading to discontinuation, and AESIs.

The causal relationship will also be assessed for other medications and study procedures. Note that for AEs that could be associated with any study procedure the causal relationship is implied as "related".

The Investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE. The AE must be characterized as unrelated, unlikely to be related, possibly related, or probably related.

- "Probably related" conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- "Possibly related" suggests that the association of the AE with the study treatment is unknown; however, the AE is not reasonably supported by other conditions.
- "Unlikely to be related" suggests that only a remote connection exists between the study treatment and the AE. Other conditions, including chronic illness, progression, expression of the disease state. or reaction to concomitant therapy appear to explain the reported AE.
- "Unrelated" is used if there is not a reasonable possibility that the study treatment caused the AE. The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration, will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure IB and/or Product Information for marketed products in his/her assessment.
- For each AE/SAE, the Investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report. However, it is very important that the Investigator always makes an assessment of causality for every event before the initial transmission of the SAE data.
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

v) Collection of Adverse Events Based on Signs and Symptoms

When entering AEs in the eCRF, the recording of diagnoses is preferred (if known) over recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not consistent with the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

vi) Collection of Adverse Events Based on Examinations and Tests

Any clinically significant abnormal laboratory result should be considered as an AE. If a clinically significant abnormal laboratory result meets at least one of the SAE criteria, then it should be reported as an SAE (Section 8.4).

Any clinically significant abnormal vital sign or other finding at a physical examination that meets at least one of the SAE criteria should also be considered and reported as an SAE.

If clinically significant abnormal laboratory results meet AESI criteria, then these results should be considered as AESI (Section 8.3).

vii) Hy's Law

Hy's Law cases represent one end of a spectrum of laboratory abnormalities that indicate liver injury. Cases where a subject has an AST or ALT ≥3×ULN and total bilirubin ≥2×ULN and international normalized ratio (INR) >1.5, if INR measured, which may indicate severe liver injury may need to be reported as SAEs.

vii) Collection of Adverse Events of Special Interest

Adverse events of special interest will be collected and recorded in the applicable eCRF. Anti-drug antibodies (ADAs) and neutralizing andibodies (Nabs) will be measured at baseline, 12-weeks and 24-weeks of therapy as well as the 4-week post treatment follow up. If any subjects develop ADAs and Nabs, we will monitor them at for an additional 3 months from the last observation to document resolution of the antibodies. Additionaly, ADA should be measured in the event of loss of efficacy, worsening metabolic parameters, and any severe and/or serious infection. If ADAs are observed, titers should be reported, and neutralizing activity should be assessed. We will continue to monitor ADAs and AEs until ADAs have cleared this 6-month intervals.

ix) Collection of Pregnancies

If a female subject, or if a female partner of a male subject, becomes pregnant while enrolled in the study, a paper Pregnancy Report Form should be completed and submitted to the Sponsor designee within 24 hours of the treating physician's/research personnel's awareness of the pregnancy. Every effort should be made to follow the pregnancy until completion or until pregnancy termination.

If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., spontaneous abortion, stillbirth, neonatal death, or congenital anomaly [including that in an aborted fetus, stillbirth, or neonatal death]), the Investigator should follow the procedures for reporting SAEs.

8.6 Reporting of Serious Adverse Events

All SAEs must be reported whether or not considered causally related to the study treatment, or to the study procedures (Section 8.4). All SAEs will be recorded in the applicable eCRF.

If an SAE occurs during the course of the study, then Investigators or other site personnel must inform the Sponsor designee per Section 8.8 immediately but no later than 24 hours of the Investigator's awareness of the event.

The investigator must report all events meeting the criteria and definition of a serious adverse event as per the local IRB reporting requirements.

If an IND is required for this study, the investigator will coordinate with the Michigan Institute for Clinical and Health Research (MICHR) IND/IDE Assistance Program (MIAP) office for reporting of any and all IND safety reports to the FDA as per the requirements outlined in 21 CFR312.32. This includes reporting of all Serious Adverse Events (SAEs) that are both unexpected and related to the drug as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. If the unexpected and related SAE is either fatal or life-threatening, then the SAE must be reported as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. A summary of all non-expedited safety reports will be submitted in the annual report.

For fatal or life-threatening SAEs where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel will inform the Sponsor designee of any follow-up information on a previously reported SAE immediately but no later than 24 hours of the Investigator's awareness of the event.

8.7 Overdose

An overdose is considered a dose of study treatment that exceeds the maximum daily dose of f 10 mg (2 mL) . An overdose is considered a medication error and should be reported on the applicable eCRF as an AESI (Section 8.3).

If an overdose fulfills the serious criteria, the SAE should be reported to the Sponsor designee (per Section 8.8) immediately but no later than within 24 hours of the Investigator's awareness of the event.

8.8 Reporting Safety Observations by the Investigator to the Sponsor

The Investigator or site personnel will be responsible for detecting, documenting, and reporting events that meet the definition of an AE, SAE, and AESI. All SAEs and applicable AESIs (as per Section 8.4) collected according to the schedule of study activities, regardless of study treatment relationship, must be reported via email or by fax on the paper Safety Event Report Form to the Sponsor or designee immediately, but no later than 24 hours, of the Investigator's awareness of the event.

The Investigator will also submit any updated SAE and applicable AESI data immediately but no later than 24 hours of the Investigator's awareness of the updated information.

Serious Adverse Event Contact Information: Email: safety-inbox.biotech@iqvia.com Fax (Worldwide Toll number): +1 919-313-1412

The paper report form will capture data surrounding the event, e.g., the nature of the symptom(s), time of onset in relation to initiation of therapy, duration, intensity, and whether or not therapy was interrupted or discontinued. The Investigator's assessment of the probable cause of the event will also be included. In addition, relevant medical history, concomitant medications, laboratory and diagnostic tests reports, and procedures as well as all pertinent medical information related to the event will be collected.

The Sponsor designee will forward SAE queries directly to the Investigator requesting incomplete or missing information. It is the Investigator's responsibility to be diligent in providing this information to Sponsor or designee as soon as it is available.

8.9 Management of the Study Treatment-related Toxicities

There have been reports of generalized hypersensitivity (e.g., urticaria or generalized rash) in subjects taking metreleptin. If a hypersensitivity reaction occurs, the Investigator should discuss the proper clinical treatment for the event, including possible discontinuation of the study treatment with the subject. Dosage adjustments, including possible large reductions of insulin or insulin secretagogue (e.g., sulfonylurea) may be necessary in some subjects to minimize the risk of hypoglycemia.

8.10 Clinical Trial Monitoring

To assure adequate protection of the rights of human subjects, per 21 CFR §312.50, 312.53, this study, if deemed to require an IND, will be monitored by the University of Michigan Institute for Clinical and Health Research (MICHR). Routine monitoring will be scheduled at appropriate intervals, with more frequent visits occurring at the beginning of the study. An initiation visit will take place, followed by routine monitoring visits. Additional visits can be scheduled at the request of the Sponsor-Investigator.

Monitoring visits may be in the form of a site visit or a review of the site records. During a monitoring visit to a site, access to relevant hospital and clinical records must be given by the site investigator to the MICHR representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. It is expected that the relevant investigational staff be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site in a timely manner.

The established monitoring plan will ensure the quality and integrity of the data through pre-investigation visits and periodic site visits to verify adherence to the protocol, completeness and accuracy of study data and samples collected, proper storage, dispensing and inventory of study medication, and compliance with regulations. Monitoring visits can be conducted onsite or remotely with appropriate electronic documentation provided to the study monitors for review.

9.0 STATISTICAL PROCEDURES

9.1 Analyses and sample size considerations

With only four subjects, we have not planned formal statistical testing, but we are planning to generate result tables.

9.2 Expected results, interpretations, and alternative approaches

With these methods, we anticipate that we will see weight loss and reduction in the truncal adiposity. We also anticipate improvements in triglyceride levels, insulin resistance and hepatic steatosis. The adipose tissue morphometric and molecular studies may show effects of leptin as well as molecular signatures of leptin action in the unique fat depots of studied patients.

10. SUMMARY AND FUTURE DIRECTIONS

With this study, we will have the opportunity to investigate the effects of Metreleptin on body composition, truncal adiposity, metabolic endpoints, adipose tissue morphometry and gene signatures. This is a pilot study and will inform us if a favorable effect of Metreleptin can be observed in these unique patients. We plan on expanding the studies if a positive signal is obtained.

11. ADMINISTRATIVE REQUIREMENTS

11.1 Ethical Considerations

The protocol will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (see Appendix). The IRB/IEC will review all appropriate protocol documentation in order to safeguard the rights, safety, and well-being of the patient.

11.2 Patient Information and Informed Consent

After the protocol has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative prior to protocol participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with all applicable regulatory requirement(s). The fat and skin biopsies in pediatric age range children are considered more than minimal risk.

Subjects < 18 years old are an integral part of the research question in Aim 3. Based on CFR 46.406, research that incurs minor increase over minimal risk (which the proposal is since the biopsy procedures are to be done with topical anesthetic) without potential direct benefit (which would be a conservative interpretation as there may be potential benefit based on what we find) can be done if research meets the following regulatory criteria:

> "*45 CFR 46.406 - Research involving greater than minimal risk and no prospect of direct benefit to the individual child subjects involved in the research, but likely to yield generalizable knowledge about the subject's disorder or condition. In order to approve research in this category, the IRB must make the following determinations:*
>
> *•the risk of the research represents a minor increase over minimal risk.*
>
> *•the intervention or procedure presents experiences to the child subjects that are reasonably commensurate with those inherent in their actual, or expected medical, dental, psychological, social, or educational situations.*
>
> *•the intervention or procedure is likely to yield generalizable knowledge about the subject's disorder or condition, which is of vital importance for the understanding or amelioration of the disorder or condition; and*
>
> *•adequate provisions are made for soliciting the assent of the children and the permission of their parents or guardians, as set forth in HHS regulations at 45 CFR 46.408.*
>
> *Studies done under this regulation require 2 parent signatures (unless documentation of only one parent with parental rights)."*

Adipose tissue biopsies performed under local anesthetic use meet the definitions set forth. The procedures will be done with a clean incision and sutures will be applied. These procedures are in the category of procedures that they can experience as pre-teens and teenagers in the usual course of their lives and are not more invasive than repair of lacerations occurring during sporting events or wisdom teeth extractions. There is the prospect of generalizable knowledge about the condition that can lead to novel therapy development. We will secure assent from the teenager and consent from two parents where applicable.

11.3 Sources of materials

Serum and plasma samples, circulating monocytes, and fat biopsy specimens obtained from the patients constitute the study materials. In addition, patients will undergo imaging studies that will be used to determine their body composition, and hepatic fat, as well as elastography.

All data collected on study participants will be obtained and managed specifically for research purposes. The types of data to be collected include demographic information, clinical and laboratory data, and pre- and post-intervention surveys. We will use Research Electronic Data

Capture (REDCap). This is a secure web-based application that provides an intuitive interface for data entry, audit trails, automated export and import procedures, and advanced features such as branching logic and calculated fields. Participants' information will only be stored on fire-walled databases, which will limit access to patient identifying information to only those assigned appropriate permissions, such as the research assistants and study coordinators. Whenever possible, research investigators will only have access to the de-identified information where the study will be identified by their unique study ID number only. This study will use the Redcap electronic data capture system, maintained by the Michigan Institute of Clinical Research (MICHR; the University of Michigan CTSA) data management Core. The study website will be available via secure access, and security will be implemented using firewalls, unique user IDs and passwords, secure socket level (SSL) encryption, trusted third party certificates, and standard operating system maintenance, backups, and patches. All completed paper forms containing data will be kept in a secure, locked filing cabinet located in the research offices at the participating sites. Study personnel and appropriate oversight organizations (including the IRB) will have access to the study databases as needed. Subject identity and confidentiality will be maintained throughout the conduct of this study.

11.4 Subject Privacy

The subject interviews during the study visits and administering of study tests will take place in a private room. Results of testing data will be shared with the participant or participant's legally authorized representative if the participant has consented to that. Informed consent documents contain detailed information on the personal information of subjects and how these will be protected.

11.5 Data security

p) Data Storage

We will use Research Electronic Data Capture (REDCap). This is a secure web-based application that provides an intuitive interface for data entry, audit trails, automated export and import procedures, and advanced features such as branching logic and calculated fields.

q) Confidentiality agreements

All users of the data will sign confidentiality agreements, and all users of the data receive training on proper procedures for working with patient-level data.

r) c) System controls to limit access to the data

Data file permissions will be set to allow access by only authorized personnel.

s) Computer system security plan

A security plan exists for this computer system providing for protection of patient-level data.

t) Virus protection software

The computer system uses UM IT selected antivirus software. The virus definitions used by the software have been updated within the last 30 days, and the software automatically checks for updates at least once a week.

u) Firewall

The organization's network system is protected by a firewall.

v) Computer locking

The computer automatically locks, requiring a password to unlock, after no more than 20 minutes of inactivity.

w) Physical security

Physical access to the servers and/or computers is restricted to authorized personnel, and computers are located in a room inaccessible to the general public (with at least one locked door) when not in use.

x) Securing electronic copies

All electronic copies of the patient-level data files on removable media (including original CDs) will be secured in a locked cabinet or room accessible only to authorized users when not in use.

y) Securing paper copies

All paper materials containing patient-level data records will be secured when not in use and otherwise protected from loss or unauthorized release while in use and will be shredded before disposal.

11.6 Publication of Protocol Findings and Use of Information

The information obtained from the clinical protocol will be disclosed to regulatory authority(ies) and may be disclosed to other Investigators or consultants as required. Subjects' confidentiality will be protected. We have every intention to disseminate these results.

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13.0 APPENDICES

Standardization of Blood Pressure Measurement

For BP measurements, the patient will remain in the sitting position **for at least 5 minutes** before any BP readings are recorded. Systolic and diastolic blood pressures will be determined by averaging three (3) consecutive measurements obtained 2 minutes apart. None of the 3 consecutive readings can be >5 mm Hg from the calculated average of the 3 readings. If this occurs, obtain additional readings, 2 minutes apart, until 3 consecutive stable measurements are obtained. Record only the 3 stable readings, in addition to the mean, the initial time of the measurement and the arm used for the measurement in the case report forms (as outlined below). The following instructions will be followed for both manual and automatic blood pressure measurements (with steps specific to manual identified by "[MANUAL]").

Please pay special attention to selecting the appropriate cuff size for this patient population, as noted below.

The accuracy and reliability of blood pressure readings will increase by following these standardized steps:

a. Situate the individual in a quiet environment with the arm resting at the heart level.
b. [MANUAL] For manual measurements, place the manometer at eye level, sufficiently close to read the calibration markings on the gauge or column.
c. Select the appropriately sized cuff. The proper cuff size will be used on the **non- dominant arm throughout the protocol.** Bladder width will be at least 40% of arm circumference; bladder will be at least 80% of arm circumference.
d. [MANUAL] Locate the brachial artery along the inner upper arm by palpation.
e. Wrap the cuff smoothly and snugly around the arm, centering the bladder over the brachial artery. The lower margin will be 2.5 cm above the antecubital space. (Do not rely on cuff marking; find the center by folding the bladder in half.)
f. For manual measurements:
    i. Determine the level for maximal inflation by observing the pressure at which the radial pulse is no longer palpable as the cuff is rapidly inflated (palpated systolic) and by adding 30 mm Hg.
    ii. Rapidly and steadily deflate the cuff. Then wait for 15 to 30 seconds before re- inflating.
    iii. For manual measurements, position the stethoscope over the palpated brachial artery below the cuff at the antecubital fossa. Earpieces should point forward. The bell head of the stethoscope will be applied with light pressure, ensuring skin contact at all points. Heavy pressure may distort sounds.
    iv. Rapidly and steadily inflate the cuff to the maximal inflation level as determined in Step f.i.
    v. Release the air in the cuff so that the pressure falls at a rate of 2 to 3 mm per second.
    vi. Note the systolic pressure at the onset of at least two consecutive beats (Phase 1 Korotkoff sounds). Blood pressure levels should always be recorded in even numbers and read to the nearest 2 mm Hg mark on the manometer.
    vii. Record the diastolic pressure at the cessation of the Korotkoff sounds (Phase V). Listen for 10 to 20 mm Hg below the last sound heard to confirm disappearance, and then deflate the cuff rapidly and completely.
g. For automatic measurement:
    i. Take automatic measurements and records.
h. Record the patient's position and the arm used for the measurement.
i. Wait 2 minutes before repeating the pressure measurement in the same arm to permit the release of blood trapped in the arm veins.

j. Note that all three readings at each time point will be captured on the case report form, as well as the average.

k. For each patient, the method used for BP determinations (manual or automatic) will be used throughout the protocol. In addition, the same size cuff will be used throughout. Care will be taken to make all measurements in the same position (sitting) for all measurements.

Rounding Rules for Blood Pressure Measurements

Blood pressure readings will be recorded to the nearest **even** mm Hg. **Do not round off to the nearest 5- or 10-mm Hg BP reading.** Therefore, a 142/94 reading should not be reported as 140/95, but will be reported as 142/94.

When calculating the **means** (average) of the readings, the following rules will be used: If the value is XX.1 to XX.4, it will be rounded down (example: A diastolic mean of 97.2 would be recorded as 97, as would a mean of 97.4).
If the value is XX.5 or greater it will be rounded up (example: A diastolic mean of 97.5 would be recorded as 98, as would a mean of 97.9).
The **mean** of the readings may be an odd number.

Special Pitfalls and Problems

The Auscultatory Gap

[MANUAL] In some subjects, particularly in patients with hypertension, the sounds heard over the brachial artery when the cuff pressure is high disappear as the pressure is reduced and then reappear at some lower level. This early, temporary disappearance of sound is called the auscultatory gap and occurs during the latter part of Phase I and Phase II. Because this gap may extend over a range as great as 40 mm Hg, one may seriously underestimate the systolic pressure or overestimate the diastolic pressure, unless its presence is excluded by first palpating for disappearance of the radial pulse as the cuff pressure is raised.

Effect of Arm Position

The pressure in the arm increases as the arm is lowered from the level of the (phlebostatic axis); conversely, raising the arm above this position lowers the pressure measurement. The effect is largely explained by hydrostatic pressure or by the effect of gravity on the column of blood.

Therefore, when measuring indirect blood pressure, the patient's arm will be positioned so that the location of the stethoscope head (preferably the bell or it is equivalent) is at the level of the heart. This location of the heart is arbitrarily taken to be at the junction of the fourth intercostal space and the lower left sternal border. **When the patient is seated, placing the arm on a nearby tabletop a little above waist level will result in a satisfactory position.**

Large Arm Size

Falsely elevated indirect pressure measurements may be obtained in patients with increased arm girth if the standard-sized bladder and technique are used. This is caused by the use of bladders that are too small, with subsequent excessive loss of cuff pressure through the thick, compressible soft tissues of the large arms. This problem may be minimized by using a bladder width that is 40 to 50% of measured arm circumference. In individuals with moderately large arm size, a large adult cuff (32 to 42 cm wide) will usually be adequate, but a larger cuff (38-50 cm) will be available, if necessary.

Creatinine Clearance Estimate by Cockcroft-Gault Equation

Male Creatinine Clearance mL/min = 1.0 * ((140 - Age) / (SerumCreat)) * (Weight (kg) / 72) Female Creatinine Clearance mL/min = 0.85 * ((140 - Age) / (SerumCreat)) * (Weight (kg) / 72)

Age in years
Serum creatinine in mg/dL

In pediatric patients younger than 12 years of age, the Cockcroft-Gault equation can also be employed, but values calculated may represent glomerular filtration rate (GFR) per body surface area and not represent creatinine clearance per se. If a more accurate creatinine clearance value is important to determine, alternative pediatric population equations can be considered as alternatives to the Cockcroft-Gault equation (Pierrat, 2003; Filler 2005).

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OBJECTIVE ANALYSIS.
EFFECTIVE SOLUTIONS.

HEALTH CARE

36-Item Short Form Survey Instrument (SF-36)

RAND 36-Item Health Survey 1.0 Questionnaire Items

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- ○ 1 - Excellent
- ○ 2 - Very good
- ○ 3 - Good
- ○ 4 - Fair
- ○ 5 - Poor

2. Compared to one year ago, how would you rate your health in general now?

- 1 - Much better now than one year ago
- 2 - Somewhat better now than one year ago
- 3 - About the same
- 4 - Somewhat worse now than one year ago
- 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day. Does your health now limit you in theseactivities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuoussports	○ 1	○ 2	○ 3
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, orplaying golf	○ 1	○ 2	○ 3
5. Lifting or carrying groceries	○ 1	○ 2	○ 3
6. Climbing **several** flights of stairs	○ 1	○ 2	○ 3
7. Climbing **one** flight of stairs	○ 1	○ 2	○ 3
8. Bending, kneeling, or stooping	○ 1	○ 2	○ 3
9. Walking **more than a mile**	○ 1	○ 2	○ 3
10. Walking **several blocks**	○ 1	○ 2	○ 3
11. Walking **one block**	○ 1	○ 2	○ 3
12. Bathing or dressing yourself	○ 1	○ 2	○ 3

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	Yes	No
13. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
14. **Accomplished less** than you would like	○ 1	○ 2
15. Were limited in the **kind** of work or other activities	○ 1	○ 2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	○ 1	○ 2

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
18. **Accomplished less** than you would like	○ 1	○ 2
19. Didn't do work or other activities as **carefully** as usual	○ 1	○ 2

20. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normalsocial activities with family, friends, neighbors, or groups?

- ○ 1 - Not at all
- ○ 2 - Slightly
- ○ 3 - Moderately
- ○ 4 - Quite a bit
- ○ 5 - Extremely

---

21. How much bodily pain have you had during the past 4 weeks?

- ○ 1 - None
- ○ 2 - Very mild
- ○ 3 - Mild
- ○ 4 - Moderate
- ○ 5 - Severe
- ○ 6 - Very severe

---

22. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

○ 1 - Not at all ○

2 - A little bit ○

3 - Moderately ○

4 - Quite a bit ○ 5

- Extremely

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
24. Have you been a very nervous person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
25. Have you felt so down in the dumps that nothing could cheer you up?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
26. Have you felt calm and peaceful?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
27. Did you have a lot of energy?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
28. Have you felt downhearted and blue?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
29. Did you feel worn out?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
30. Have you been a happy person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
31. Did you feel tired?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6

32. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

○ 1 - All of the time

○ 2 - Most of the time ○

3 - Some of the time ○ 4 -

A little of the time ○ 5 -

None of the time

How TRUE or FALSE is each of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	○ 1	○ 2	○ 3	○ 4	○ 5
34. I am as healthy as anybody I know	○ 1	○ 2	○ 3	○ 4	○ 5
35. I expect my health to get worse	○ 1	○ 2	○ 3	○ 4	○ 5
36. My health is excellent	○ 1	○ 2	○ 3	○ 4	○ 5

Disease Distress Scale (adapted for Lipomatosis)

DIRECTIONS: Living with chronic diseases, such as lipomatosis can sometimes be tough. There may be many problems and hassles concerning lipomatosis and they can vary greatly in severity. Problems may range from minor hassles to major life difficulties. Listed below are 14 potential problem areas that people with lipomatosis may experience. Consider the degree to which each of the 14 items may have distressed or bothered you DURING THE PAST MONTH and circle the appropriate number.

Please note that we are asking you to indicate the degree to which each item may be bothering you in your life, NOT whether the item is merely true for you. If you feel that a particular item is not a bother or a problem for you, you would circle "1". If it is very bothersome to you, you might circle "6".

	Not a Problem	**A Slight Problem**	**A Moderate Problem**	**Somewhat Serious Problem**	**A Serious Problem**	**A Very Serious Problem**
1. Feeling that lipomatosis is takingup too much of my mental and physical energy every day.	1	2	3	4	5	6
2. Feeling that my doctor doesn't know enough about lipomatosis andlipomatosis care.	1	2	3	4	5	6
3. Not feeling confident in my day-to-day ability to deal with lipomatosis.	1	2	3	4	5	6
4. Feeling angry, scared and/or depressed when I think about living with lipomatosis.	1	2	3	4	5	6

5. Feeling that my doctor doesn't give me clear enough directions on how to deal with my lipomatosis.	1	2	3	4	5	6
6. Feeling that I will end up with serious long-term complications, no matter what I do.	1	2	3	4	5	6

	Not a Problem	A Slight Problem	A Moderate Problem	Somewhat Serious Problem	A Serious Problem	A Very Serious Problem
7. Feeling that friends or family are not supportive enough of self-care efforts (e.g. planning activities that conflict with my schedule).	1	2	3	4	5	6
8. Feeling that lipomatosis controlsmy life.	1	2	3	4	5	6
9. Feeling that my doctor doesn't take my concerns seriously enough.	1	2	3	4	5	6
10. Feeling that I am not sticking closely enough to a good lifestyle plan.	1	2	3	4	5	6
11. Feeling that friends or family don't appreciate how difficult living with lipomatosis can be.	1	2	3	4	5	6
12. Feeling overwhelmed by the demands of living with lipomatosis.	1	2	3	4	5	6
13. Feeling that I don't have a doctor who I can see regularly enough about my lipomatosis.	1	2	3	4	5	6
14. Feeling that friends or family don't give me the emotional support that I would like.	1	2	3	4	5	6

DECLARATION OF HELSINKI

World Medical Association Declaration of Helsinki:

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:

- 29th WMA General Assembly, Tokyo, Japan, October 1975
- 35th WMA General Assembly, Venice, Italy, October 1983
- 41st WMA General Assembly, Hong Kong, September 1989
- 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
- 52nd WMA General Assembly, Edinburgh, Scotland, October 2000

- 53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
- 55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
- 59th WMA General Assembly, Seoul, Republic of Korea, October 2008
- 64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that “A physician shall act in the patient’s best interest when providing medical care.”

It is the duty of the physician to promote and safeguard the health, well-being, and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfilment of this duty.

Medical progress is based on research that ultimately must include studies involving human subjects.

The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic, and therapeutic interventions (methods, procedures, and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility, and quality.

Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

Physicians must consider the ethical, legal, and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal, or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

Medical research should be conducted in a manner that minimizes possible harm to the environment.

Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training, and qualifications. Research on patients or

healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

In medical practice and in medical research, most interventions involve risks and burdens.
Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.
All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.
Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed, and documented by the researcher.
Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.
When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.
All vulnerable groups and individuals should receive specifically considered protection.
Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.
The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.
In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

The research protocol must be submitted for consideration, comment, guidance, and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards, but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

When a potential research subject who is deemed incapable of giving informed consent is able to

give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

The benefits, risks, burdens, and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

In advance of a clinical trial, sponsors, researchers, and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

Researchers, authors, sponsors, editors, and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness

and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health, or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.