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Phase 2 Study of Orlistat and SLx-4090 for the Treatment of Type 1 Hyperlipoproteinemia

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Abstract

Type I hyperlipoproteinemia is a rare, autosomal recessive metabolic disorder characterized by extreme hypertriglyceridemia due to a deficiency in lipoprotein lipase or related proteins. Treatment of these patients is challenging as triglyceride-lowering medications are ineffective. A low fat diet is helpful, however, despite good dietary compliance, some patients continue to have severe hypertriglyceridemia and recurrent pancreatitis which can be life threatening. Therefore, we wish to investigate whether inducing dietary fat malabsorption or inhibiting chylomicron formation will cause further lowering of serum triglycerides (TG) beyond the effect of limiting dietary fat intake.

We will study the efficacy and safety of an inhibitor of intestinal lipase (Orlistat) and an intestinal-specific inhibitor of microsomal triglyceride transport protein (MTP) involved in the assembly and secretion of chylomicrons (SLx-4090), alone and in combination, for reducing serum triglyceride levels in patients with Type I hyperlipoproteinemia. We plan to enroll 20 patients with Type I hyperlipoproteinemia in a randomized, double-blind, placebo-controlled, cross-over trial. After a baseline evaluation, the subjects will be randomly assigned to placebo/placebo, Orlistat/placebo, SLx-4090/placebo or Orlistat/SLx-4090 for the duration of four weeks followed by a one week wash out period. During the last week of each study period, fasting blood samples will be drawn for three consecutive days for serum lipids and chemistry panel. The primary endpoint will be serum triglycerides; the secondary endpoint variables will be fasting and postprandial serum chylomicron-TG levels, postprandial serum TG levels during a meal tolerance test and retinyl palmitate levels during a meal tolerance test, **and episodes of acute pancreatitis**. Repeated measures analysis of variance will be used for statistical comparisons.

Our results may help in designing novel therapeutic approaches for patients with type 1 hyperlipoproteinemia.

SPECIFIC AIMS:

Type I hyperlipoproteinemia (T1HLP) is a rare, autosomal recessive condition detected in early childhood and characterized by recurrent pancreatitis due to extreme hypertriglyceridemia as a result of accumulation of chylomicrons. In most of the patients, T1HLP is due to lipoprotein lipase (LPL) or apolipoprotein CII (APOC2) deficiency, however, recently mutations in lipase maturation factor 1 (LMF1) and glycosyl-phosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) have also been reported. In some patients, the genetic basis of T1HLP remains unknown. Patients present with eruptive or tuberous xanthomas, pancreatitis, lipemia retinalis, and hepatosplenomegaly. Most importantly, acute pancreatitis is often a cause for significant morbidity and even mortality in these patients. Treatment of these patients poses a significant challenge as the current medications for hypertriglyceridemia such as fibrates, niacin and omega-3 fatty acids are ineffective. Some investigators try invasive procedures such as plasmapheresis to lower TG and others are exploring gene therapy using intramuscular injection of AAV1-Lipoprotein lipase^{S447X} in patients with LPL deficiency. The only effective therapy is a low fat diet (<20% of total energy). Since the basic defect in T1HLP is the reduced clearance of chylomicrons due to impaired lipolysis of triglyceride (TG), reduction in dietary fat by reducing chylomicron formation can lower serum TG. However, some patients continue to have severe triglyceridemia and acute pancreatitis despite following a low fat diet. Therefore, we propose to test novel therapeutic approaches for the management of hypertriglyceridemia in patients with T1HLP.

The absorption of dietary TG from the small intestine occurs in a stepwise fashion. In the intestine, lipases (in the presence of bile salts) hydrolyze TG into monoacylglycerol (MAG), diacylglycerol (DAG), fatty acids and glycerol, and form mixed micelles for absorption at the brush border of the enterocytes. After entering the enterocyte, MAG, DAG and glycerol are reacylated. Finally, microsomal triglyceride transfer protein (MTP) assembles chylomicrons using TG, cholesterol esters and apolipoprotein B-48 (ApoB48) and chylomicrons are secreted in the lymph. We wish to investigate whether inhibitors of intestinal lipase (Orlistat) or intestinal-specific inhibitor of MTP (SLx-4090, Surface Logix Inc.) will reduce serum triglyceride concentrations in patients with T1HLP when used alone or in combination. Thus, the hypothesis to be tested and the specific aim are:

Hypothesis: Inhibiting dietary fat absorption using lipase inhibitor, and chylomicron formation using an intestinal-specific MTP inhibitor, will lower serum TG concentrations in patients with T1HLP.

Specific Aim: To investigate the efficacy and safety of Orlistat and SLx-4090, alone and in combination, for reducing serum TG levels in patients with T1HLP in a double-blind, randomized, placebo-controlled, cross-over study.

We expect that Orlistat or SLx-4090 alone will markedly reduce serum triglycerides in patients with T1HLP. We also expect that the combination of the two drugs will be more efficacious than each drug used alone. Therefore, the results of this study may help in designing a rational therapeutic approach to extreme hypertriglyceridemia in patients with T1HLP. This study will be the first clinical trial in patients with T1HLP and the proposed research will find novel therapies for patients with T1HLP.

RESEARCH STRATEGY:

Significance:

Type I hyperlipoproteinemia (T1HLP) is a rare, autosomal recessive disorder detected in early childhood and characterized by recurrent pancreatitis due to extreme hypertriglyceridemia as a result of accumulation of chylomicrons (1-2). Four loci have been identified so far for T1HLP: lipoprotein lipase (LPL); apolipoprotein CII (APOC2); lipase maturation factor 1 (LMF1); and glycosyl-phosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) (3-4). The deficiency of LPL, APOC2, LMF1 or GPIHBP1 impairs the ability to hydrolyze chylomicron triglycerides in circulation and the patients develop recurrent eruptive or tuberous xanthomas, abdominal pain due to acute pancreatitis, lipemia retinalis and hepatosplenomegaly. **Some patients with LPL deficiency have been reported to develop premature atherosclerosis (5).** Acute pancreatitis is the most serious complication and can cause significant morbidity and even mortality. Unfortunately, most of the currently approved triglyceride lowering medications such as fibrates, niacin and omega-3 fatty acids are ineffective (1-2). In some cases, physicians have used invasive techniques such as plasmapheresis to lower TG (6). On the other hand, gene therapy trials using intramuscular injection of AAV1-Lipoprotein lipase^{S447X} have been ongoing in patients with LPL deficiency, however, long term efficacy has been poor perhaps related to an immune response against AAV1-capsid proteins (7). The only effective and accepted therapy is a low fat diet (<20% of total energy). Since the basic defect in T1HLP is the reduced clearance of chylomicrons due to impaired lipolysis of triglyceride (TG), reduction in dietary fat by reducing chylomicron formation can lower serum TG. However, some patients continue to have severe triglyceridemia and acute pancreatitis despite following a low fat diet (1). This could be due to the presence of invisible fat in diet (8).

There have been no formal clinical trials conducted in patients with T1HLP so far and most of the evidence about efficacy or lack of efficacy of medications or dietary intervention is anecdotal. Lack of availability of enough patients with T1HLP at a single academic institution has been a critical barrier in the progress in this field. Nonetheless, there is a need to develop novel therapies for patients with T1HLP. We will recruit patients locally as well as from other academic institutions in North America. We have successfully employed this model to investigate efficacy and safety of leptin and cholic acid therapy for rare patients with lipodystrophies (9-10). Therefore, we propose to test two drug therapies for the management of hypertriglyceridemia in patients with T1HLP based upon the current understanding of dietary fat absorption and chylomicron formation (Fig. 1) (11).

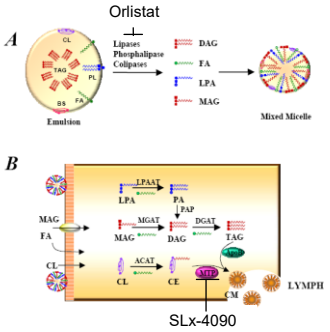


Fig. 1. The process of dietary lipid digestion and absorption. *A,* The digestion of dietary lipids begins with partial digestion by gastric lipase, forming large fat globules with a triacylglycerol (TAG) core surrounded by phospholipids (PL), free cholesterol (CL), fatty acids (FA), and ionizing proteins. In intestine, fat globules are mixed with bile salts (BS) and pancreatic lipases. Monoacylglycerol (MAG), lysophosphatidic acid (LPA), diacylglycerol (DAG), and FA that are released from lipid and PL hydrolysis join bile salts, CL, and fat-soluble vitamins to form mixed micelles for dietary fat absorption at the brush border of the enterocytes. *B,* After entering the enterocyte, MAG, LPA, and CL are reacylated. MAG is sequentially acylated by MGAT and DGAT enzymes to form TAG. LPA is acylated by LPA acyltransferase (LPAAT) to phosphatidic acid (PA) followed by dephosphorylation by PA phosphorylase (PAP) to yield DAG. Dietary cholesterol (CL) is acylated by acyl-CoA:cholesterol acyltransferase (ACAT) to cholesteryl esters (CE). Facilitated by microsomal triglyceride transfer protein (MTP), TAG joins CE and apolipoprotein B-48 (ApoB48) to form chylomicrons that are secreted in the lymph for delivery to circulation. Modified from Shi and Cheng (11). The steps that are inhibited by Orlistat (an intestinal lipase inhibitor) and by SLx-4090, an intestinal-specific MTP inhibitor are shown.

Orlistat is an inhibitor of gastric and pancreatic lipases and can reduce dietary fat absorption by 30% (12). Thus, Orlistat may reduce serum TG levels in patients with T1HLP by decreasing the substrate available for chylomicron formation. Another step that is amenable to pharmaceutical intervention involves chylomicron formation by microsomal triglyceride transfer protein (MTP) (13-18). At present, several MTP inhibitors are being developed for lowering lipids, some are non-specific and cause generalized inhibition at the liver and intestine [CP-346086 (13), BAY13-9952 (14), 8aR (15), and BMS-201038/AEGR-733 (16)] whereas some cause more specific inhibition of intestinal MTP [SLx-4090 (17, 19), JTT-130 (18), and dirlotapide (20)]. SLx-4090 (Surface Logix Inc.) targets enterocytes specifically and avoids the side effect of hepatic TG accumulation seen with nonspecific MTP inhibitors (17, 19). SLx-4090 has been shown to reduce post-prandial TG by about 50% (17, 19). In the proposed study, we wish to investigate the efficacy and safety of Orlistat and SLx-4090 alone and in combination for reducing serum TG concentrations in patients with T1HLP.

T1HLP qualifies for the Orphan Product Development program as it is a rare condition. The prevalence of LPL deficiency (the most common type of T1HLP) is estimated to be 1 in a million. To date, there are less than 1000 patients reported in the world with T1HLP (Table 1).

Table 1: Reported cases of different types of T1HLP in the World

Type of T1HLP	Approximate N
LPL deficiency	300
Apo CII deficiency	18
LMF 1 deficiency	2
GPIHBPI mutations	4
Unknown genetic basis	600

Source OMIM and literature review (1)

This study for the first time aims to develop new therapeutic strategies for patients with T1HLP. If the proposed aims are achieved, Orlistat, SLx-4090 or a combination of the two drugs may become standards of therapy for patients with T1HLP. **In our clinical experience and those of the others, T1HLP patients develop chylomicronemia-associated acute pancreatitis only when serum TG concentration is > 2000 mg/dL. We will expect that if the drug therapies are able to reduce TG concentration < 2000 mg/dL and preferably < 1000 mg/dL that these will be effective in reducing the risk of acute pancreatitis. We certainly plan to follow this study with a multicenter study to determine long-term efficacy and safety of these therapies in patients with T1HLP. This multicenter study will also include acute pancreatitis as an end point variable. In the current study, we will capture episodes of acute pancreatitis and include them as a secondary end point variable.**

Innovation:

Conventional pharmacologic therapies alone are ineffective in patients with T1HLP and there is great need for alternative treatments for T1HLP since they face potentially life-threatening complication of acute pancreatitis. Orlistat is not approved for treatment of hyperlipidemia and this protocol will test its efficacy in patients with T1HLP. The SLx-4090 is currently under development for hyperlipidemias. The choice of SLx-4090 is unique to these patients with T1HLP as it is intestinal-specific and is devoid of hepatotoxicity seen with other non-specific MTP inhibitors. Thus, this will be the first well-designed trial testing novel therapeutic options for patients with T1HLP.

Approach:

We plan to conduct a pilot and feasibility study involving 20 patients with T1HLP selected according to the following criteria. **The study has been approved by the UT Southwestern IRB and an IRB approved consent form is attached (see appendix).**

Subjects:

Inclusion Criteria:

1) Type I hyperlipoproteinemia.
2) Fasting serum triglyceride levels of greater than 1000 mg/dL.
3) Age > 18 years or 12-18 years with a previous history of acute pancreatitis

Exclusion criteria:

1) Secondary hypertriglyceridemias due to diabetes, renal disease, hypothyroidism, alcoholism and drug therapy such as estrogens and estrogen analogues, steroids, HIV-protease inhibitors, retinoic acid derivatives and interferons.
2) Pregnant or lactating women
3) Significant liver disease (elevated transaminases > 2 times upper limit of normal)
4) Alcohol abuse (> 7 drinks or 84 g per week for women and > 14 drinks or 168 g per week for men)
5) Severe anemia (hematocrit < 24%)

6) Drug use (cocaine, marijuana, LSD, etc.)
7) Major surgery in the past three months
8) Congestive heart failure
9) Serum creatinine greater than 2.5 mg/dL
10) Cancer within the past five years
11) Gastrointestinal surgery in the past
12) Current therapy with anti-coagulants, digoxin and anti-arrhythmics
13) Chronic malabsorption syndromes
14) Cholestasis
15) Acute illnesses such as acute pancreatitis in the last 8 weeks

Recruitment: The study subjects will be recruited from the lipid clinics at the Parkland Memorial Hospital, Veterans Affairs North Texas Health Care System, and UT Southwestern Aston Ambulatory Center. We will also advertise the study among local physicians. We will post the study on our website and at clinicaltrials.gov. We will also enroll patients from all over the U.S. and Canada (see letters of support). We have previously been very successful in recruiting patients from other cities in the US and Canada for other therapeutic trials in patients with lipodystrophies and will use the same strategy. We will also contact authors of recent papers on T1HLP for patient referral. We have already identified **10** patients with T1HLP eligible for the study (Table 2).

Table 2: Eligible Subjects with T1HLP for the Study

Pt ID	Sex	Country of Origin	Age (y)	Acute Pancreatitis	Age of onset (y)	Hepatosplenomegaly	Anemia	Highest TG (mg/dl)	Current Therapy
T1HLP 100.8	F	El Salvador	37	Yes	24	Yes	Yes	9600	Gemfibrozil Fish oil
T1HLP 200	F	Colombia	41	Yes	13-15	No	No	8730	Fenofibrate Fish oil Insulin
T1HLP 400	F	Hispanic	52	No	42	No	No	1548	Fenofibrate Statin, Fish oil
T1HLP 500.3	F	India	33	Yes	6	Yes	Yes	2476	Gemfibrozil Fish oil
T1HLP 600	F	Mexico	26	Yes	15-16	No	Yes	2000	None
T1HLP 900	F	US	48	Yes	44	No	Yes	12,000	Fenofibrate fish oil
T1HLP 1100.4	**F**	**India**	**18**	**Yes**	**birth**	**Yes**	**Yes**	**>19,000**	**Tricor Lovaza**
T1HLP1200.4	**M**	**Hispanic**	**42**	**No**	**42**	**No**	**No**	**3597**	**None**
T1HLP1500.3	**M**	**Indian**	**39**	**No**	**29**	**No**	**No**	**4840**	**Fenofibrate L-thyroxine Omacor**
T1HLP 1600	**F**	**Mexico**	**22**	**Yes**	**21**	**N/A**	**N/A**	**>5000**	**Fenofibrate Fish oil, Niacin**

Study design: A randomized, double-blind, placebo-controlled, cross-over study design will be employed. Each treatment period will be of 4 wk duration and will be followed by a wash out period of one week (Fig. 2).

Screening visit: Fasting blood samples will be obtained from the patients for complete blood counts, serum lipid profile, chylomicron-TG, serum chemistry and aminotransaminases, serum TSH and free thyroxine. A routine urinalysis will be conducted. A full history will be obtained and a physical examination will be performed to determine eligibility.

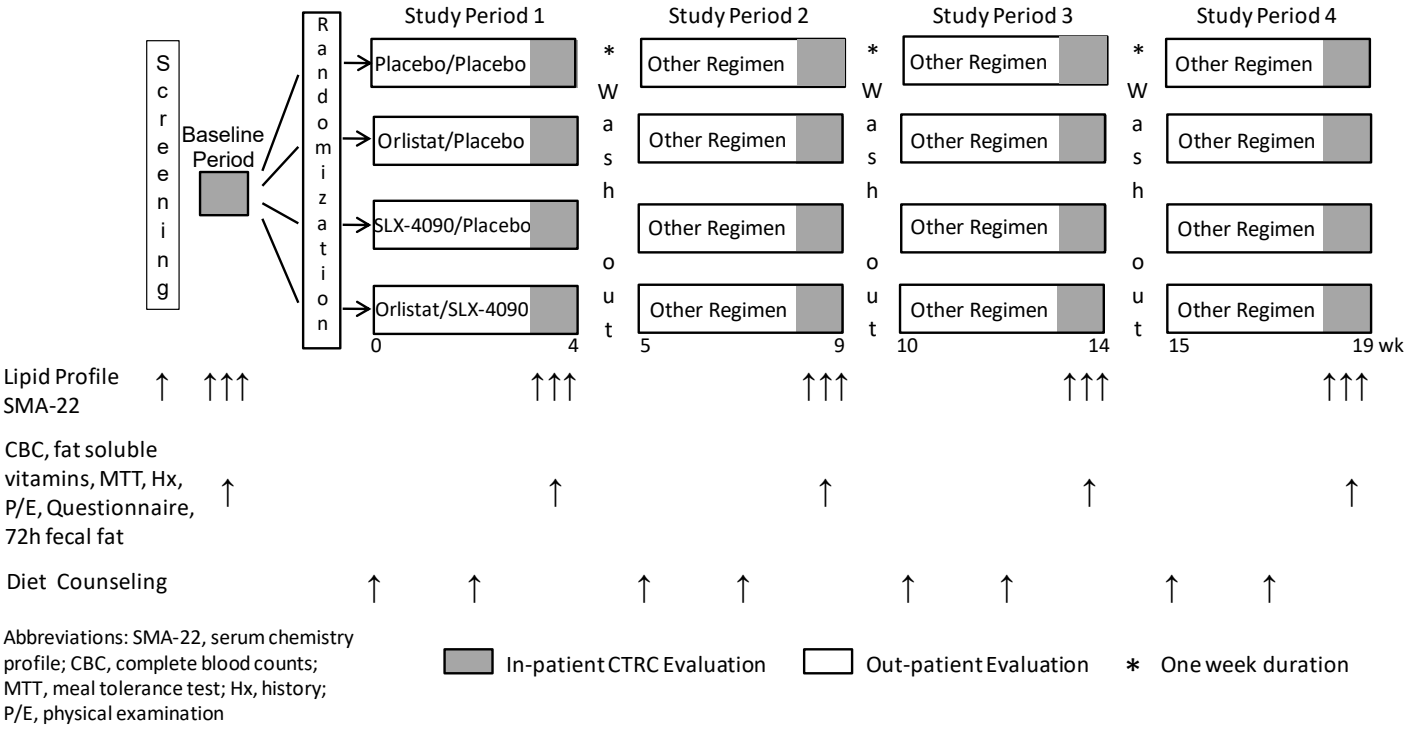
Baseline period: All patients will be admitted to the inpatient Clinical and Translational Research Center (CTRC) for a period of four to five days. During this time, they will consume a metabolic isoenergic diet prepared in CTRC kitchen containing 15% of total energy from fat, 70% from carbohydrate and 15% from protein. The same diet will be used during subsequent admissions to the CTRC. This is to ensure that serum lipid evaluation is made when patients are taking a low fat diet to avoid confounding effects of variability in

dietary fat content. The subjects will also be asked to complete a 3-day food recall before admission to the CTRC to assess energy intake as well as dietary composition. All patients will then be asked to follow a low fat diet (< 20% energy from fat) for the remainder of the study period. The diet counseling will be done by a trained research fellow or CTRC dietician immediately after the baseline period and at the beginning each new treatment period and at 2 wk duration for the remainder of the study. The counseling will be done in person at baseline and at subsequent follow up visits and by phone in the intervening intervals.

During the last three consecutive days of the inpatient stay at the CTRC, fasting blood samples will be obtained for serum lipid profile, chylomicron-TG, serum chemistry, and aminotransaminases. Blood will also be drawn once for determination of fat soluble vitamin levels (A, D, E, and K). Patients will collect 72 hour stool for determination of fecal fat. On the last day of the baseline period, a meal tolerance test will be performed.

Study phase: The patients will then be randomized to receive any of the four regimens in a double-blind, cross-over fashion: placebo/placebo, placebo/Orlistat, placebo/SLx-4090, or Orlistat/SLx-4090. The randomization will be done using 4X4 Williams Latin squares where each treatment follows every other treatment once within each square. Each subject will be assigned to take each regimen for 4 weeks followed by a one week wash out period. We will give Orlistat/placebo at a dose of 2 capsules (each containing 60 mg of active drug or placebo) three times a day with each meal (a total dose of 360 mg daily). SLx-4090/placebo will be given at a dose of 4 tablets (each containing 50 mg of active drug or placebo) three times a day with each meal. SLx-4090/Orlistat at a dose of 4 tablets each containing 50 mg active drug three times a day with each meal/ 2 capsules each containing 60 mg active drug three times a day with each meal. Placebo/placebo at a dose of 4 tablets (each containing placebo) and 2 capsules (each containing placebo) taken three times per day with each meal. In the first week of each phase of the study, patients will be given half of the total dose of both Orlistat/Placebo (1 capsule three times a day) and SLx-4090/Placebo (2 tablets three times a day). If they are able to tolerate this without any serious side effects, they will be advised to take the full dose for the next three weeks. In case of side effects or poor tolerance to the drugs during the last three weeks of each study phase, the dose may be reduced to 1 capsule three times a day for Orlistat/placebo and 1 tablet three times a day for SLx-4090/placebo or lower at the Investigators' discretion. The patients will also take a daily multivitamin supplement (Centrum, Wyeth) containing vitamin A 3,500 IU (71% as retinol and 29% as β-carotene), vitamin D 400 IU (as cholecalciferol), vitamin E 30 IU (as dl-α-tocopheryl acetate) and vitamin K 25 μg (as phytonadione). All laboratory studies enlisted during the baseline period will be repeated during the four study periods when patients will be admitted to CTRC for four to five days each.

Fig. 2 Flow Diagram for the study



We will proactively inform the subjects of common anticipated drug side effects prior to enrollment in the study. We will ask the Clinical and Translational Research Center (CTRC) dietician to provide nutritional counseling under the guidance of Dr. Shah (co-investigator). The CTRC nurses and the study research nurse will be aware of the anticipated side effects and their management and will be available 24 hours, 7 days a week.

Withdrawal criteria:

1. Acute pancreatitis
2. Aminotransferases greater than three times upper limit of normal
3. Intolerability of medications due to side effects

Procedures:

Serum lipoproteins: Serum cholesterol, TG and high density lipoprotein cholesterol will be measured by Quest Diagnostics. Serum chylomicron TG will be measured according to the Lipid Research Clinics method after ultracentrifugation (21) using enzymatic kits.

Serum chemistry, complete blood counts and urinalysis: Complete blood counts will include hemoglobin, hematocrit, MCV, MCH, MCHC, WBC count, RBC count, Platelet count and differential WBC count. Serum chemistry will include SMA-22 panel with electrolytes (Na, K, Cl, carbon dioxide, urea nitrogen, Creatinine, Ca, Mg, Phosphate), liver function tests (alanine aminotransferase, asparatate aminotransferase, Bilirubin, alkaline phosphatase, gamma glutamyl transferase), glucose, hemoglobin A1c, creatine phosphokinase, total protein, albumin, globulin, uric acid and lactate dehydrogenase. Urinalysis will include color, appearance, specific gravity, pH, glucose, bilirubin, ketones, occult blood, protein, nitrite, leukocyte esterase, WBC, RBC, Squamous epithelial cells, bacteria and hyaline cast. These tests will be done as indicated in the flow diagram. **Serum TSH and free T4 will be measured during each CTRC admission for those patients who are taking thyroid hormone.**

Plasma Levels of SLx-4090: Fasting blood samples will be drawn for measuring plasma levels of SLx-4090 during each study period. The determinations will be made by Surface Logix, Inc.

72 Hour Fecal Fat: Stool will be collected for 72 hours as an inpatient in the CTRC during each study period. The measurement will be performed by Quest Diagnostics. Oral carmine will be used as a marker to ensure 72 hour collection of the stools.

Fat soluble vitamin levels: Serum retinol, α-tocopherol, 25-OH Vitamin D and vitamin K will be measured once at baseline and during each study period. The measurements will be performed by Quest Diagnostics.

Three-Day Food Record: Dietary intake will be assessed by 3-day food record (two weekdays and one weekend day), a valid and reliable method (23). The records will be taken at baseline and at two week intervals. The participants will be instructed on how to record in detail all the food and drink consumed in the 3-day food record book provided. The booklet will be available in English and Spanish. The participants will also be provided with weighing scales and a two-dimensional visual chart of food portions to aid them in estimating portion sizes. Any questionable input or incomplete food entries recorded will be promptly addressed when the subjects return their food records. The food records will be analyzed for nutrient content using the University of Minnesota Nutrient Data System (NDS) for research.

Gastrointestinal Symptoms Questionnaire: All patients will complete a gastrointestinal symptoms questionnaire (see appendix) during admission to the CTRC (24). This questionnaire includes questions about diarrhea and steatorrhea, the expected side effects of Orlistat and SLx-4090.

Justification for the choice of study design and medications:

Orlistat: Orlistat is an inhibitor of gastric and pancreatic lipases and at a dose of 120 mg three times daily reduces dietary fat absorption by 30% (12). Several studies in the past ten years have shown a beneficial effect of Orlistat on postprandial lipids in healthy volunteers and in patients with diabetes (25-28). In healthy volunteers, Orlistat decreased postprandial chylomicrons by 19% after 8 weeks in doses of 10 mg, 30 mg, 60 mg or 120 mg three times daily (26). A single dose of Orlistat (120 mg) has also been shown in healthy men to reduce postprandial chylomicron levels and reduced the time at which postprandial lipemia returned to fasting levels (27). In obese diabetic patients, 120 mg of orlistat in a single dose reduced total triglycerides and free fatty acids (28). In a 52 week study involving obese type 2 diabetic men, Orlistat 120 mg three times daily reduced triglycerides and apolipoprotein B levels significantly (25). Furthermore, 32 obese, nondiabetic women with metabolic syndrome were randomized to receive Orlistat 120 mg three times daily or placebo for 10 days. The area under the curve for postprandial triglycerides was significantly reduced by 17.1% in the Orlistat group and did not change significantly in the placebo group (29). In five patients with type 5 HLP, on maximum triglyceride lowering therapy, three to six months of therapy with Orlistat 120 mg three times daily provided an additional 35% decrease in triglycerides (30). Thus, we expect Orlistat to be effective in lowering serum TG levels in T1HLP patients as well. The dose in children 12-18 years is the same as that for adults, i.e., 120 mg three times a day.

SLx-4090: The supporting evidence for the contention that inhibiting MTP can lower serum TG and chylomicrons, comes from patients with MTP deficiency who develop abetalipoproteinemia and have an inability to secrete apo-B containing lipoproteins, chylomicrons from the intestine and very low density lipoproteins from the liver (31). These patients have extremely low serum TG and do not form chylomicrons even in response to a dietary fat load. Pharmacological MTP inhibition can induce increased fecal fat loss. For example, an intestinal specific MTP inhibitor, dirlotapide, when given in doses of 0.4 to 0.5 mg/kg to dogs caused 4 to 5 fold increase in fecal fat excretion compared to placebo (20). SLx-4090 has been chosen for its enterocyte specific non-systemic actions and thus has been proposed to avoid the toxicities of MTP inhibition in the liver (17, 19). Non-specific MTP inhibitors such as AEGR-733 have been associated with increased serum transaminase levels and hepatic steatosis (16). SLx-4090 is currently being tested in Phase 2 trials for the treatment of dyslipidemia. Preliminary data show ~50% reduction in post-prandial TG area under the curve with SLx-4090 and it was tolerated well (17, 19).

Since SLx-4090 is an investigational drug, the following section provides a summary of information from preclinical and clinical studies available so far.

SLx-4090 is 6-(4'-trifluoromethyl-6-methoxy-biphenyl-2-ylcarboxamido)-1,2,3,4tetrahydroisoquinoline-2-carboxylic acid phenyl ester. The compound is a free base.

- Molecular formula: C31H25F3N2O4
- Molecular weight: 546.5

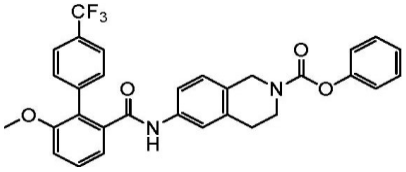


Fig. 3 Chemical structure of SLx-4090

NONCLINICAL STUDIES

- SLx-4090 inhibited MTP-mediated triglyceride (TG) transfer between lipid vesicles with an IC50 of 11 ± 1 nM when determined using purified bovine MTP. The M1 metabolite, SLx-4099, has an IC50 of 430 nM which is 40-fold less potent than SLx-4090.
- SLx-4090 inhibited MTP-mediated TG transfer in human intestinal microsomes with an IC50 of 15 ± 3 nM.
- SLx-4090 inhibited basolateral secretion of apolipoprotein B (ApoB) in an *in vitro* intestinal absorption assay (differentiated Caco-2) with an IC50 of 9.6 ± 1.6 nM.
- SLx-4090 inhibited the postprandial plasma increases in TG with an ED50 of 7 mg/kg and approximately 10 mg/kg PO in rats and dogs, respectively.
- SLx-4090 (30 mg/kg PO) effectively reduced the postprandial increases in plasma TG up to 6 h post-dosing in rats.
- In ApoE-/- mice maintained on a high fat diet, SLx-4090 (10, 30 and 100 mg/kg) administered for 10 weeks dose-dependently reduced body weight gain, plasma TGs, total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C). There was no effect on food consumption.
- In a 24 day study in mice (C57BL6/J) with diet induced obesity (DIO), SLx-4090 inhibited weight gain at a dose of 30 mg/kg and caused weight loss at a dose of 100 mg/kg in comparison with placebo.
- Once daily dosing of SLx-4090 for up to 4 days had no effect on the plasma levels of vitamins A, E and C.
- SLx-4090 (10 μM) had no effect on 25 gastrointestinal targets including neurotransmitters, ion channels, second messengers, brain/gut peptides and enzymes. SLx-4090 produced a slight inhibition of binding at the L-Type calcium channel (dihydropyridine site; 67% at 10 μM).

PHARMACOKINETICS AND PRODUCT METABOLISM STUDIES

- Systemic oral bioavailability (%F) in rats following single oral doses of 10 and 30 mg/kg was 0%.
- SLx-4090 was rapidly metabolized *in vitro* by human hepatic (half-life < 20 min) and intestinal (half-life < 20 min) microsomes. It was rapidly metabolized by rat hepatic microsomes (half-life < 20 min) but slowly metabolized by rat intestinal microsomes (half-life > 60 min). Metabolism in dog intestinal and hepatic preparations was slow (half-life > 60 min).
- SLx-4090 did not inhibit CYP P450 isozymes 2C9, 2D6, 2C19 and 1A2 (IC50 > 10 μM). The IC50 for CYP 3A4 using testosterone and midazolam as substrates were 4.3 μM and > 30 μM, respectively.
- SLx-4090 has a single identified M1 metabolite, SLx-4099, in rats, dogs and humans.
- SLx-4090 and SLx-4099 were highly (> 99.7%) protein bound in human plasma.

TOXICOLOGY

- SLx-4090 did not affect potassium currents in HEK293 cells at concentrations from 0.03 to 3 μM *in vitro*. Inhibition (26%) of potassium currents was observed at 10 μM. SLx-4090 was not detected in human plasma following a single oral dose of 800 mg in a first in man study (SLX-4090-06-01) indicating a significant margin of safety.
- SLx-4090 did not alter general physiology, pulmonary or cardiovascular function in safety pharmacology testing at dose up to 2000 mg/kg.

- SLx-4090 was negative in the core ICH genotoxicology testing battery.
- The no observed adverse event level (NOAEL) for SLx-4090 administered orally to Beagle dogs for 90 consecutive days was at least 100 mg/kg for both males and females, and was based on a reduction in cholesterol levels and decreases in body weight gain consistent with the mechanism of action of SLx-4090. Both male and female dogs showed an increase in soft feces. The prevalence of soft feces was highest at doses of 100 mg/kg.
- The toxicokinetic (TK) measures of plasma levels for SLx-4090 in dogs dosed orally for 90 days at the NOAEL of 100 mg/kg were as follows: males Cmax = 37.0 ng/mL; AUC0-last = 630 h*ng/mL; females Cmax=26.8 ng/mL; AUC0-last = 379 h*ng/mL.
- The TK measures of plasma levels for SLx-4099 in dogs dosed orally for 90 days at the NOAEL of 100 mg/kg were as follows: males Cmax = 23.6 ng/mL; AUC0-last = 418 h*ng/mL; females Cmax=21.9 ng/mL; AUC0-last = 365 h*ng/mL.
- The NOAEL for SLx-4090 administered orally to rats for 90 days was > 1000 mg/kg. There were no changes in clinical chemistry measures including TC and liver function tests (LFTs).
- Dose levels up to 1000 mg/kg for SLx-4090 administered orally to rats for 90 days did not produce pulmonary phospholipidosis and no biologically or toxicologically relevant changes were seen in Troponin T levels.
- The TK measures of plasma levels for SLx-4090 in rats dosed orally for 90 days at the NOEL of 1000 mg/kg were as follows: males Cmax = 2.65 ng/mL; AUC 0-last = 20.9 h*ng/mL; females Cmax = 14.6 ng/mL; AUC 0 – last = 144 h*ng/mL.
- The TK measures of plasma levels for SLx-4099 in rats dosed orally for 90 days at the NOEL of 1000 mg/kg were as follows: males Cmax = 3.73 ng/mL; AUC 0-last =14.0 h*ng/mL; females Cmax =7.18 ng/mL; AUC 0 – last =55.4 h*ng/mL.
- SLx-4090 had no phototoxic liability at a dose of 2000 mg/kg in pigmented rats.

EFFECTS IN HUMANS

The safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of SLx-4090 have been evaluated in 182 healthy subjects at increasing single doses ranging up to 800 mg, and at multiple doses up to 200 mg tid (600 mg daily) for up to 14 days.

- SLx-4090 given as single or repeat doses was well tolerated and there were no clinically significant adverse events (AEs) reported. There was no effect of SLx-4090 on LFTs.
- SLx-4090 was not detected in the plasma at any dose level.
- SLx-4099, the M1 metabolite of SLx-4090, appeared in a small number of samples at levels just slightly above the lower limit of quantification (LOQ) of the analytical assay when subjects were dosed with 400 mg, 600 mg and 800 mg.
- Statistically significant reductions in postprandial serum TG were observed at 5 to 8 hours postdose when subjects were administered the three highest single doses, 400 mg, 600 mg and 800 mg.
- SLx-4090 was well tolerated in healthy male volunteers at doses up to 200 mg tid for 14 days. There was no evidence of plasma levels of SLx-4090 at any dose
- Administration of SLx-4090 reduced postprandial serum triglyceride levels and decreased both TC and LDL-C after 14 days.
- In patients with mixed dyslipidemia SLx-4090 200 mg tid for 14 days reduced peak postprandial serum TG, reduced the AUC for postprandial serum TG and resulted in a significant reduction in serum LDL-C (-31 mg/dL).

Study Design: A cross-over design is chosen to achieve maximum power with a small number of participants. Single dose of Orlistat can reduce postprandial TG levels (27-28). Orlistat in varying doses for 9 to 14 days causes a significant increase in fecal fat within two weeks (32). In addition, two weeks of therapy with SLx-4090 resulted in significant reductions of serum TG (17). Therefore, we believe that four weeks of therapy in each arm is adequate to observe a reduction in serum TG and chylomicrons. Orlistat 120 mg three times daily will be used given that this dose appears to be the maximum effective dose tolerated by most patients and is the recommended dosage approved by the FDA (33). SLx-4090 will be used in an attempt to minimize

the side effect of increased hepatic fat seen with nonspecific MTP inhibitors (16). The half life of Orlistat is known to be 1 to 2 hours (33) and the since SLx-4090 is not absorbed in any appreciable quantity from the intestinal tract, the effects of these drugs are not expected to last long and we do not expect any carry over effects to confound the design. We have also included a wash out period in between the study periods to avoid carry over effects. We have previously used similar design to assess efficacy of gemfibrozil alone or in combination with lovastatin for hypertriglyceridemia (34).

Most of the patients with T1HLP present with recurrent abdominal pain and even acute pancreatitis (a potentially life threatening complication) along with extreme hypertriglyceridemia during childhood. Since there is no established therapy for T1HLP, these children are at high risk of acute pancreatitis. Therefore, we have included children age 12-17 years. However, to improve the risk-benefit ratio, we will include only those children with T1HLP who have a previous history of acute pancreatitis. We will carefully monitor each child during participation in the study for any possible side effects. Since variability in glycemic control could be a confounding factor and patients with poorly controlled DM can develop secondary hypertriglyceridemia, we have excluded patients with T2DM from the study.

End Point Variables: Fasting serum TG is chosen as the primary end point variable as this determination is made during clinical follow up of patients with T1HLP. Since both Orlistat and SLx-4090 are expected to affect chylomicron formation, fasting and postprandial levels of chylomicron TG, serum TG and retinyl palmitate (as a measure of chylomicron remnant clearance) are included as secondary endpoint variables. **We will also capture any episodes of acute pancreatitis and include them as a secondary endpoint variable.** Tertiary end point variables include fat soluble vitamin levels to assess the potential effects of Orlistat and SLx-4090 on fat soluble vitamin absorption and fecal fat excretion to assess the potency of these drugs in blocking dietary fat absorption.

Statistical Analysis Plan:

This pilot study will generate data to provide more precise estimates of the magnitude and variability of the effects of Orlistat and SLx-4090 on serum TG in patients with T1HLP and to refine hypothesis. The geometric mean triglyceride level was 2800 mg/dL in 38 patients with T1HLP published in the literature (35) and the standard deviation of the triglyceride response in $\log_e$ transformed units is estimated at 0.4. From these data, we calculate that a decrease (or difference between regimens) of 25-30% would be detectable with 15 subjects (Table 2). To allow for 20% attrition during the study, we estimate that 20 subjects need to be randomized.

Table 2

Variable	SD of differences	% change between study phases	Alpha*	Power	Sample size
Log_e Triglycerides, mg/dL	0.4	30%	0.05	0.80 0.90	 10
	0.4	25%	0.05	0.80 0.90	15

Summary statistics (mean, SD, geometric means, ranges) and confidence intervals will be used to describe the distributions of serum TG and continuous biochemical variables for each study phase, and for differences between phases. The crossover study phases will be analyzed using repeated measures mixed model which incorporates a sequence factor to assess interactions due to treatment order. The study phases will be compared from the least squares means estimates from the mixed linear model. Potential confounders such as weight change or diet changes will be examined. **Whether broad age range will confound the response to therapy is not clear but If there is an apparent difference in the response to therapy related to age, we will further assess age as a factor in our statistical analysis.** The data, which are likely to be skewed, will be log transformed, as appropriate. Nonparametric tests will be implemented, if necessary, to meet assumptions of normality. A two-sided p value of <0.05 will be considered significant for omnibus tests and for pairwise phase comparisons. Adjustments Adverse event frequencies will be summarized for all study periods and compared between phases with McNemar's test. SAS (SAS Institute, Cary, NC) statistical software will be used. Particularly, SAS PROC MIXED will be used for repeated measures analyses.

Primary Endpoint Variable:

1) Fasting serum triglyceride levels

Secondary Endpoint Variables:

1) Serum chylomicron-TG levels (Fasting and postprandial)
2) Postprandial serum TG levels during meal tolerance test
3) Postprandial retinyl palmitate levels during meal tolerance test
4) 72 hour fecal fat
5) Fat soluble vitamin levels

Potential Untoward Effects:

1) Orlistat has been approved by the Food and Drug Administration (FDA) for the use in the US as monotherapy for the management of obesity when used in conjunction with a reduced energy diet. The most common side effects are gastrointestinal such as oily stools, anal leakage and flatus with discharge (25, 36-37). The major risks associated with Orlistat therapy are drug-drug interactions and reduction in levels of cyclosporine and thyroid hormone levels in those taking thyroid supplementation (33). Also, fat soluble vitamin deficiencies due to fat malabsorption have been shown (25, 33, 36-38). The deficiencies are most notable with vitamins D, E and beta-carotene (33, 39). The safety of Orlistat is not established in the pediatric population under the age of 12 years. However, it is deemed to be safe in the pediatric population aged 12-16 years. Adverse effects in children were similar to those found in adults (33).

2) SLx-4090 is an intestinal specific, non-systemically available MTP inhibitor currently in development for the treatment of dyslipidemia (17, 19). BMS-201038, a nonspecific MTP inhibitor, has been used in patients with familial hypercholesterolemia (FH) in an attempt to lower LDL levels (16). In FH patients, it caused an increase in hepatic fat levels (16). Intestinal specific MTP inhibitors such as SLx-4090 do not cause hepatotoxicity. SLx-4090 was well tolerated in healthy male volunteers at doses up to 200 mg tid for 14 days with no impact on liver function tests. Nonetheless, we plan to monitor serum transaminase levels at every visit and will discontinue the drugs if elevations greater than three times the upper limit of normal are seen. MTP inhibitors also tend to cause GI side effects such as diarrhea and the patients will be interviewed for these side effects.

3) Since the patients will be on a reduced fat diet throughout the study, we anticipate less gastrointestinal side effects of both, Orlistat and SLx-4090 when given alone or even during combination therapy.

Expected Results:

We expect that Orlistat and SLx-4090 alone will be able to reduce both fasting and postprandial serum TG and chylomicron TG levels significantly in patients with T1HLP. It is difficult to predict which may be better in this setting. We expect that both may lower serum TG equally. We also expect that the combination of the two agents will be synergistic and further reduce serum TG and chylomicron TG levels beyond the effects of single agents. We expect that patients with T1HLP who will be consuming a low fat diet will have only minimal gastrointestinal side effects related to fat malabsorption when taking each drug alone or in combination.

Potential Problems and Solutions:

It is possible that some may not tolerate the study medication and may drop-out. However, since patients will be on low fat diet, we expect less gastrointestinal side effects of Orlistat and SLx-4090. In case of intolerable side effects, we will reduce the dose of the medications by half.

Recruitment may also be a potential problem. However, we have experience of recruiting patients with rare diseases such as lipodystrophies for clinical trials. We will use the same model of recruiting patients both from local and nearby lipid clinics and from University based Lipid Clinics all over the US and Canada.

We will continue to recruit the patients throughout the duration of the project according to the following ways:

a. <u>Direct Referrals to the P.I.</u>: In the past, several patients have been referred to the P.I. for diagnosis, clinical evaluation and management.

b. Contact authors of published papers: In the past, we have successfully recruited several patients of interest by directly corresponding with the senior authors of papers or abstracts. We will regularly monitor the recent publications reporting patients of interest to us.

c. Referrals through organizations for rare diseases: We have received referrals from the National Organization of Rare Disorders (NORD), U.S., and from the Children Living with Inherited Metabolic Diseases (CLIMB), U.K., and expect to continue this association.

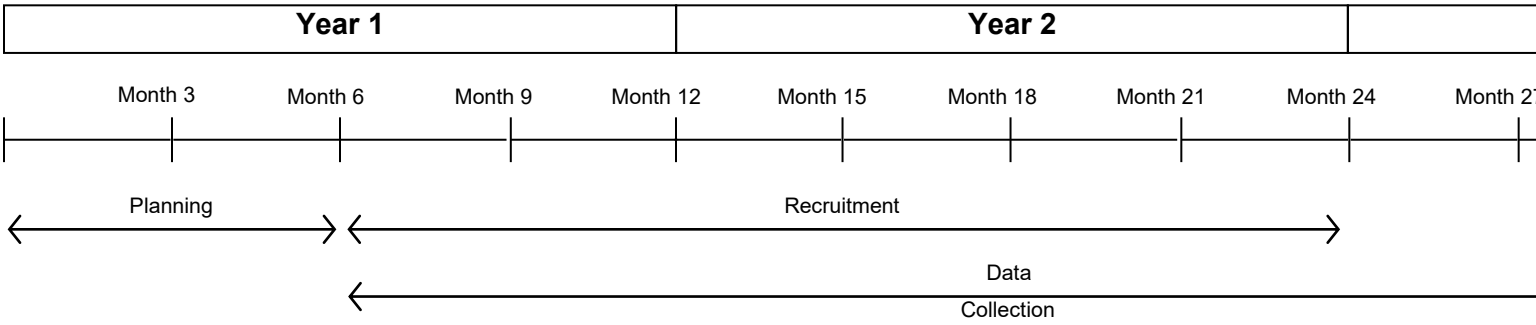
d. WebSite: We will establish an Internet web site at www.UTSouthwestern.edu, which provides latest clinical and research information to the physicians, patients and public with access to the current literature about T1HLP.

e. Referrals from other Clinics: We will recruit patients from other University based Lipid Clinics (see Letter of collaboration).

4. Timeline and Milestones:

The timeline for the study is shown in Figure 4. We will continue to evaluate enrollment of the subjects in the study at 3 month interval. If needed, we will increase our recruitment efforts to find 20 eligible patients for this trial through announcements in Journals such as Journal of Clinical Endocrinology and Metabolism and Pediatrics. We have used this strategy successfully previously to find patients with genetic lipodystrophies for our clinical trials. We do not intend to conduct an interim analysis as the study is of short duration.

Fig. 4 Timeline for the study.



5. Resource Sharing Plan

The PI will comply by the NIH policy of data sharing. We will share the final data after publication of the manuscripts in a timely fashion. The privacy and the rights of the participants will be protected.

E. Human Subjects Research:

E.1 Protection of Human Subjects:

E.1.1 Risks to the Subjects.

E.1.1.1 Human subjects' involvement and characteristics:

Approximately 20 subjects with hyperlipoproteinemia, ages over 12 years, will participate in the study. Patients of all races and ethnic groups will be eligible.

We will exclude subjects younger than 12 years of age as the safety of Orlistat has not been established for pediatric subjects younger than 12 years. We will also exclude those subjects with alcohol abuse or substance abuse. Also excluded will be subjects with significant liver disease, gastrointestinal surgery, significant renal compromise, cholestasis, congestive heart failure, chronic malabsorption syndromes and cancer in the past 5 years. Women who are pregnant or lactating will be excluded. Additionally, because of the potential problem with vitamin K absorption, people with recent major surgery, and those on anti-coagulants, digoxin and anti-arrhythmics will be excluded.

The rationale for including pediatric subjects age over 12 years is that subjects with type 1 hyperlipoproteinemia are typically identified as children. These children have limited success with the currently available treatment options and face life-threatening complication of acute pancreatitis. Thus, they could potentially benefit from this therapy which is expected to have minimal side effects. Since SLx-4090 has not been used in children yet, we will include only those children with T1HLP who have had acute pancreatitis in the past.

E.1.1.2 Sources of Materials:

Research material will consist of questionnaires, blood and stool specimens, physical examination, and 3-day food records obtained from the subjects according to the protocol.

E.1.1.3 Potential Risks:

All subjects will undergo a history and physical examination Subjects will be asked to collect stool samples and undergo blood drawing for which there is a minimal risk of psychological or physical discomfort, the inconvenience of time spent and the unlikely risk for bruising, fainting or infection. Phlebotomy volumes for pediatric subjects will be adjusted based on the child's weight so that no more than 5% of their total volume will be drawn in any 24 hour period and no more than 10% in any 2 month period, based on the guidelines of The Federal Children's Nutrition Research Center.

The potential risks and discomforts of the evaluation methods being used are: *3 Day Food records*: no risk, the inconvenience of the brief time spent.

The potential risks of orlistat include: gastrointestinal side effects such as: diarrhea, oily stools, flatulence with discharge and anal leakage. The major risks for Orlistat are: drug-drug interactions and the reduction of cyclosporine and thyroid hormone levels in subjects taking thyroid supplementation, and fat soluble vitamin deficiencies due to malabsorption. We will provide vitamin supplementation daily during the study and monitor the fat soluble vitamin levels periodically.

The potential risks for SLx-4090 include: GI side effects such as diarrhea. SLx-4090 is an intestinal-specific MTP inhibitor and does not cause hepatotoxicity. However, we will measure serum transaminase levels to monitor any hepatic toxicity.

E.1.2 Adequacy of Protection Against Risks

E.1.2.1 Recruitment and Informed Consent:

Subjects will be recruited from the lipid clinics at Parkland Memorial Hospital, Veterans Affairs Medical Center and UT Southwestern Aston Ambulatory Center as well as from advertising among local physicians and from our website. We will also recruit patients from all over the US. All subjects will be informed of the nature and purpose of the research by the Investigators and will give informed consent as required by the UT Southwestern Institutional Review Board. Investigators will obtain informed consent after explaining the study and the subjects having read or been read the consent form. Consent from the parents or legal representatives will be obtained for pediatric age subjects. Consent will be obtained prior to any study procedures being performed. A copy of the consent form will be given to the subjects and the original consent will be maintained in the subject's research records, which will be maintained in the Clinical Translational Research Center.

E.1.2.2 Protection Against Risk:

Potential risks will be minimized by utilizing study codes to identify subjects, all files will be kept locked and all information on computers will be password protected. Access to research data is restricted to key personnel directly involved with the study who have been trained in the protection of human subjects and signed statements assuring their compliance with University policies protecting the privacy of research subjects. The physical risks will be minimized by the inclusion and exclusion criteria and a complete physical examination prior to initiating study procedures with careful monitoring throughout. Additionally, pregnant or lactating women are excluded from the study. Subjects will have close monitoring of their liver enzymes. In the event of adverse effects or events, the study subjects will be referred to an appropriate physician for treatment. Study subjects who become pregnant during the course of the study will be discontinued from the trial. Blood tests including chemistry, lipids and liver enzymes will be obtained periodically to ensure safety of the subjects.

Plan for data and safety monitoring: We propose the creation of an internal institutional data and safety monitoring committee.

E.1.3 Potential Benefits of the proposed research to subjects and others

The potential benefit of Orlistat and SLx-4090 therapy is an improvement in serum triglycerides, and well as improvement in serum chylomicron-TG levels and postprandial serum triglyceride levels. If the therapies are effective, they will reduce the severe hypertriglyceridemia and decrease the incidence of acute pancreatitis these patients ordinarily faces.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED The knowledge to be gained by these studies will have important implications in our understanding of how to effectively treat the rare Type 1 hyperlipoproteinemia.

5. DATA AND SAFETY MONITORING PLAN

We propose the creation of an internal Data and Safety Monitoring Committee composed of experts in statistics and lipid metabolism. Identified for the necessary expertise are individuals who are not associated with this trial and not collaborators of the principal investigator. 1. Alan Elliott, Ph.D., Faculty Associate, Department of Clinical Sciences. 2. Scott Grundy, M.D., Ph.D. Professor of Internal Medicine 3. Perrin White, M.D., Professor of Pediatrics 4. Fredrick Dunn, M.D., Associate Professor of Internal Medicine.

The Committee will be asked to: a) Review the research protocol, informed consent documents and the plans for data and safety monitoring. b) Evaluate the progress of the study including periodic assessments

of data quality and timeliness, recruitment, retention, and risk versus benefit. c) Report on the safety and scientific progress of the trial. d) Make recommendations to the PI, and if needed to the IRB, NIH, CTRC and FDA concerning continuation, termination or modifications of the trial based on the observed beneficial or adverse effects of the interventions under study. e) Conduct or review the interim analysis with regards to safety in accordance with stopping rules, which should be defined in advance of the data analysis. f) Ensure data integrity. g) Ensure the confidentiality of the data and results of monitoring. h) Maintain study integrity by commenting on any problems with study conduct, enrollment, statistics or data collection.

The PI will hold the primary oversight responsibility for this trial and as such he will be responsible for surveying the medical literature for scientific or therapeutic developments that may impact the safety of participants or the ethics of the study. He will insure that: subjects are fully informed of the study requirements throughout the trial, insure that study subjects receive a study calendar upon enrollment, are updated on any new information relevant to their continued participation or change in the risk versus benefit ratio of the interventions. The PI will be responsible for reporting adverse events to the IRB, NIH, CTRC and FDA in accordance with IRB policies and time frames. The PI is responsible for submitting continuing review reports to the IRB, NIH and FDA.

Inclusion of Women

The PI has had excellent success in recruiting and retaining women for clinical trials in the past. Women have made up greater than 50% of our enrollment in the past and will likely continue to do so. We do not expect to have any trouble recruiting women for this trial and already have identified three female candidates.

Inclusion of Minorities

In previous studies by the PI, the efforts to enroll minorities have been very successful. In a recent clinical trial involving leptin, the enrollment for minorities and those with Hispanic ethnicity accounted for 54% of the study participants. **The targeted/planned enrollment with mixed ethnicity may not be a requirement for such a rare disease for which data on ethnicity and racial distribution is not known. We will recruit any patient who qualifies for the study.**

Inclusion of Children

Pediatric subjects (>12 years of age) will be included in this research to find a safe and effective intervention to help manage the devastating hypertriglyceridemia and prevent the recurrent episodes of pancreatitis that these children currently face. Dr. Garg and other co-investigators have worked with children with genetic and other types of acquired lipodystrophies and metabolic derangements for the past 15 years. The Clinical Translational Research Center at UT Southwestern Medical Center has extensive experience in dealing with children with diabetes and rare metabolic disorders characterized by hypertriglyceridemia such as lipodystrophies and lipoprotein lipase deficiency.

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