Study Title: Targeting Inflammation With Salsalate in Type 1 Diabetes Neuropathy (TINSAL-T1DN)

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CONFIDENTIAL

CLINICAL PROTOCOL

TARGETING INFLAMMATION WITH SALSALATE IN TYPE 1 DIABETES NEUROPATHY- (TINSAL -T1DN STUDY)

A phase II-III, multi-site randomized, double-blind, placebo-controlled clinical trial

US IND Number: 113650

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Version and Date: Version 2.3, September 19, 2022

Protocol Committee:
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NOTE: To effectively manage study medication supply chain disruptions, changes to protocol-items are to be made to minimize disruptions and ensure participate safety. These changes are listed in Appendix A of the protocol (Contingency Operation Plan).

SYNOPSIS

Indication: Prevention of diabetic neuropathy (DN) progression or improvement in patients with Type I diabetes (T1DM) and mild DN

Rationale: DN is the most common chronic complication of diabetes, ultimately affecting half of patients with T1DM and leading to severe morbidity, high mortality, major physical disability and poor quality of life. Attainable intensive glycemic control, although necessary, is insufficient to prevent adverse nervous system effects, justifying a therapeutic need to identify new drug targets to treat DN early in its course. Evidence for an important role of low-grade inflammation and of nuclear factor kappa B (NF-κB) activation in the pathogenesis of DN is emerging from both experimental and clinical studies. Salsalate, a nonacetylated prodrug of salicylate, is an FDA approved treatment for osteoarthritis and other rheumatologic conditions. It is a highly effective drug in blocking the inhibitor of κB kinase (IKKβ)/NF-κB pathway, with a large margin of safety, and low cost.

Objectives:

Primary: Determine whether a 12-month course of oral salsalate (up to 3 grams daily) will prevent the progression of DN, as measured by intraepidermal nerve fiber density (IENFD) at the proximal thigh and distal leg in subjects with T1DM and mild DN.

Secondary: Determine whether a 12 month course of up to 3 grams of salsalate daily will have an effect on:

1. The change from baseline to months in IENFD at the distal thigh;

2. The proportion of subjects with confirmed clinical neuropathy at baseline, and 12 months defined as the combination of:

a) Definite clinical neuropathy requiring at least two positive responses among neuropathic symptoms (pain, numbness, paresthesias), sensory signs, and abnormal ankle reflexes (diminished or absent) consistent with a distal symmetrical polyneuropathy and without causal explanation aside from diabetes, as assessed clinically using a standardized neurological examination; and

b) NC abnormalities involving ≥ 2 nerves among the sural, peroneal, and ulnar nerves.

3. The proportion of subjects with definite small fiber neuropathy, defined as neuropathic symptoms and signs (decreased pinprick or temperature sensation) consistent with small fiber predominant dysfunction, and an abnormality in IENFD (one standard deviation below normal for age/gender/weight) or abnormal cardiovascular autonomic testing.

4. Measures of cardiovascular autonomic neuropathy derived from cardiovascular reflex testing (R-R interval ratio during deep breathing, postural change, and Valsalva) at baseline and 12 months; resting HR will be obtained at baseline and 3 month intervals through 12 months of follow-up.

5. Measures of quality of life as assessed by the NeuroQOL domain and overall scores at

baseline, 6, and 12 months.

6. Autonomic symptoms will be assessed using the Survey of Autonomic Symptoms (SAS).

7. Measures of pain as assessed by the Neuropathy Pain Scale

8. Functional Assessments: BERG Balance Scale; Falls Efficacy Scale, and 8 Foot Get-Up-and-Go assessments.

Exploratory: Assessment of expression of pain-related molecules in skin biopsies, including sodium channels, TrkA receptors, neuropeptides, such as substance P and CGRP and TNFα. Serum inflammatory markers TNFα, IL1β, IL6 and CCL2 will also be measured.

Trial Design: This is a phase II-III, multi-site randomized, double-blind, placebo-controlled clinical trial to evaluate the effect of salsalate on measures of small fiber neuropathy in 70 subjects with T1DM and mild DN. Subjects will be randomized in a 3:2 fashion to (up to) 3 grams/day salsalate or matched placebo and receive study medication for 12 months. Randomization will be stratified by baseline HbA_{1C} ((≤8.0 or >8.0%)). Subjects will be evaluated every 3 months for a total of 12 months.

Endpoints:

Primary outcome: Change from baseline to month 12 in the intraepidermal nerve fiber density (IENFD) at the distal thigh.

Secondary outcomes:

<u>IENFD at the ankle</u> at baseline and 12 months

<u>Electrophysiological measures</u>

- *Nerve Conduction Studies* of the sural, peroneal, and ulnar nerves will be obtained at screening and 12 months
- *Quantitative Sensory Testing:* Cold and vibration thresholds are assessed at screening and 12 months.
- *Measures of CAN* will comprise cardiovascular reflex testing using the DCCT/EDIC protocol *(*R-R interval ratio during deep breathing, postural change, and Valsalva).

<u>Clinical measures of DN</u> will be assessed at baseline and 12 months and will include:

a) DN symptoms as evaluated by the *Neuropathy Total Symptom Score-6 (NTSS-6)*
b) *Survey of Autonomic Symptoms* (SAS)
c) *The DCCT/EDIC Structured Neurological Examination*

<u>Measures of DN- specific pain</u> will include the pain domain part of the *NeuroQOL* and the Neuropathic Pain Scale that will be administered at baseline, 6, and 12 months.

Functional scales will be assessed at baseline and 12 months and include:
a*) The Berg Balance Scale* which measures balance in 14 separate activities of daily living such as going from sitting-to-stand and standing on one leg; the unipedal stance portion that had been shown to be particularly reflective of neuropathy-related mobility loss;
b) The *8 Foot Up and Go Test*, a test of functional mobility that assesses the time needed for a subject to arise from sitting position, walk 8 ft and turn 180 degrees around a cone and return sitting;
c*) The Modified Falls Efficacy Scale* assessing patient's self-reported ability to perform activities of daily living (e.g. get dressed/ undressed, walking, shopping).

Quality of life measures will be administered at baseline, 6, and 12 months and include *the NeuroQOL*, a validated measure of quality of life specific to peripheral neuropathy.

Exploratory outcomes: will be assessed at baseline and 12 months and include:

a) Assessment of expression of *pain-related molecules in distal leg skin biopsies*, including the ion channel TRPV1, TrkA receptors, and neuropeptides, e.g. substance P and CGRP, using our published protocol;

b) *skin nerve fiber inflammatory markers* including TNFα, IL1β, CD68c, using our published protocol; and

c) a complex panel of serum inflammatory markers using the Luminex 200 platform (Luminex Corporation, Austin, TX) and Millipore multiplex immunoassay panels (Millipore Corporation, Chicago, IL, which offers customization of 40 different cytokines including TNFα, sTNF receptor, IL1β, IL6, MCP-1, adiponectin, IL8, IL17, m-CSF, sVCAM-1, s-ICAM-1, IL10; and

d) urine inflammatory markers using the Luminex 200 platform which offers customization for urinary cytokines as outlined above

Trial Treatments:

Oral administration of salsalate up to 3 grams/daily for 12 months, or

Oral administration of matched placebo daily for 12 months.

Statistical Methods:

We reported that the average IEFND in the distal leg in T1DM subjects (mean disease duration of 27 years) was 2.2 fibers/mm. We assume that the targeted diabetic subjects have the average IENFD for ages 20-29 shown by published normatives at the start of their disease, and the decline due to diabetes is 10 fibers/mm or an average of 0.25 fibers/mm /year. If the decline is more rapid as neuropathy progresses, then the average decline in IEFND may be as high as 0.5 fibers/mm/year. Based on our assumption about the mechanism by which salsalate will influence IEFND (reduction of inflammation), and published effect of other interventions, we believe that an increase of 1.65 fibers/mm (0.25 + 1.4 = 1.65) in fiber density is possible with treatment with salsalate. The standard deviation

of the change is approximately 2 fibers/mm. With 25 subjects per group there will be 81% power to reject the null hypothesis of no difference between groups using a two-tailed t-test at a 5% level of significance. No formal interim analysis for stopping for efficacy benefit or futility is planned, given the projected duration of follow-up required for the assessment of the primary endpoint. Rather, safety will be assessed periodically and reviewed by a Data and Safety Monitoring Board.

A general linear model will be fitted to the change in IENFD score with factors for treatment group, baseline HbA1c (≤8.0 or >8.0%), and baseline IEFND as fixed covariates. If the distributional assumptions are not met, transformations of IENFD or the use of nonparametric methods will be employed. Sensitivity analyses will be performed to assess how subjects who withdrew may affect conclusions of the analysis. An intent-to-treat analysis set (including subjects who are randomized, treated and have a baseline IENFD) will be used to assess treatment effects with multiple imputation methods employed to impute outcomes for subjects with missing post-baseline data.

SUMMARY OF CHANGES:

Protocol Version 1.2
July 13, 2016:

Section 4.1 Exclusion Criteria: Updated to allow continued low-dose (81 mg daily) aspirin therapy.

Section 2, 6.1, 6.2, Table 1: Updated to move quantitative sensory testing to the screening visit, rather than baseline visit.

Section 2: Corrected to indicate that nerve conduction studies are done at the screening visit.

Section 2, 4.1, 6.1, 7.1.2: Updated to indicate that the ulnar nerve will be tested, rather than median nerve.

Protocol Version 1.3
October 19th, 2016:

Table 1: Proposed visit schedule corrected for spelling and timing of events.

Section 1 & 10.1: Corrected to indicate that this is a single center (not multi-center) study.

Section 4.1: Added history of keloid scarring to list of exclusions.

Section 6.2: Removed language related to subject medication log

Section 10.6: Added reference that MICHR will provide study monitoring.

Throughout protocol provided clarification that research pharmacy used is Belmar Pharmacy (not UMHS Research Pharmacy).

Minor typographical, spelling and grammatical errors fixed throughout.

Protocol Version 1.4
December 6th 2016

Section 3: Added that randomization will be by gender as well as by HbA_{1c}.

Section 4.1: Clarified inclusion and exclusion criteria as follows;

-Severe hypoglycemia is defined by hypoglycemia resulting in coma or seizure.

-Active Hepatitis C is an exclusion.

-Active foot ulcers are an exclusion.

-Exclusion related to DKA clarified to a history of recurrent DKA or any DKA in the prior 3 months.

-Lithium use added to list of exclusions

-Upper age for eligibility increased from 65 to 70 years.

Protocol Version 1.5
February 28, 2017

Section 4.1: Inclusion criteria amended follows;

-Inclusion criteria # 4 amended to include provision to use peroneal motor nerve conduction velocity of ≥ 35 m/second as inclusion criteria if sural nerve amplitude is 0 μV (unrecordable).

Section 4.1: Clarified exclusion criteria as follows;

-Exclusion # 9 clarified with addition of chronic immunosuppressive therapy to long-term glucocorticoid therapy, exception specified for those using steroid inhalers for asthma management.

-Exclusion # 15 corrected to reflect treatment period of 12 months rather than 24 months.

-Exclusion # 16 added (exclusions related to hypersensitivity to salsalate, aspirin and other NSAIDS). Language related to hypersensitivity is

included in section 9.3 of protocol, but has now been added to the exclusion list for clarity.

-added language indicating that people with known hypersensitivity to lidocaine or epinephrine may not be able to participate as these agents are used for local anesthesia during skin biopsies.

Protocol Version 1.6
May 01, 2017

Section 4.1: Clarified exclusion criteria as follows;

- Exclusion # 6 clarified to include “lung” transplant to match ICD

Section 5.2.3 and 6.2: Revised study drug titration schedule.

Section 9.3: Added language re: increased risk for fluid retention and edema to be consistent with risks for NSAIDs that are listed in the Salsalate package insert.

Protocol Version 1.7
August 10, 2017

Section 3: Trial Design. Amended to reflect increased enrollment to 70 subjects and to modify randomization distribution from 1:1 (Salsalate:Placebo) to 3:2 (Salsalate:Placebo).

Section 5.1: Removed reference to 1:1 randomization ratio.

Throughout:

-Reference to enrollment of 60 subjects changed to 70 subjects

-Reference to salsalate dose clarified from “3 grams per day” to “up to 3 grams per day” to better reflect intent to use a lower dose for participants who experience dose-dependent, treatment related side effects.

-Clarified the randomization stratification by A1c from <, 8% to >8% to ≤ 8.0% to >8.0%.

Protocol Version 1.8
December 4th, 2017

Table 1: Amended to increase the allowable visit window between screening (visit 1) and baseline (visit 2)

Section 6.1: Amended last paragraph to reflect increase in allowable duration between screening (visit 1) and baseline (visit 2).

Throughout: Minor formatting changes and spelling/grammar corrections.

Protocol Version 1.9

July 11th, 2019

Section 2.0: Sudoscan removed from study procedures due to the bankruptcy of the manufacturing company and the subsequent inability to obtain supplies

Section 4.1: Clarified exclusion criteria as follows;

- Amputation Exclusion - edited exclusion criteria to allow patients who have had a lower limb amputation from a cause other than neuropathy

- Cancer Exclusion - edited exclusion criteria to only exclude patients with active cancer (other than basal cell or squamous cell skin cancer) rather than also excluding those who have had it in the last five years.

- Macroabluminuria Exclusion - clarify that a history of persistent macroalbuminuria is checked by random urine microalbumin creatinine ratio rather than a 24-hour test.

Section 5.3: Pill Disposal - edited the method of pill disposal in the protocol such that the pills will be disposed of in a U of M pharmaceutical black bin upon patient return and pill counting, rather than returning them to Belmar.

Section 3.0 and 10.1: Changed single center to multi-site to reflect the addition of Henry Ford Health System as a performance site. In section 10.1, it is also noted that the previous version of this protocol contained an error that stated that the purpose of this single center study was the justify the conduction of single center study. This should have stated that the purpose of this single center study was the justify the conduction of multi-center study. Thus, this has now been edited to acknowledge that this is now a multi-site study that would justify a larger multi-site study.

Protocol Version 2.0
December 16th, 2019

Table 1: Proposed Visit Schedule for TINSAL-T1DN Study: Removed Sudomotor testing row that was missed in the changes made for protocol version 1.9.

Section 6.2: Removed Sudomotor testing from visit 2 tests as that was missed in the changes made for protocol version 1.9.

Section 6.3: Removed Sudomotor testing from visit 7 tests as that was missed in the changes made for protocol version 1.9.

Protocol Version 2.1
December 18th 2020

Hold over reference to 1:1 randomization changed to 3:2 on page 3.

Section 8.8.1 updated to reflect SAE reporting in accordance with current IRB reporting guidelines.

Table one updated to reflect that visits 3-7 windows are based on medication start following visit 2 due to the shipping of the medication from Belmar pharmacy directly to the participant.

Protocol Version 2.2
January 11, 2022

Section 6 was updated to include option for conducting visit number 4 (3 months) and 6 (9 months) remotely.

Table one updated to include a footnote indicating that visit number 4 and number 6 may be conducted remotely.

Protocol Version 2.3
September 19, 2022

Appendix A was added. Appendix A provides a contingency plan in case of disruption to supplies of medication (salsalate or placebo)

Table 1: Proposed Visit Schedule for TINSAL-T1DN Study

Visit Number	Visit 1	Visit 2	Visit 3	Visit 4**	Visit 5	Visit 6**	Visit 7
Visit Description	Screening	Baseline	Monitoring	Monitoring	Monitoring/ Outcomes	Monitoring	Monitoring/ Outcomes
Time point	Day -180 to 0	Day 0	Month 1	Month 3	Month 6	Month 9	Month 12
Visit window		NA	± 7 days	± 14 days	± 14 days	± 14 days	± 14 days
Informed Consent	X						
Medical History /Physical Examination†	X	X		X	X	X	X
Laboratory Measures*	X			X	X	X	X
Skin Biopsies (proximal/distal leg)		X					X
Neurological History /Physical Examination†	X						X
Nerve Conduction Studies	X						X
Neuropathy Total Symptom Score	X	X			X		X
Cardiovascular Autonomic Studies		X					X
Resting Heart Rate		X	X	X	X	X	X
Quantitative Sensory Testing	X						X
NeuroQOL		X			X		X
Survey of Autonomic Symptoms		X			X		X
Neuropathy Pain Scale		X			X		X
Functional Assessments Berg Balance Scale 8-foot Get Up and Go Test Modified Falls Efficacy Scale		X					X
Medication Dispensing§		X		X	X	X	
Medication Compliance			X	X	X	X	X
Medication Return				X	X	X	X
Adverse Events Monitoring		X	X	X	X	X	X

*† Baseline history and examination will include diabetes duration, diabetes complications, diabetes treatment, concurrent medical problems, relevant past surgical history, height, weight, blood pressure and pulse, and foot examination. Follow up visits will include changes in medical or surgical history, weight, blood pressure, pulse and foot examination * HbA1c, Metabolic Comprehensive Panel, Complete Blood Count with Platelets, TSH, Lipid Profile, Cystatin–C, Uric Acid and Urine Albumin performed at screening visit (unless results for these tests are documented in the medical record as being done in the prior 3 months for all except HbA1c (1 month) and Urine Albumin (6 months). Urine pregnancy test at screening for all women of childbearing potential regardless of contraceptive practiced. HbA1c, Metabolic Comprehensive Panel, CBCP, Cystatin-C, Uric Acid and Urine Albumin will be repeated at 3,6,9 and 12 months of follow-up. Lipids will be repeated at 3, 6 and 12 months of follow-up. Luminex inflammatory marker assays on blood and urine performed at screening or baseline, and again at study end.*

*Visit windows for visit 3-7 are based off when the patient starts the study medication following visit 2 due to the shipping of the medication from Belmar Pharmacy directly to the participant.

*** Visits 4 and 6 may be conducted remotely with laboratory samples collected at a Michigan Medicine affiliated lab.*

§Medication dispensing,and return may be modified if study drug supplies (salsalate, placebo) are disrupted (Appendix A).

TABLE OF CONTENTS

1. INTRODUCTION

For patients with type 1 diabetes (T1DM) and diabetic neuropathy (DN), no disease modifying treatment other than glycemic control is available. The IKKβ/NF-κB pathway is unique in that it is implicated in both the pathogenesis of nerve fiber loss, pain and other symptoms in DN [1-4]. This will be the first study to confirm beneficial effects of the anti-inflammatory agent, salsalate, on preventing DN progression and on DN pain. The proposed study design employs a quantifiable early measure of DN, intraepidermal nerve fiber density (IENFD) of the thigh, allowing for accurate assessment of actual nerve fiber density over time[5], while also incorporating traditional large fiber DN outcomes, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) primary DN endpoint [6,7], measures of pain and quality of life.

1.1. Background

Diabetic neuropathy is the most common chronic complication of diabetes, ultimately affecting half of patients with T1DM and leading to severe morbidity, high mortality, major physical disability, poor quality of life [8]; and estimated total annual costs of $22 billion (www.diabetes.org). Due to the complex structure and anatomy of the peripheral nervous system, DN presents with a very broad spectrum of clinical symptoms and deficits, including severe pain, sensory deficits, foot ulcers and amputations. Despite the high morbidity associated with DN, most randomized clinical trials evaluating therapies for established DN have been disappointing. To date there is no pathogenetic treatment for this condition [8-10]. The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive control designed to achieve near-normal glycemia is essential in reducing the risk of DN development in type 1 diabetes [6,7]. However, attainable intensive glycemic control, although necessary, is insufficient to prevent adverse nervous system effects [11], justifying a therapeutic need to identify new drug targets to treat DN early in its course.

1.2. Rationale

Evidence for an important role of low-grade inflammation and of nuclear factor kappa B (NF-kB) activation in the pathogenesis of DN and in the pain syndrome associated with DN is emerging from both experimental [1,2,12] and clinical studies [13]. This suggests that agents with known anti-inflammatory properties, such as salicylates, may prevent the development of DN and the pain associated with DN. Salsalate, a pro-drug form of salicylate, is indicated for relief of the signs and symptoms of rheumatoid arthritis, osteoarthritis and related rheumatic disorder. It is a highly effective drug in blocking the IKKβ/NF-κB pathway, and can be obtained at a low cost. Salsalate has recently been shown to have glucose lowering effects.

The recently published NIDDK-funded “Targeting Inflammation Using Salsalate in Type 2 Diabetes (TINSAL-T2D)” trial confirmed salutary effects of 3.5 gram/day salsalate on markers of inflammation, glucose control and overall safety after 48 weeks in patients with type 2 diabetes (T2DM).

Our initial NIDDK funded R03 (DK 094499) grant confirmed the safety and feasibility of targeting inflammation with salsalate treatment in T1DM subjects with DN (26). Our current study builds upon and expands our initial promising results and will either confirm or refute the therapeutic efficacy of salsalate in a larger T1DM cohort.

Traditionally the diagnosis of DN is made by the presence of abnormal nerve conductions (NC), and a combination of symptoms and signs of distal sensorimotor polyneuropathy. However, NC studies assess only large myelinated nerve fiber function and in early DN, these conventional measures can be normal and if abnormal, change slowly. Recent data have also described minimal worsening [14] or improvement in NC [15] in placebo and epidemiological cohorts with little relation to other measures of small fiber nerve function in diabetic patients. This was further confirmed by the relative modest changes in all NC studies in the DCCT/EDIC cohort during 13-14 years of follow-up while adhering to current standard of care [16].

In T1DM, DN is a diffuse, symmetrical injury to the entire peripheral nervous system (5, 30-33). The smallest Aδ thinly-myelinated fibers and the unmyelinated C-fibers are likely the earliest to undergo damage in the natural history of DN. These fibers mediate pain, temperature discrimination, touch and autonomic responses, and constitute 80% to 90% of all peripheral nerve fibers (34). Current evidence on DN natural history point to a progressive course leading to later stage phenotypes that include abnormality in the morphology and function of larger nerve fibers (as documented by changes in NC studies).

Therefore, when studying a population with earlier stages of disease *which would be most amenable to a therapeutic intervention*, it is important to utilize outcome measures that best identify these patients (in this case a measure that sensitively capture small fiber deficits).

IENFD is endorsed by several societies and authorities in the field including the Toronto Consensus Panel on Diabetic Neuropathy as a reliable, efficient and valid outcome measure to assess early neuropathy in clinical trials [5,8,17,18]. IENFD, is a continuous measure of small fiber neuropathy, with high positive and negative predictive values along with a high diagnostic efficiency in differentiating between subjects with and without neuropathy [5,19,20].The morphometric quantification of IENFD, most commonly expressed as the number of IENFs per length of section (IENF/mm e.g. density) is possible with skin biopsy, a *minimally invasive procedure,* with less than 1% of mild adverse events such as bleeding, swelling or erythema. Inter-observer variability for the assessment of IENFD demonstrates good agreement, especially with assessment at the thigh, and a large normative data base was recently created by an international consortium [5,19,20]. Patient refusal rate for this procedure is minimal (less than 2 % in several past and ongoing trials of the two Principal Investigators).

2. TRIAL OUTCOMES

Primary outcome: Change from baseline to month 12 in the intraepidermal nerve fiber density (IENFD) at the distal thigh.

Secondary outcomes:

IENFD at the ankle at baseline and 12 months
Electrophysiological measures

- *Nerve Conduction Studies* of the sural, peroneal, and ulnar nerves will be obtained at screening and 12 months

- *Quantitative Sensory Testing:* Cold and Vibration thresholds are assessed at screening and 12 months.

- *Measures of CAN* will comprise cardiovascular reflex testing using the DCCT/EDIC protocol *(*R-R interval ratio during deep breathing, postural change, and Valsalva).

Clinical measures of DN will be assessed at baseline and 12 months and will include:
a) DN symptoms as evaluated by the *Neuropathy Total Symptom Score-6 (NTSS-6)*
b) *Survey of Autonomic Symptoms* (SAS)
c) *The DCCT/EDIC Structured Neurological Examination*

Measures of DN- specific pain will include the pain domain part of the *NeuroQOL* and the Neuropathic Pain Scale that will be administered at baseline, 6, and 12 months.

Functional scales will be assessed at baseline and 12 months and include:
a*) The Berg Balance Scale* which measures balance in 14 separate activities of daily living such as going from sitting-to-stand and standing on one leg; the unipedal stance portion that had been shown to be particularly reflective of neuropathy-related mobility loss;
b) The *8 Foot Up and Go Test*, a test of functional mobility that assesses the time needed for a subject to arise from sitting position, walk 8 ft. and turn 180 degrees around a cone and return sitting;
c*) The Modified Falls Efficacy Scale* assessing patient's self-reported ability to perform activities of daily living (e.g. get dressed/ undressed, walking, shopping).

Quality of life measures will be administered at baseline, 6, and 12 months and include *the NeuroQOL*, a validated measure of quality of life specific to peripheral neuropathy.

Exploratory outcomes: will be assessed at baseline and 12 months and include:

a) Assessment of expression of *pain-related molecules in distal leg skin biopsies*, including the ion channel TRPV1, TrkA receptors, and neuropeptides, e.g. substance P and CGRP, using our published protocol;

b) *skin nerve fiber inflammatory markers* including TNFα, IL1β, CD68c, using our published protocol; and

c) a complex panel of serum inflammatory markers using the Luminex 200 platform (Luminex Corporation, Austin, TX) and Millipore multiplex immunoassay panels (Millipore Corporation, Chicago, IL, which offers customization of 40 different cytokines including TNFα, sTNF receptor, IL1β, IL6, MCP-1, adiponectin, IL8, IL17, m-CSF, sVCAM-1, s-ICAM-1, IL10; and

d) urine inflammatory markers using the Luminex 200 platform which offers customization for urinary cytokines as outlined above

3. TRIAL DESIGN

This is a phase II-III, multi-site randomized, double-blind, placebo-controlled clinical trial to evaluate the effect of salsalate on measures of small fiber neuropathy in 70 subjects with T1DM and mild DN. In the original study design we proposed to randomize up to 60 subjects in a 1:1 fashion to up to 3 grams of salsalate or matched placebo. In August 2017 the protocol was modified by recommendation of the DSMB to address differential study medication discontinuation rates and to increase the number of subjects exposed to study drug. Specifically, the protocol will enroll up to 70 subjects and randomize them in a 3:2 fashion to

salsalate or placebo. All subjects enrolled subsequent to the approval of the revised protocol will be randomized in 3:2 fashion, up to 3 grams per day of salsalate or placebo respectively. Treatment duration will be 12 months. Randomization will be stratified by baseline HbA_{1c} (≤8.0 or >8.0%) and by gender within the HbA_{1c} groups. Subjects will be evaluated every 3 months for a total of 12 months.

4. STUDY POPULATION

70 subjects with type 1 diabetes mellitus (T1DM) will be enrolled in this study.

This clinical trial can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject.

4.1. Inclusion Criteria

1. T1DM;

2. age 18-70;

3. mild DN as defined by symptoms and/or signs, confirmed by at least one abnormality in electrophysiology studies (abnormality of at least one attribute among conduction velocity, latency, amplitude or F-Wave in at least one nerve among sural sensory, ulnar sensory, or peroneal motor);

4. sural nerve amplitude > 0 μV. If sural nerve amplitude is 0 μV (unrecordable) peroneal motor nerve conduction velocity must be ≥ 35 m/second*;

5. on a stable insulin regimen for the 3 months prior to enrollment;

6. be willing and capable of signing the IRB approved consent form and willing and able to cooperate with the medical procedures for the study duration;

7. be willing to accept random treatment assignment to salsalate or placebo; and

8. women of childbearing age agree to use an appropriate contraceptive method (hormonal, IUD, or diaphragm) for the duration of the study and must have a negative urine pregnancy test at screening.

Exclusion Criteria

1. history of severe DN, active lower limb ulceration or lower limb amputation directly caused by diabetic neuropathy, or risk factors for any other causes of neuropathy (e.g. active hepatitis C, end stage renal disease, systemic lupus erythematosus or a known hereditary neuropathy) as determined through medical history, family history, history of medications, occupational history, history of exposure to toxins, physical and neurological examinations);

2. history of recent severe hypoglycemia (within prior 6 months) as defined by hypoglycemia resulting in coma or seizure or a history of recurrent diabetic ketoacidosis (DKA) or any diabetic ketoacidosis within the last three months.

3. history of persistent macroalbuminuria [random urine microalbumin creatinine ratio (ACR) >300 mg/gm]. ACR up to 300 mg/gm is acceptable if serum creatinine is <1.4 for women, <1.5 for men AND estimated GFR (eGFR) is > 60;

4. serum creatinine >1.4 for women and >1.5 for men or eGFR <60 [possible chronic kidney disease stage 3 or greater calculated using the Modification of Diet in Renal Disease (MDRD) equation];

5. pregnancy or lactation, or intention to become pregnant in next 12 months;

6. history of previous lung, kidney, pancreas, liver, cardiac or bone marrow transplant;

7. history of drug or alcohol abuse within the previous 5 years, or current weekly alcohol consumption >10 units/week;

8. use of warfarin (Coumadin), clopidogrel (Plavix), dipyridamole (Persantine), heparin or other anticoagulants, probenecid (Benemid, Probalan), sulfinpyrazone (Anturane) or other uricosuric agents; Subjects must agree to not use high-dose aspirin during the course of the study. Daily low-dose aspirin treatment (not more than 81 mg per day) may be continued if currently prescribed.

9. requiring long-term glucocorticoid therapy or chronic immunosuppressive therapy; Inhaled steroid use for management of asthma is not an absolute exclusion.

10. use of lithium

11. participation in an experimental medication trial within 3 months of starting the study;

12. current therapy for malignant disease other than basal- cell or squamous-cell skin cancer;

13. history of gastrointestinal bleeding or active gastric ulcer; screening laboratory abnormalities including AST (SGOT) and or ALT (SGPT) > 2.5 x the upper limit of normal (ULN), total bilirubin > 1.5 x ULN, platelets < 100,000;

14. developed keloid scarring in the past. Keloids are large, thick masses of scar tissue. These are more common among dark-skinned people.

15. presence of any condition that, in the opinion of the investigator would make it unlikely for the subject to complete 12 months of study participation, e.g., history of non-adherence to therapeutic regimens, presence of conditions likely to limit life expectancy, living situation that would interfere with study visit schedules such as a job requiring frequent or extended travel

16. known hypersensitivity to salsalate. Patients who have experienced asthma, hives, or other allergic-type reactions to aspirin or other NSAIDs are excluded from participation.

Patients with known or suspected aspirin or NSAID-sensitive asthma are excluded.

In addition, subjects with concurrent chicken pox, influenza, flu-like symptoms or other symptomatic viral illnesses should not be enrolled in the study until the illness or condition has resolved.

Subjects with known or suspected hypersensitivity to lidocaine or epinephrine may not be able to participate as these agents are used for local anesthesia during skin biopsies. The study investigators should consider the nature and severity of past reported reactions to these agents, and may consider alternative anesthesia options for local anesthesia on a case by case basis.

**Because obtaining the sural nerve response can be technically challenging, the absence of response does not necessarily indicate advanced neuropathy. Criteria for peroneal motor nerve conduction velocity is added as a secondary measure of neuropathy severity so that otherwise eligible candidates are not excluded based solely on inability to obtain the sural response.*

5. TRIAL TREATMENTS

5.1. Allocation to Treatment

Randomization to Salsalate or Placebo: After signing informed consent, confirming eligibility and completion of baseline assessments (visit 2), subjects will be randomized to salsalate, up to 3 grams/daily or matched placebo for 12 months. Randomization will be stratified by baseline HbA1c (≤8.0% or >8.0%).

Procedure for Subject Treatment Assignment: Subjects will be randomized to treatment with either Salsalate or Placebo. The procedures for randomization will be determined by the study biostatistician.

Breaking the Blind
In the event of an emergency and a need to know study medications, the investigator will contact Belmar Research Pharmacist (the research pharmacy contracted for this study) and request identification of subject's test article assignment. The Belmar Pharmacist will indicate that the blind was broken on the Pharmacy Randomization Log and will document the date of the request, the name of the research personnel making the request, and the pharmacist's signature. The research coordinator will report the emergent event on the Adverse Event CRF and the unmasking event on the Unmasking Report CRF and then signed by the investigator.

5.2. Study Drug

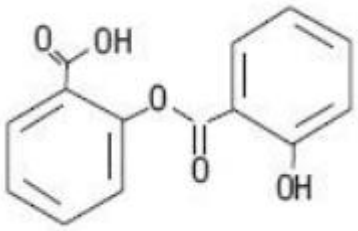
This study will be conducted under IND 113650 (Dr. Pop-Busui, Sponsor-Investigator).

Neither subjects nor their insurance will incur any additional cost from their participation in this study. Salsalate and matching placebo will be provided by Belmar Research Pharmacy.

5.2.1. Description of drug product

Salsalate tablets 500mg

Salsalate is a nonsteroidal anti-inflammatory agent for oral administration. Chemically, salsalate (salicylsalicylic acid or 2 hydroxy-benzoic acid, 2-carboxyphenyl ester) is a dimer of salicylic acid; its structural formula is shown below:



5.2.2. Preparation and Dispensing

The Belmar Research Pharmacy will act as the central pharmacy for this study, and will provide salsalate and matching placebo to each participant.

5.2.3. Administration

Each subject will be given sufficient study medication to last until the next scheduled visit.

Subjects will be instructed to take one tablet with breakfast for the first four days beginning with the first breakfast after receipt of the study medication. The study medication dose will be increased slowly to the maximum tolerated dose (2 tablets, 3 times daily) using the following schedule: Beginning on day 5, take 1 tablet with breakfast and 1 tablet with lunch. Beginning on day 9, take 1 tablet at breakfast, 1 tablet at lunch, 1 tablet at dinner. Beginning on day 23, take 2 tablets at breakfast, 1 tablet at lunch, 1 tablet at dinner. Beginning on day 27 take 2 tablets at breakfast, 2 tablets at lunch, 1 tablet at dinner. The maximum dose of study medication, 2 tablets at breakfast, 2 tablets at lunch, and 2 tablets at dinner, may be started no sooner than the 31st day from receiving the first supply of study medication. Should side effects (e.g., stomach upset, tinnitus) develop, the subject will be instructed to contact the study staff for guidance re: reducing the dose of study medication.

5.2.4. Compliance

The number of tablets dispensed at each visit will be recorded in the case report form. Subjects are reminded to return all unused study medication at each visit in the original container. The study staff will collect all unused medications and record the number of tablets remaining. Discrepancies between expected versus actual study medication returned will be evaluated by the local study staff to address issues related to non-adherence to the study protocol. Subjects will be encouraged to continue on the maximally tolerated dose of study medication for the duration of the treatment period. Consistent with the intention to treat model, all subjects will be followed for the duration of the study treatment period according to the treatment schedule, unless consent is withdrawn, or, in the opinion of the investigator, it is unsafe for the subject to continue participation.

5.3. Drug Storage and Drug Accountability

The Belmar Research Pharmacy shall be responsible for storage and dispensing of study drug for the cohort of subjects followed at the University of Michigan and Henry Ford Health System. The clinical coordinator will be responsible for drug accountability. Upon the completion of drug accountability, the coordinator will discard the remaining drug in a University of Michigan pharmaceutical bin.

5.4. Concomitant Medication(s)

The research staff will record in the source documents all concomitant drugs used by the subject. This is to include prescription, non-prescription, homeopathic medications and dietary/vitamin supplements. At the screening visit, medication use is to be carefully reviewed to ensure that no contraindicated medications as described in the protocol are being used (e.g., blood thinning agents, immune-modulating medications, NSAIDS). At all subsequent visits, subjects will be asked to report any changes to their reported medication use and the source document updated accordingly. Queries regarding medication exclusions and contraindications are directed to the study co-chairs.

6. STUDY VISITS

The schedule of study assessments is shown in Table 1. After obtaining informed consent, verifying eligibility at screening and completing all required baseline assessments, subjects are randomized, receive study medication and are followed every 3 months for one year. The staff should query the subject regarding any changes to their health, changes to their medication regimen, including changes in their insulin treatment, and frequency and severity of hypoglycemia.

Visits 4 and 6 may be conducted remotely if the study investigator agrees that a remote visit is appropriate for the participant, and the participant has access to a University of Michigan/Michigan Medicine affiliated laboratory. The remote visit option for Visits 4 and 6 will reduce the burden of study participation for those participants who live at a distance from the study site, or have limited ability to take time off from work or have other limiting obligations, or who are concerned about travel during the COVID-19 pandemic. Study staff will conduct the visit via telephone or site-approved and HIPPA compliant electronic conferencing platform. Study staff will place an order for required laboratory assessments and instruct the participant to have the laboratory measures obtained at the nearest Michigan Medicine affiliated laboratory.

6.1. Recruitment/Screening

<u>Visit 1: Screening/Eligibility.</u>

An eligibility visit will be scheduled for subjects who, based on preliminary screening (telephone, email, medical chart review) meet the general entry criteria (age, T1DM, diabetes duration, symptomatic peripheral neuropathy, no contraindications for salsalate, etc.). At visit 1 the investigator or her designee will obtain written informed consent, after which all required eligibility examinations will be performed. The eligibility examination will include the following laboratory assessments: HbA1c, metabolic comprehensive panel (METCOMP), complete blood count with platelets (CBCP), thyroid stimulating hormone (TSH), lipid profile, cystatin-C, uric acid, urine albumin assessment, and in all women of child bearing age and potential, a urine

pregnancy test, even if an accepted method of contraception is being used. If any of the required laboratory measures are documented in the subject's medical record as being completed in the prior 3 months (1 month for HbA1c, and 6 months for urine albumin), they will not need to be repeated at the screening visit. Additional blood and urine will be obtained for Luminex assays of inflammatory markers. The Luminex assays though may be postponed until the baseline examination.

Weight, height and seated blood pressure and pulse will be obtained.

A structured neurologic history and physical examination and nerve conduction studies will be performed at the screening visit to verify and document the presence of signs or symptoms of peripheral neuropathy, the presence of a sural nerve response, and abnormality of at least one abnormal nerve attribute (amplitude, latency, conduction velocity or f-wave) in the sural, peroneal or ulnar nerve. In the absence of a sural nerve response (e.g., sural amplitude is 0 μV, response is unobtainable, then the presence of a peroneal motor nerve conduction velocity of ≥ 35 meters per second will be required for inclusion. Quantitative sensory testing will also be conducted at the screening visit.

All visit 1 measures are ideally to be completed within a 4-week time frame but may take up to 6 months (180 days) if repeated measures are needed, and may be done at one, or over several visits if that is more convenient for the subject.

6.2. Baseline

Visit 2: Baseline (Day 0).

The key activities of the baseline visit are to obtain the initial skin biopsies and to initiate treatment with either Salsalate or placebo. Cardiovasular autonomic neuropathy (CAN) assessment, as well as quality of life and symptom questionnaires, and a short battery of functional assessments, will also be performed at the baseline visit. Two skin biopsies are obtained; one at the proximal thigh, and one at the distal leg. These will be done on the subjects' dominant side unless contraindicated (e.g., presence of a cast).

Upon satisfactory completion of all eligibility and baseline assessments, including the skin biopsy, a 3 month + 14-day supply of Salsalate or placebo will be provided, and written instructions for use of the medication reviewed with and provided to the subject. Subjects will be instructed to take one tablet with breakfast for the first four days beginning with the first breakfast after receipt of the study medication. The study medication dose will be increased slowly to the maximum tolerated dose (2 tablets, 3 times daily) using the following schedule: Beginning on day 5, take 1 tablet with breakfast and 1 tablet with lunch. Beginning on day 9, take 1 tablet at breakfast, 1 tablet at lunch, 1 tablet at dinner. Beginning on day 23, take 2 tablets at breakfast, 1 tablet at lunch, 1 tablet at dinner. Beginning on day 27 take 2 tablets at breakfast, 2 tablets at lunch, 1 tablet at dinner. The maximum dose of study medication, 2 tablets at breakfast, 2 tablets at lunch, and 2 tablets at dinner, may be started no sooner than the 31st day from receiving the first supply of study medication.

Subjects are to be reminded to return any unused study medication at their next visit.

Study staff will contact the subject within one week of the baseline visit to assess medication compliance and tolerance and to inquire as to the status of the biopsy sites.

6.3. Follow-up Visits

Visit 3: Safety Monitoring (Month 1).

At this visit, the study staff will measure the weight, blood pressure, and resting heart rate, will observe and note the condition of the baseline biopsy sites, and will ask the subject about any changes in health status or physical symptoms and record the findings in the source documents. The staff will assess medication compliance and tolerance. Adverse events will be similarly recorded and reported as required.

Visit 4: Safety Monitoring and Drug Dispensing (Month 3).

At this visit, the study staff will measure the weight, blood pressure, and resting heart rate, will observe and note the condition of the baseline biopsy sites, and will ask the subject about any changes in health status or physical symptoms and record the findings in the source documents. Adverse events will be similarly recorded and reported as required. Laboratory assessments (HbA1c, METCOMP, CBCP, cystatin-C, lipids, uric acid and urine albumin) will be obtained. Urine pregnancy testing may be performed at the discretion of the investigator for all women of childbearing potential. The study staff will collect any unused study medication, and will dispense 3 months + 14 day of medication. The number of unused salsalate tablets will be recorded in the source document and unused medication secured until returned for final drug disposition.

Visit 5: Safety Monitoring and Drug Dispensing (Month 6).

At this visit, the study staff will measure the weight, blood pressure and resting heart rate, and will ask the subject about any changes in health status or physical symptoms. Laboratory assessments (HbA1c, METCOMP, CBCP, cystatin-C, lipids, uric acid and urine albumin) will be obtained. Urine pregnancy testing may be performed at the discretion of the investigator for all women of childbearing potential. The study staff will collect any unused study medication, and will dispense a 3 month + 14-day supply of study medication. The number of unused salsalate tablets will be recorded in the source document and unused medication secured until returned for final drug disposition. The subjects will be asked to complete quality of life and neuropathy symptom surveys.

Visit 6: Safety Monitoring and Drug Dispensing (Month 9).

Weight, blood pressure and resting heart rate are recorded and inquiries made re: changes in health status or physical symptoms. Laboratory assessments (HbA1c, METCOMP, CBCP, cystatin-C, uric acid and urine albumin) will be obtained. Urine pregnancy testing may be performed at the discretion of the investigator for all women of childbearing potential. The study staff will collect any unused study medication, and will dispense a 3 month + 14-day supply of medication. The number of unused salsalate tablets will be recorded in the source document and unused medication secured until returned for final drug disposition.

Visit 7: Final Visit (Month 12).

Weight, blood pressure and resting heart rate are recorded and inquiries made re: changes in health status or physical symptoms. Laboratory assessments (HbA1c, METCOMP, CBCP, cystatin-C, lipids, uric acid and urine albumin) will be obtained as will blood and urine samples

for Luminex assays of inflammatory markers. Urine pregnancy testing may be performed at the discretion of the investigator for all women of childbearing potential. The study staff will collect any unused study medication. The number of unused salsalate tablets will be recorded in the source document and unused medication secured until returned for final drug disposition.

The structured neurological history and examination and nerve conduction studies performed at screening will be repeated.

Cardiovascular autonomic neuropathy (CAN) assessment, quantitative sensory tests, as well as quality of life and symptom questionnaires, and battery of functional assessments, will be performed at the final visit. Two skin biopsies are obtained; one at the proximal thigh, and one at the distal leg. These will be done on the subjects' dominant side unless contraindicated (e.g., presence of a cast). The biopsies will be obtained from skin which is adjacent (within 1 cm) to the original biopsy sites.

Within 1 week of the final visit, the study staff will contact the subject by phone or email to inquire about any changes in health status, and to as inquire as to the status of the skin biopsy sites.

6.4. Scheduled Interim Contact

Within 1 week of Visit 2 (Baseline, initial biopsy) the study staff will contact (telephone, email, in-person) the subject to inquire about any problems at the biopsy sites and to inquire about medication compliance and any side effects encountered. This contact will be recorded in the source document.

6.5. Subject Withdrawal

A subject may withdraw from the trial at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioral, or administrative reasons. If a subject does not return for a scheduled visit, every effort should be made to contact the subject. In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data; however, they may be questioned regarding their reason for withdrawal and asked to return all unused investigational products.

7. ASSESSMENTS

7.1. Efficacy

7.1.1. Interaepidermal Nerve Fiber Density (IENFD)

Traditionally the diagnosis of DN is made by the presence of abnormal nerve conductions (NC) studies, and a combination of symptoms and signs of distal sensorimotor polyneuropathy. However, NC studies assess only large myelinated nerve fiber function and in early DN, these conventional measures can be normal and if abnormal, change slowly. Recent data have also described minimal worsening [14] or improvement in NC [15] in placebo and epidemiological cohorts with little relation to other measures of small fiber nerve function in diabetic patients. This was

further confirmed by the relative modest changes in all NC studies in the DCCT/EDIC cohort during 13-14 years of follow-up while adhering to current standard of care [16].
Therefore, when studying a population with earlier stages of disease which would be most amenable to a therapeutic intervention, it is important to utilize outcome measures that best identify these patients (in this case, a measure that sensitively captures small fiber deficits).

In patients with confirmed or more advanced DN, the prevalence of abnormal NC, quantitative sensory testing and IENFD was comparable [5,18]. Data from several trials show also that IENFD was significantly reduced in patients with normal NC studies and that there is an inverse correlation between IENFD and the severity of DN, defined by the Neurological Disability Score and the Neuropathy Impairment Score [18], suggesting early damage to small nerve fibers. These data suggest that IENF loss is an early feature of DN, progresses with increasing neuropathic severity and may improve with appropriate intervention. Additionally, IENFD was endorsed by several societies and authorities in the field including the Toronto Consensus Panel on Diabetic Neuropathy as a reliable, efficient and valid outcome measure to assess small fiber neuropathy in clinical trials [5,8,17,18].

IENFD is a continuous measure of small fiber neuropathy, with high positive and negative predictive values along with high diagnostic efficiency in differentiating between subjects with and without neuropathy [5,19,20].The morphometric quantification of IENFD, most commonly expressed as the number of IENF per length of section (IENF/mm e.g. density) is possible with skin biopsy, a minimally invasive procedure, with less than 1 % of mild adverse events such as bleeding, swelling or erythema. Intra- and inter-observer variability for the assessment of IENFD demonstrates good agreement and a large normative data base was recently created by an international consortium [5,19,20]. Patient refusal rate for this procedure is minimal.

The change in IENFD at the distal thigh and ankle will be assessed by skin biopsies obtained at baseline and 12 months (as described in detail in the Manual of Operations.

7.1.2. Additional Measures of Neuropathy

Considering the complexity of nerve fiber involvement in patients with diabetes, we have included in our trial design several additional outcomes including the change in the composite measure of large fiber neuropathy (the endpoint used in the DCCT/EDIC study) as a secondary outcome and definite small fiber neuropathy.

1. The change from baseline to months in IENFD at the distal thigh;

2. The proportion of subjects with confirmed clinical neuropathy at baseline, and 12 months defined as the combination of:

a) Definite clinical neuropathy requiring at least two positive responses among neuropathic symptoms (pain, numbness, paresthesias), sensory signs, and abnormal ankle reflexes (diminished or absent) consistent with a distal symmetrical polyneuropathy and without causal explanation aside from diabetes, as assessed clinically using a standardized neurological examination and,

b) NC abnormalities involving ≥ 2 nerves among the sural, peroneal, and ulnar nerves.

3. The proportion of subjects with definite small fiber neuropathy, defined as neuropathic symptoms and signs (decreased pinprick or temperature sensation) consistent with small fiber

predominant dysfunction, and an abnormality in IENFD (one standard deviation below normal for age/gender/weight) or abnormal cardiovascular autonomic testing.

4. Measures of cardiovascular autonomic neuropathy derived from cardiovascular reflex testing (R-R interval ratio during deep breathing, postural change, and Valsalva) at baseline and 12 months; resting HR will be obtained at baseline and 3 month intervals through 12 months of follow-up.

5. Measures of quality of life as assessed by the NeuroQOL domain and overall scores at baseline, 6, and 12 months.

6. Autonomic symptoms will be assessed using the Survey of Autonomic Symptoms (SAS). 7. Measures of pain as assessed by the Neuropathy Pain Scale

8. Functional Assessments: BERG Balance Scale; Falls Efficacy Scale, and 8 Foot Get-Up-and-Go assessments.

7.2. Safety

Safety will be assessed through the collection of adverse events and laboratory parameters.

7.3. Exploratory

Assessment of expression of pain-related molecules in skin biopsies, including sodium channels, TrkA receptors, neuropeptides, such as substance P and CGRP and TNFα. Serum and urine inflammatory markers TNFα, IL1β, IL6 and CCL2 will also be measured

8. ADVERSE EVENT REPORTING

8.1. Adverse Events

All observed or volunteered adverse events regardless of treatment group or suspected causal relationship to the investigational product(s) will be recorded on the adverse event page(s) of the case report form (CRF).

For all adverse events, the investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification to the IRBMED. For all adverse events, sufficient information should be obtained by the investigator to determine the causality of the adverse event. The investigator is required to assess causality and indicate that assessment on the CRF. Follow-up of the adverse event, after the date of therapy discontinuation, is required if the adverse event or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator and the IRBMED.

8.2. Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a product or medical device; the event need not necessarily have a causal

relationship with the treatment or usage. Examples of adverse events include but are not limited to:

- Abnormal test findings;
- Clinically significant symptoms and signs;
- Changes in physical examination findings;
- Hypersensitivity;
- Progression/worsening of underlying disease;

8.3. Abnormal Laboratory Findings

The criteria for determining whether an abnormal objective test finding should be reported as an adverse event are as follows:

- Test result is associated with accompanying symptoms, and/or
- Test result requires additional diagnostic testing or medical/surgical intervention, and/or
- Test result leads to a change in trial dosing outside of protocol-stipulated dose adjustments, discontinuation from the study, significant additional concomitant drug treatment, or other therapy, and/or
- Test result is considered to be an adverse event by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an adverse event. Any abnormal test result that is determined to be an error does not require reporting as an adverse event.

8.4. Serious Adverse Events

A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- Results in death;
- Is life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Results in congenital anomaly/birth defect.

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and may require intervention to prevent one of the other outcomes listed in the definition above, the important medical event should be reported as serious.

8.5. Hospitalization

Adverse events reported from clinical trials associated with hospitalization or prolongation of hospitalization are considered serious. Any initial admission (even if less than 24 hours) to a healthcare facility meets these criteria.

Hospitalization does not include the following:

- Rehabilitation facilities;
- Hospice facilities;
- Respite care (e.g., caregiver relief);
- Skilled nursing facilities;
- Nursing homes;
- Routine emergency room admissions;
- Same day surgeries (as outpatient/same day/ambulatory procedures).

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse event is not in itself a Serious Adverse Event. Examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new adverse event or with a worsening of the preexisting condition (e.g., for work-up of persistent pre-treatment lab abnormality);
- Social admission (e.g., subject has no place to sleep);
- Administrative admission (e.g., for yearly physical exam);
- Protocol-specified admission during a clinical trial (e.g., for a procedure required by the trial protocol);
- Optional admission not associated with a precipitating clinical adverse event (e.g., for elective cosmetic surgery);
- Pre-planned treatments or surgical procedures should be noted in the baseline documentation for the entire protocol and/or for the individual subject.

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an adverse event. For example, an acute appendicitis that begins during the adverse event reporting period should be reported as the adverse event, and the resulting appendectomy should be recorded as treatment of the adverse event.

8.6. Severity Assessment

If required on the adverse event case report forms, the investigator will use the adjectives MILD, MODERATE, or SEVERE to describe the maximum intensity of the adverse event. For purposes of consistency, these intensity grades are defined as follows:

MILD	Does not interfere with subject's usual function.
MODERATE	Interferes to some extent with subject's usual function.
SEVERE	Interferes significantly with subject's usual function.

Note the distinction between the severity and the seriousness of an adverse event. A severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with subject's usual function) but would not be classified as serious unless it met one of the criteria for serious adverse events, listed above.

8.7. Discontinuations (See also Subject Withdrawal, Section 6.5)

The reason for a subject discontinuing from the trial will be recorded in the CRF. A discontinuation occurs when an enrolled subject ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The investigator must determine the primary reason for discontinuation. Withdrawal due to adverse event should be distinguished from withdrawal due to insufficient response, according to the definition of adverse event noted earlier, and recorded on the appropriate adverse event CRF page.

When a discontinuation is due to a serious adverse event, the serious adverse event must be reported in accordance with the reporting requirements defined below.

8.8. Eliciting Adverse Event Information

The investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the trial subject.

8.8.1. Reporting Requirements (Serious and Nonserious Adverse Events)

Each adverse event is to be classified by the investigator as serious or nonserious. This classification determines the reporting procedures to be followed. If a serious adverse event occurs, expedited reporting will follow local and international regulations, as appropriate. SAEs are reportable from the time that the subject provides informed consent, which is obtained prior to the subject’s participation in the clinical trial, i.e., prior to undergoing any trial-related procedure and/or receiving investigational product.

8.8.2. Reporting Serious Adverse Events

Serious adverse events will be reported to the UMIRBMED in accordance with IRB Reporting guidelines.

In the event that the investigator does not become aware of the occurrence of a serious adverse event immediately (e.g., if an outpatient trial subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it and document the time of his/her first awareness of the adverse event.

The investigator's assessment of causality must be provided. An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an adverse event. If the investigator's final determination of causality is unknown and the investigator does not know whether or not study drug caused the event, then the event will be handled as "related to study drug" for reporting purposes. If the investigator's causality assessment is "unknown but not related to study drug", this should be clearly documented on study records. In addition, if the investigator determines the adverse event is associated with trial procedures, the investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with the serious adverse event reporting requirements, if applicable.

8.8.3. Reporting Non-Serious Adverse Events

Adverse events reported by subjects that are unrelated or unlikely related to study participation, regardless of expectedness, will not routinely be reported, unless the severity and duration of the event reaches the threshold for defining a serious adverse event. Local reporting guidelines should be followed.

Adverse events reported by subjects that are related (definitely related, probably related or possibly related) to study participation and are expected based on known risks of participation, will not be routinely reported, unless the severity and duration of the event reaches the threshold for defining a serious adverse event. For example, an episode of mild, self-treated hypoglycemia would be expected because of the underlying diagnosis of type 1 diabetes and expected as a known side-effect of salsalate. This would be classed as a mild expected adverse event possibly related to study treatment and would not be reported

9. EXPECTED RISKS ASSOCIATED WITH THE PROTOCOL

9.1. Risks Associated with Study Participation

Risks associated with study participation in general are related to the time commitment for the study, and risk for loss of privacy. There will be 7 "in-person" visits required for the study, as well as 2-3 "remote" visits conducted by phone or email. The study staff will try to minimize the burden of the visits to the extent practical by scheduling on days and at times that are most convenient for the patient. Privacy risks will be reduced by maintaining a separate research record that uses a coded identifier, rather than personal identities. Links to the coded identifier and personal identity shall be maintained in a secure electronic file by the study coordinator and the links will ultimately be destroyed. Destruction of the links will occur no sooner than 2 years after the study has been acknowledged as terminated by the University of Michigan IRB (UM IRBMED).

9.2. Risks Associated with Blood Collection

Risks associated with blood collection are pain, discomfort, bleeding, bruising or infection. There is also the risk of dizziness, light-headedness, nausea or even fainting. Blood sampling will be performed by staff with skill and experience in venipuncture.

9.3. Risks Associated with Study Medication

The study is a randomized, double blind study and so neither the subject nor the study staff will know whether the medication is salsalate or a placebo. Not knowing what the actual study medication is may cause anxiety. Subjects who are not willing to accept random treatment assignment will not be accepted into the study.

The Belmar Research Pharmacy will maintain a master list of treatment assignments and in the event of a medical emergency, may be contacted to "unmask" the subject treatment.

Once the study is completed in all subjects, subjects may request their treatment assignment.

The common side effects of salsalate are nausea, loss of appetite, heartburn and feeling of ringing in the ears and some degree of deafness. These effects usually subside with ongoing treatment but if they persist, the dose of salsalate may be reduced. Subjects will be instructed to take the medication at mealtimes to reduce the GI side effects of the medication. While the GI and otologic side effects of salsalate tend to subside, if they persist or are troubling, the dose of salsalate may be reduced or salsalate may be stopped.

Salsalate may cause allergic reactions. People with known allergy to salsalate, aspirin, or to other NSAIDS are excluded from participating in the study. Subjects will be reported to stop salsalate treatment should they develop a rash, itching, hives, or difficulty breathing and to contact the study team or their usual health care provider right away. Emergency care may be required if the subject has breathing difficulties.

Salsalate and other salicylate medications in high doses can cause gastrointestinal bleeding, especially in patients with previous history of gastric ulcers. Patients with a history of GI bleeding or gastric ulcers are excluded from participation. Subjects will be advised of the risk for GI bleeding and provided information regarding signs and symptoms of GI bleeding and are asked to report these symptoms to the study team or to their regular health care provider.

Salsalate and other drugs in the class of salicylates can, especially in high doses, cause the blood sugar to drop, which may increase the risk of hypoglycemia in this population of people with type 1 diabetes. Subjects will be made aware of the risk for low blood sugar and advised to check blood glucose levels frequently, and to carry treatment for low blood sugar. Frequent monitoring and carrying treatment for low blood sugar is consistent with routine advice given to people with type 1 diabetes and people with type 2 diabetes on insulin or on other hypoglycemic agents. The subjects' insulin dose may be adjusted to prevent hypoglycemia.

Salsalates, like other NSAIDs can cause cardiovascular side effects including heart attack or stroke. Subjects will be informed of the symptoms of stroke and heart attack and instructed to seek emergent evaluation and treatment should symptoms occur.

Fluid retention and edema has been associated with NSAIDS, and so these should be used with caution in people with fluid retention or heart failure.

Salsalate, like other NSAIDs can cause serious skin reactions such as exfolliative dermatitis and Stevens-Johnson Syndrome. Subjects will be instructed to report any rash, blistering, hives, fever or itching that occurs while on salsalate, and to stop using salsalate and contact the study doctor or their own health care provider should a rash or other skin changes appear.

Salsalate may cause hepatotoxicity; subjects will be asked to report nausea, fatigue, itchiness, jaundice, abdominal pain and discomfort and or flu-like symptoms to the study staff or to their usual doctor and, to discontinue the medication until an evaluation of the symptoms as been done.

Salsalate is not approved for use in pregnant or nursing women. Pregnant and nursing women (and women who are planning pregnancy) are excluded from participation. Subjects are asked about current contraceptive practices during screening and those with child-bearing potential must agree to use an acceptable form of contraception during the study [oral contraceptive pills, injectable contraception (e.g. depo-provera) IUDs, barrier contraceptives (diaphragm/condoms), or surgical contraception (tubal ligation)]. A urine pregnancy test will be performed on all women of childbearing potential at baseline, regardless of contraceptive practices and will be repeated during the study if indicated. Women who suspect pregnancy are asked to contact the study staff and may be asked to have a pregnancy test. Women who suspect pregnancy or who are known to be pregnant are instructed to stop study medication immediately and to contact the study staff.

9.4. Risks Associated with Nerve Conduction Studies

Risks associated with nerve conduction studies are the discomfort associated with these studies. The subjects will be alerted that they will feel a sensation similar to a “carpet shock” or snap from a rubber band during the test and will be reassured that the sensation will be temporary. A skilled electromyographer will perform the studies and care will be taken to minimize the discomfort associated with the studies.

9.5. Risks Associated with Skin Biopsies

The risks associated with skin biopsies are pain, bleeding, bruising, or infection. Subjects may be left with a small scar or discolored area at each biopsy site. Four sites are biopsied in the course of the study, each about 3 mm in diameter. The skin biopsies will be done by members of the staff who have training and experience in doing them. The areas biopsied will be cleaned with antiseptics prior to the biopsy and a local anesthetic used to reduce pain. Subjects will be given written information about wound care and how and when to report any signs or symptoms of infection. The study staff will call the subject within one week each biopsy to check on how it is healing.

9.6. Risk Associated with Cardiovascular Autonomic Testing

Participants will be asked to fast in preparation for testing and are therefore at heightened risk for hypoglycemia. The study team will advise the subject regarding adjustments to insulin dosing to reduce the risk for hypoglycemia. Testing will be scheduled in the morning to shorten the duration of fasting. The test itself has few risks associated with it, although some patients may become dizzy or lightheaded when moving from supine to standing, or after performing valsalva maneuvers. Subjects will be reminded to report any dizziness or lightheadedness immediately to the examiner and will be instructed to return to a seated or lying position as needed to relieve symptoms.

10. DATA ANALYSIS/STATISTICAL METHODS

Detailed methodology for summary and statistical analyses of the data collected in this trial will be documented in a Statistical Analysis Plan, which will be prepared during the R34 and finalized prior to breaking of the blind.

10.1. Sample Size Determination

The purpose of this clinical trial is to provide the preliminary data necessary to design a larger

multi-site clinical trial. There are two assumptions that are necessary to justify the larger trial. The first is that salsalate will cause an increase in nerve fiber density (re-innervation) and the second is that the natural progression of DN, even with treatment for diabetes, leads to loss of nerve fibers. The primary outcome is an assessment of IENFD at the distal thigh after 12 months of placebo or salsalate treatment.

In the largest study of IENFD, Lauria et al[57] provided a table of median IEFND values in the distal leg as a function of age and gender. In the 20-29 age range the average IEFND was 12.2 fibers/mm with an average decline of 0.1 fibers/mm per year. We reported that the average IEFND in the distal leg in T1DM subjects (mean disease duration of 27 years) was 2.2 fibers/mm. If we assume that our diabetic subjects have the average IENFD for ages 20-29 shown by Lauria et al at the start of their disease, then the decline due to diabetes was 10 fibers/mm or an average of 0.25 fibers/mm /year. If the decline is more rapid as neuropathy progresses, then the average decline in IEFND may be as high as 0.5 fibers/mm/year.

In a study of the effect of physical exercise Smith et al found an increase in the IENFD at the thigh of 1.4 fibers/mm after one year of intervention. Based on our assumption about the mechanism by which salsalate will influence IEFND (reduction of inflammation), we believe that a similar increase in fiber density is possible with treatment with salsalate. In multiple studies that reported a change in IEFND over time (including one of our own studies), the standard deviation of the change was approximately 2 fibers/mm. With the above assumptions, there will be an expected difference of 1.65 fibers/mm (0.25 + 1.4 = 1.65) in the change between the two groups at the thigh with a standard deviation of 2.0. With 25 subjects per group there will be 81% power to reject the null hypothesis of no difference between groups using a two-tailed t-test at a 5% level of significance. Prior data are insufficient to perform a power calculation for the distal leg.

The trial will be considered a success if the primary endpoint demonstrates a significant difference between the two groups and one other secondary endpoint trends in a similar direction. This trial will also be considered a success if there is sufficient evidence to show that IEFND increases with salsalate but is likely to decrease with usual treatment. With 25 subjects per group, the standard error of the estimate will be approximately 0.4 [=2/square root (25)] and therefore the confidence interval for the mean change in each group will be the estimated mean change ± 0.8 fibers/mm; i.e. an increase of greater than 0.8 fibers/mm with salsalate and an observed decrease with placebo (since the expected decrease with placebo is not expected to be large). With these results, this trial would provide the preliminary data needed for a larger trial to validate the effect of salsalate. Assuming 15-20% attrition rates, 70 subjects will be recruited to attain the necessary 50.

10.2. Efficacy Analysis

The two treatment groups will be compared with respect to demographic and baseline variables (e.g., age, BMI, smoking, etc.). If a significant difference is found in any variable, the models described below will be fitted both with and without the variables that differ at baseline.

Analysis of Primary Outcome:

The primary outcome is change from baseline to 12 months in IENFD at the distal thigh. A general linear model will be fitted to the change in IENFD score with factors for treatment group, baseline HbA1c (≤8.0% or >8.0%), and baseline IEFND as fixed covariates. If the distributional

assumptions are not met, transformations of IENFD or the use of nonparametric methods will be employed. Sensitivity analyses will be performed to assess how subjects who withdrew may affect conclusions of the analysis. An intent-to-treat analysis set (including subjects who are randomized, treated and have a baseline IENFD) will be used to assess treatment effects with multiple imputation methods[101] employed to impute outcomes for subjects with missing post-baseline data. Depending on the extent and pattern of missingness, other simpler sensitivity analyses may be used: for example, change from baseline to month 12 for subjects completing the study (completers) may be analyzed using an analysis of variance model. The model will include the same covariates that are included in the primary analysis.

Analyses of Secondary Outcomes:
Due to multiple tests, formal statistical testing won't be performed; p-values will be reported to allow the reader to assess the result. For analysis of repeated measures of continuous data (change from screening/baseline to 12 months in NC studies, quantitative sensory testing, CAN measures, Neuropathy Symptoms, Pain, and NeuroQOL domain scores), a longitudinal approach will be employed. Specifically, each outcome will be analyzed using a mixed effect model for repeated measures with the treatment group and baseline HbA1c (≤8.0 or >8.0%)) as fixed covariates. Under the model, time of assessment will be a continuous variable and the outcome will be modeled as a linear function of time. Appropriate non-linear parametric or non-parametric tests may be applied if assumptions of the model are not satisfied.

Logistic regression will be used to compare the proportion of subjects who have confirmed clinical neuropathy with NC and those who had normal NC and therefore required an assessment of IENFD for trial entry, between the salsalate and placebo groups, with covariates for treatment group, and baseline $HbA1_C$ (≤8.0 or >8.0%). The components of this composite endpoint will also be assessed. Rank-transformation analysis of covariance will be used on each of the NC values, and an overall treatment group differences in the multiple NC values will be assessed using the nonparametric Wei-Lachin rank test of stochastic order[102]. The impact of additional covariates on the relationship between IENFD and confirmed clinical neuropathy and treatment will be explored by adding age, sex, height, weight, BMI, cigarette smoking, cardiovascular risk factors and concomitant medications into the models described above. A forward stepwise selection procedure will be used, with $p<0.10$ used to retain covariates. Interaction terms between treatment groups and select covariates will be investigated. In addition, we will adjust for the effect of changes in glucose on outcomes during the course of treatment by including HbA1c as a time-varying covariate in models.

10.3. Analysis of Exploratory Endpoints

Several exploratory analyses will be employed to investigate phenotypic characteristics associated with response to intervention, and the association of biomarkers with outcomes, to maximize the scientific knowledge gained from this study. For example, exploiting the longitudinal measures of the phenotypic characteristics, we will jointly model longitudinal and event time (e.g., response to treatment) as a function of treatment group and other potential prognostic factors [25,26]. We will also use more sophisticated statistical models, such as linear and/or non-linear mixed effects models or nonparametric regression, to explore the relationship of phenotype and response to treatment, and biomarkers with clinical outcomes [25,27,28].

10.4. Safety Analysis

Adverse events and laboratory parameters will be summarized by treatment group. Chi-square or Fisher's exact tests will compare salsalate to placebo for discrete outcomes, and two-sample

t tests (on appropriately transformed continuous outcomes, if necessary) or nonparametric wilcoxon tests will assess treatment differences for continuous outcomes. Serious adverse events (SAEs), unexpected adverse events and all AEs will also be summarized descriptively by body system and treatment group, regardless of relatedness to study treatment.

10.5. Interim Analysis

Given the expected enrollment period of 2 years and the 1-year follow-up period, an interim analysis to stop study enrollment for benefit or futility is not feasible. Rather, periodic assessment of the safety of study subjects will be undertaken by an independent Data and Safety Monitoring Board (see below). Although salsalate is an approved medication (off-label for this indication), if there were safety concerns in this study, the DSMB could request a formal analysis of safety and efficacy data to delineate the risk-benefit profile of salsalate in this study, with the potential to stop the study.

10.6. Data and Safety Monitoring Board

Study monitoring will be done by the Michigan Institute for Clinical and Health Research (MICHR). A Data and Safety Monitoring Board (DSMB) will oversee this study. Members of the DSMB are independent of the study investigators and represent Endocrinology, Neurology, Biostatistics, as well as possibly a lay member. The DSMB will meet every three months or more frequently if requested by the Chairs either in person or by teleconference. This protocol will be approved by the DSMB prior to initiation of recruitment.

11. DATA HANDLING AND RECORD KEEPING

11.1. Case Report Forms / Electronic Data Record

As used in this protocol, the term case report form (CRF) should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this trial.

A CRF is required and should be completed for each included subject. It is the investigator's responsibility to ensure completion and to review and approve all CRFs. CRFs must be signed by the investigator or by an authorized staff member. These signatures serve to attest that the information contained on the CRFs is true. At all times, the investigator has final personal responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the CRFs. Subject source documents are the physician's subject records maintained at the trial site. In most cases, the source documents will be the hospital's or the physician's chart. In cases where the source documents are the hospital or the physician's chart, the information collected on the CRFs must match those charts.

In some cases, the CRF may also serve as the source document. In these cases, the DCC and the investigator must prospectively document which items will be recorded in the source documents and for which items the CRF will stand as the source document.

12. ETHICS

12.1. Institutional Review Board (IRB)

It is the responsibility of the investigator to obtain prospective approval of the trial protocol, protocol amendments, informed consent forms, and other relevant documents, e.g., advertisements, if applicable, from the IRB. All correspondence with the IRB should be retained in the Investigator File.

12.2. Ethical Conduct of the Trial

The trial will be performed in accordance with the protocol, International Conference on Harmonization Good Clinical Practice guidelines, and applicable local regulatory requirements and laws.

12.3. Subject Information and Consent

The informed consent form must be agreed to by the Protocol Committee and the IRB and must be in compliance with ICH GCP, local regulatory requirements, and legal requirements.

The investigator must ensure that each trial subject, or her legally acceptable representative, is fully informed about the nature and objectives of the trial and possible risks associated with participation. The investigator will obtain written informed consent from each subject or the subject's legally acceptable representative before any study-specific activity is performed. The informed consent form used in this trial, and any changes made during the course of the trial, must be prospectively approved by the IRB before use. The investigator will retain a copy of each subject's signed consent form.

12.4. Subject Confidentiality

Study records with the study participant’s information for internal use at the clinical sites will be secured at the study site during the study. At the end of the study, all records will continue to be kept in a secure location. There are no plans to destroy the records.

Study participant data, which is for reporting purposes, will be stored at the Coordinating Center. Data will identify participants by the unique study identification number. The study data entry and study management systems used by clinical sites and by Coordinating Center research staff will be a secured, password protected computer.

12.5. Sample and Data Storage

Data collected for this study will be analyzed and stored at the Coordinating Center. After the study is completed, de-identified data will be stored at the Coordinating Center for use by researchers for future analyses with the participant’s approval. The results of these future analyses will not be made known to the participant.

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Appendix A: Contingency Operation Plan in Case of Reduced Availability of Study Medication

The following changes may be implemented and/or adapted in response to disruptions in study medications (salsalate and/or placebo) availability. Information explaining any departures from the current protocol due to unavailability, or limited availability of study medication will be documented in the subject's research record. Changes to study medication dose and visit schedules will be documented on the protocol deviation log with a notation that the change in dose or schedule is because of change in medication availability. The notation will permit the study team to censor those protocol changes that were made because of medication shortages (versus, non-adherence for example) and these events will ultimately be reported separately from other protocol deviations.

1. New subject enrollment

Enrollment of new subjects (Screening Visit 1) may be paused until the investigator is notified that adequate supplies of study medications are available. Eligible participants who have not yet completed Visit 2 (randomization, assignment, and initiation of study medication) will have Visit 2 postponed. The protocol currently allows for Visit 2 to take place up to 180 days after Visit 1. If Visit 2 must be delayed for more than 180 days due to unavailability of study medication, then baseline laboratory measures performed at Visit 1 will be repeated at Visit 2. A delay of over 365 days would require that all Visit 1 screening measures be repeated prior to Visit 2. Reconsent of the participant would be required in this case.

2. Treatment and visit modifications for enrolled participants (Visits 2 to 7)

The overarching goal of treatment modifications in the setting of limited supplies of study medications will be to keep all participants on at least a minimal daily dose of study medication during their remaining time in the study. The study site staff will collaborate with the research pharmacy to achieve this goal on a case-by-case basis.

The current protocol indicates that participants will be provided with sufficient study medication to last until the next scheduled visit. If this cannot be achieved based on participants' current maximally tolerated dose of the study medication, then the shipment schedule, or amount of medication shipped may be adjusted. Examples include sending less than the full number of tablets (e.g., sending a 2 month versus 3 month supply), or sending fewer tablets to provide a lower dose over a period of 3 months. Similarly, changes to treatment (dosing of study medications) and visit schedules will be individualized with consideration to a number of factors including:

- the current dose of study medication being taken
- the number of doses of study medication currently in the participant's possession
- the number of study visits completed/number of study visits remaining
- the expected time to resolution of the study medication supply shortage

Modifications in the event that supplies of study medication are limited or unavailable include, but are not be limited to the following:

- Participants will NOT return unused study medication after each visit/receipt of new study medication. Instead, they will be permitted to continue to use study medications

until the bottles are empty. This instruction will be documented in the participant's research record.

- To avoid gaps in exposure to study medication and to increase the likelihood of reaching 12 months of exposure to study medication the dose of study medication may be reduced, generally to ½ of the maximally tolerated dose, (up to 6 tablets per day), with further reductions as necessary to the minimum of 1 tablet per day).
- When study medication becomes available, the dose will be titrated back to the prior maximally tolerated dose according to the current study medication titration schedule (beginning with the participants' current dose and up-titrating to the maximally tolerated dose). Participants will be reminded that study medication can cause changes in blood glucose levels and changes to the dose of study medication may require adjustments to their insulin doses. Regular glucose monitoring will be encouraged. These instructions are consistent with the current protocol and consent document.
- If participants are without study medication at any dose for a period of 3 consecutive months, at any point between visit 2 and 6, the study team will consult with the DSMB to determine whether the participant should be scheduled for an early final visit (visit 7), or whether the participant should simply be withdrawn from further participation.
- Participants who have completed visit 6, and who do not have an adequate supply of study medication to remain on their maximally tolerated dose until visit 7 will either
    - reduce their daily dose of study medication so that the number of tablets available extends to the opening of the Visit 7 window. Visit 7 should be scheduled as early in the window as possible
    - complete visit 7 prior to the opening of the visit 7 window. This could include completing all Visit 7 measures at Visit 6 if it is clear that NO study medication will be available to the participant after completing visit 6

3. Alternate Source of Study Medication

The research pharmacy (Belmar) will identify alternate suppliers of study medication. Once a new manufacturer/supplier is identified, appropriate regulatory authorities (FDA/IRB) will be notified and the research pharmacy will obtain the study medication. The research pharmacy will contact the study team to establish an appropriate shipping schedule (timing, number of doses necessary to last until the next scheduled visit) to re-supply participants. All bottles of study medication currently include a label indicating that the appearance of medication may be different, so a change of manufacturer and possible change in the appearance of the study drug is unlikely to raise concerns among the study participants. Procedures are already in place at the site to reduce the risk of inadvertent unblinding of the study team to treatment assignment based on appearance of the study medication.

4. Safety and Protocol Deviation Reporting

All current safety and protocol deviation reporting for the study remains in place, per protocol requirements.

5. Reporting Study Changes to Participants

The study staff will be in regular contact with participants to make them aware of changes in the study, their dose of study medication, or any changes to their visit follow-up schedule.

Participants have the option to opt out of continued participation in the study. Any subject withdrawals will be reported to the IRB as required.

The study team will be in contact with participants as well, to notify them of expected shipments of study medication, alert them to changes in the amount of study medication they may receive, provide instruction for reducing (and ultimately increasing) their dose of study medication, and review instructions related to dose timing, drug monitoring and return. Participants who have had their dose of study medication reduced will be asked to follow the initial dose titration schedule once study medication becomes available, with the goal to return them to their prior maximally tolerated dose.

Communications between the study team and participants will generally be accomplished by telephone call and documented in the study source documents/case-report forms. Participants have the option to opt out of continued participation in the study. Communications with participants will be summarized in the source documents. Any subject withdrawals will be reported to the IRB as required.