TINSAL-T2D

Targeting INflammation using SALsalate for Type 2 Diabetes Stage II

Protocol

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Protocol Amendment: Version 2.4

Page 6, Section 1.2 Overall Design and Study Interventions – added W56 visit
Page 23, Section 4.2 Visit Schedule – added W56, visit 13
Page 26, Section 5.1 Data Collection - added a column for W56 visit 13 procedures
Page 28, Section 6.2 Safety Monitoring and Risk Management - added criteria for a W56 post dosing visit.

Protocol Amendment: Version 2.3.1

Page 29, Section 6.2.1.1 Hypoglcemia Management – clarified management plans that only 1 criteria needs to be met, that a BG < 70 ≥ 2x/week leads to a 0-50% reduction, and a BG < 70 ≥ 3x/week leads to a 50-100% reduction, and that plan A covers weeks 0 – 24 and plan B covers weeks 24 – 50.

Protocol Amendment: Version 2.3

Page 17, Section 2.3.2 Vascular Ultrasonography of the Brachial Artery to Measure Vascular Function (Flow Mediated Vasodilation) – Replaced Thomas Jefferson with University of Michigan
Page 18, Section 3.1 Recruitment – change 1.5 year period to 1.5 – 2 year period
Page 19, Section 3.2 Eligiblity – add information allowing participants with a calculated eGFR below 60 but with a measured 24 hour urine creatinine clearance above 60 to be eligible, change history of malignancy from less than 10 years to less than 5 years, and specify that alcohol or substance abuse within 5 years is exclusionary.
Page 20, Section 3.5 RUN-IN – clarify patient use and validity check on blood glucose meter.
Page 21, Section 3.6 Randomization and Baseline Visit – remove EKG collection statement for Visit 3 and clarify that height is only collected at Visit 3.
Page 23, Section 4.4 Withdrawal of Participant – Removed new diagnosis of an exclusionary condition.
Page 25, Section 5.1 Data Collection – clarify physical exam (full vs brief) and clarify drug dispensing on Schedule of Assessments.
Page 29, Section 6.2.1.1 Hypoglycemia Management - added section, clarifying definition and management of hypoglycemia and the revised role of the site investigator to manage diabetes concomitant medications for patient safety.
Page 40, Appendix Recommended Dosing of Diabetic Agents – Added saxagliptin, bromocriptine mesylate, liraglutide

Protocol Amendment: Version 2.2

Page 18, Section 3.2 Eligibility Criteria – modified the number of eligible inclusion medication combinations from two to three.
Page 24, Section 4.5 Rescue Therapy – added third medication and/or insulin therapy
Page 25, Section 5.1 Data Collection - corrected typo in 24 hour urine and ambulatory BP measurement schedules.

Protocol Amendment: Version 2.1

Two eligible medication combinations.

Two medications and/or insulin therapy.
24 hour urine and ambulatory BP measurement marked for week 0, 16, and 48.

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Abbreviations Used

AE	Adverse Event
BP	Blood Pressure
DCC	Data Coordinating Center
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
IRB	Institutional Review Board
MOP	Manual of Procedures
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
SAE	Serious Adverse Event
T2D	Type 2 Diabetes

CHAPTER 1: INTRODUCTION AND RATIONALE

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) has sponsored a collaborative agreement to conduct a clinical treatment trial, entitled Targeting Inflammation Using Salsalate for Type 2 Diabetes (TINSAL-T2D). The trial is conducted in two stages. Stage I was completed and presented to the DSMB on July 24, 2008. This protocol describes the background, design and organization of Stage II of the trial.

The protocol was written by the members of the TINSAL-T2D Study Group, approved by an External Advisory Board, and approved by the Institutional Review Boards (IRB's) of each participating clinical center prior to the initiation of recruitment.

The Steering Committee for the TINSAL-T2D Study Group is composed of investigators at Joslin Diabetes Center, Tulane University, the George Washington University Biostatistics Center, and the NIDDK project office. Detailed study procedures are provided in the study's manual of procedures (MOP).

1.1 SPECIFIC AIMS AND OBJECTIVES

The primary objective of the study is to determine whether salicylates represent a new pharmacological option for diabetes management. The study is conducted in two stages. The primary objective of the first stage was to select a dose of salsalate that was both well-tolerated and demonstrated a trend toward improvement in glycemic control. The primary objective of Stage II of the study is to evaluate:

- the effects of salsalate on glycemic control in diabetes;
- the tolerability of salsalate use in patients with type 2 diabetes (T2D); and
- the effects of salsalate on measures of inflammation, the metabolic syndrome, and cardiac risk.

1.2 OVERALL DESIGN AND STUDY INTERVENTIONS

The TINSAL-T2D study is a multi-center, randomized, double-masked, placebo-controlled, parallel-group clinical trial, conducted in two stages. Stage I consisted of a 1 week screening period, a 6 week single-masked placebo run in to ensure compliance, and a pre-treatment baseline evaluation. In Stage I, there was a 14 week double-blind treatment period with a total of 7 visits (1 screening, 1 run-in, baseline/randomization, and 4 post-randomization at 2, 4, 8, and 14 weeks). A post-treatment safety visit occurred at 16 weeks. In Stage II there is a 48 week double-blind treatment period with a total of 12 visits (1 screening, 1 run-in, baseline/randomization, 8 post-randomization at 2,4,8,12,16,24,36, and 48 weeks) and two safety, post-treatment visits at week 50 and week 56. Note that visit 4 (week 2) is by phone only.

The purpose of the first stage was to select a dose of salsalate that was well-tolerated and demonstrated a trend toward improvement in glycemic control. Participants were randomized 1:1:1:1 to placebo or salsalate dosed at 3.0, 3.5 or 4.0 g/d, such that 3 out of 4 participants received an active dose. The study drug or placebo was administered for a total of 14 weeks. Safety and efficacy of all doses were considered and an optimal dose of 3.5 g/d was selected for Stage II. The dose of 3.5 g/d is selected for further evaluation in the second stage of the trial.

During the second stage, a second cohort of participants are randomized 1:1 to placebo or salsalate at the dose of 3.5 g/d. The primary endpoint is change in HbA1c from baseline to 48 weeks.

1.3 BACKGROUND AND SIGNIFICANCE

The ongoing national and worldwide epidemics in obesity and type 2 diabetes (T2D) are now well-established [1, 2, 3, 4, 5]. Central questions being asked relate to "what is it about weight gain, Western diet and sedentary lifestyle that leads to insulin resistance?" Posed more scientifically, it is possible that a discrete molecular mechanism might be identified that connects obesity to such chronic health consequences as insulin resistance, T2D, the metabolic syndrome, and coronary heart disease (CHD). As a corollary, the identified mechanism(s) might be used to delineate potential drug targets to treat or prevent these disorders. The DPP (Diabetes Prevention Program) results clearly showed that lifestyle and pharmacological interventions are useful to prevent or delay disease onset in predisposed individuals [6] and provide increased impetus to these types of investigations.

Insulin resistance occurs when target tissues, including muscle, fat and liver, fail to respond normally to circulating insulin. While genetic traits clearly play roles both in the development of insulin resistance and the ability of β cells to compensate, there is also a connection that links weight gain to insulin resistance and other features of the metabolic syndrome. According to the National Health and Nutrition Examination Survey III, up to 24% of adult Americans have the metabolic syndrome, and this increases with age to a prevalence of 43% in men and women >60 years of age [7]. Individuals with the metabolic syndrome have significantly increased risks of developing CHD and T2D.

Recent studies suggest that a state of chronic, subacute inflammation, specifically mediated by the IKKβ/NF-κB pathway, might be both involved in the pathogenesis of insulin resistance and provide new targets for its reversal [8,9,10,11]. While basic research independently implicated inflammation, the findings closely parallel recent epidemiological results looking at correlates and potential causes of type 2 diabetes [12, 13, 14]. Perhaps even more interestingly, epidemiologists have identified potential relationships between insulin resistance and mediators of inflammation, including the proinflammatory cytokines, IL-6 and IL-1β and possibly TNF-α (see below) [13, 14].

Treating diabetes using high-dose salicylates is an old method and one that is accompanied by insulin sensitization [11, 15]. Furthermore, it has been shown during short-term trials that salicylate therapy markedly lowers circulating glucose, triglycerides, free fatty acids and cholesterol, and that all of these effects are mediated by the IKKβ/NF-κB pathway.

1.3.1 Inflammation and NF-κB Overview

NF-κB is a central integrator of proinflammatory signals and master regulator of genes involved in inflammation, innate immunity, and apoptosis. Numerous inputs that activate NF-κB in addition to proinflammatory cytokines include bacterial cell wall and viral products, dsDNA, mitogens, and oxidative stress [16, 17] (Fig. 1). Toll receptors, the gatekeepers of innate immunity, activate NF-κB. IκB proteins sequester NF-κB in the cytoplasm of resting cells. Activation of the kinase IKKβ promotes IκBα phosphorylation, ubiquitination, and proteasomal degradation. This releases NF-κB to translocate into the nucleus and mediates transcription of large numbers of target genes. The partial list in Fig. 1 includes many that have been implicated in the pathogenesis of insulin resistance, T2D and CHD.

1.3.2 Inflammation in Type 2 Diabetes

Growing evidence over recent years supports a potential role for cytokine-associated, subacute inflammation in the pathogenesis of insulin resistance and type 2 diabetes [18, 19]. Inasmuch as insulin resistance is an underlying feature of the metabolic syndrome, subacute

inflammation may also play a role in the development of associated dyslipidemias and hypertension. Together, insulin resistance (with or without hyperglycemia), dylipidemia, and hypertension all increase risk for atherosclerosis, which is itself increasingly thought to be a disease of chronic subacute inflammation [20, 21]. These interrelationships suggest that inflammation may be the basis of a "common soil" [22] involved in the pathogenesis of both type 2 diabetes and atherosclerosis.

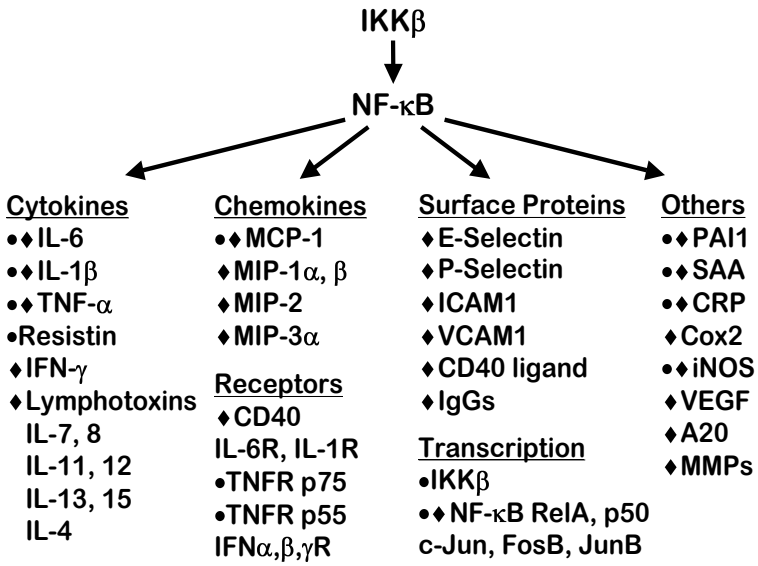


Fig. 1. Selected NF-κB responsive genes with potential roles in insulin resistance and T2D (•) or CHD (♦). Collectively these could be considered mediators of the metabolic syndrome.

Proinflammatory cytokines, including IL-1β, IL-6 and TNF-α, are produced in cells involved in immunity and inflammation, such as macrophages and monocytes, and in adipose tissue and liver, particularly in response to over-nutrition. The cytokines (IL-1β, IL-6 and TNF-α) also act on liver to produce a characteristic dyslipidemia associated with T2D, increased VLDL and decreased HDL, and to promote the release of acute-phase proteins which are atherosclerotic risk factors, such as fibrinogen. Circulating cytokines may act on the endothelium to promote atherogenesis and they may impair β cell insulin secretion.

Elevations in components of the acute-phase response and of inflammation more generally occur in type 2 diabetes and predict risk for its occurrence [13, 18, 19, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34]. Elevated markers include PAI1, CRP, fibrinogen, leukocyte count, sialic acid, IL-6 and IL-1β. However, the big question in the field has been whether these are simply correlative markers for the process or causatively involved in its pathogenesis. It is clear that proinflammatory cytokines such as IL-6 and TNF-α can cause insulin resistance in model systems [35, 36, 37], but it has been difficult to make the leap to what actually occurs in patients with the disease.

Our findings with anti-inflammatory salicylates, which inhibit IKKβ and NF-κB, have provided new impetus to the field [11, 38]. We hypothesized that as a central mediator of inflammatory responses, NF-κB activation might be a root of insulin resistance. Indeed, many of the gene products regulated by NF-κB have been implicated as markers or mediators of insulin resistance and T2D (Fig. 1). Specifically, those that are bulleted with •
have actually been reported to promote insulin resistance in animal models or human conditions [24, 26, 30, 31, 34, 36, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55]. Since any or all of these might promote resistance, decreased expression through NF-κB

inhibition should sensitize in each case. We hypothesize that NF-κB orchestrates the transcription of a constellation of genes, some known and others yet to be discovered, that coordinately mediate obesity-induced insulin resistance [8, 9]. The corollary is that NF-κB inhibition coordinately down-regulates an entire constellation of putative mediators [11, 13, 38].

1.3.3 NSAIDs

Anti-inflammatory drugs are conveniently separated into the glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs). The actions of the glucocorticoids are mediated by the glucocorticoid receptor, a member of the nuclear receptor family. Since glucocorticoids promote insulin resistance, these do not provide much promise as potential treatments for T2D or CHD. NSAIDs are chemically distinct and among the most commonly used drugs. Nearly all of the NSAIDs inhibit one or both of the cyclooxygenase enzymes, Cox1 and Cox2.

Aspirin (ASA, acetylsalicylic acid), the acetylated form of salicylate, is the prototypical NSAID. Aspirin very efficiently inhibits the Cox enzymes through irreversible, covalent transacetylation of an active site residue – *i.e.* aspirin's acetyl group covalently modifies Cox. Aspirin is antithrombotic even at low doses of 80-100 mg/day, because it irreversibly modifies Cox1 in platelets. Since platelets are non-nucleated and cannot resynthesize Cox1, this inactivates platelets for their lifetimes. Aspirin at the typical analgesic/antipyretic/anti-inflammatory dose of 650 mg inhibits both Cox1 and Cox2 in most cells and tissues. In this case aspirin is dosed more frequently, every 4-6 hours up to 4 doses per day. Other non-selective NSAIDs, including ibuprofen, naprosyn, ketoprofen and indomethacin, also inhibit Cox1 and Cox2 in most cells and tissues.

Salicylate has a distinct mechanism of action. At the high doses necessary for therapeutic efficacy (3-7 g/day), salicylates inhibit NF-κB [56], apparently by binding IKKβ and inhibiting IκB phosphorylation [57]. **Salicylate is a very weak inhibitor of Cox1 or Cox2**, because it lacks the acetyl group necessary for transacetylation and covalent modification. Aspirin, on the other hand, appears to be equivalent to salicylate in inhibiting NF-κB. However, GI toxicity and risk of life-threatening bleeds limit aspirin's use at high doses.

We have chosen to use salsalate (Disalcid), a prodrug form of salicylate, commonly used to treat osteoarthritis and other rheumatologic conditions. Due to efficacy, margins of safety, and low cost, salsalate and generic ibuprofen are at the top of the class of agents recommended for treating osteoarthritis. This is despite their having distinct molecular targets, NF-κB and Cox1/2, respectively. Salsalate is made of two covalently esterified salicylate molecules (Fig. 2), which renders it insoluble at the acidic pH of the stomach and non-irritating. Salsalate is cleaved into two salicylate molecules and absorbed in the duodenum. In contrast to aspirin, salsalate does not alter bleeding time [58].

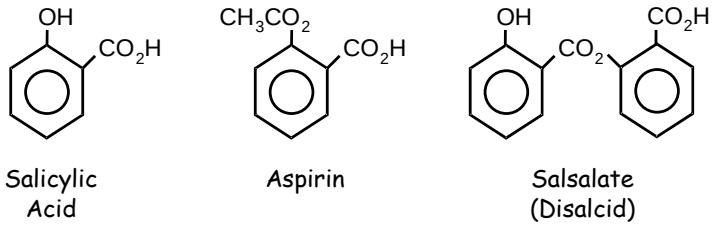


Fig. 2. Salicylate chemical formulas. Salsalate is a covalent, head-to-tail dimer between two salicylic acid moieties. Salsalate is insoluble at acidic pH, so it doesn't irritate the stomach.

1.3.4 Salicylates and Diabetes

In addition to the molecular rationale for targeting inflammation in the treatment of insulin resistance, a historical clinical experience supports the use of salicylate in treating diabetes. Sodium salicylate was used to treat patients with diabetes in studies dating as far back as 1875 [59, 60, 61] (Fig. 3).

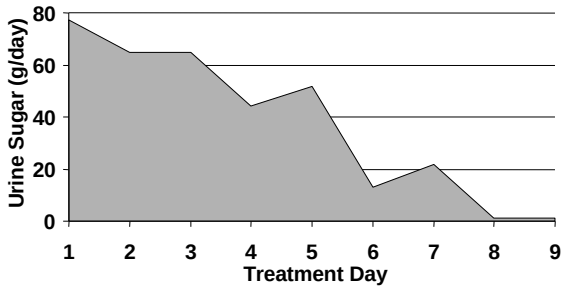


Fig. 3. Correction of glycosuria during salicylate therapy in the year 1875 [59].

A report in 1957 showed beneficial 'side effects' of high-dose aspirin used to treat a diabetic with acute rheumatic fever [62]. This prompted the authors to study 7 additional diabetic patients prospectively (Fig. 4). **Every patient responded to high-dose aspirin.**

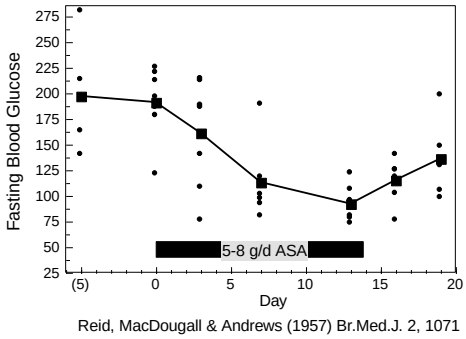


Fig. 4. Seven diabetic patients treated by diet alone (days -5 to 0), 5-6 g/day ASA during days 1-14, then off ASA [62].

The average fasting blood glucose value fell from ~200 mg/dl to less than 100 mg/dl during 14 days of treatment 5-8 g/day ASA. Fasting blood glucose levels crept back up after treatment was discontinued. Another study in 1960 similarly reported improvements in glycemia [63]. Six hospitalized diabetics were taken off insulin, placed on a 1800-2000 kcal/d diet, and then treated with ASA (Fig. 5). **Again, blood glucose values improved in every patient.** Mean fasting blood sugar fell from 371 ± 116 mg/dl to 128 ± 28 mg/dl.

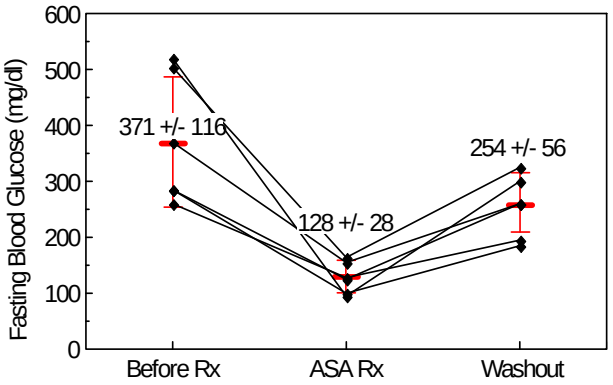


Fig. 5. Six hospitalized diabetics were taken off insulin and placed on a 1800-2000 kcal/d diet. Data were collected after 7 d diet alone, following ASA treatment (6 g/d for 10 d), and after a 5 day washout [63].

Thus several historical studies clearly demonstrated that high doses (4-7 g/day) of

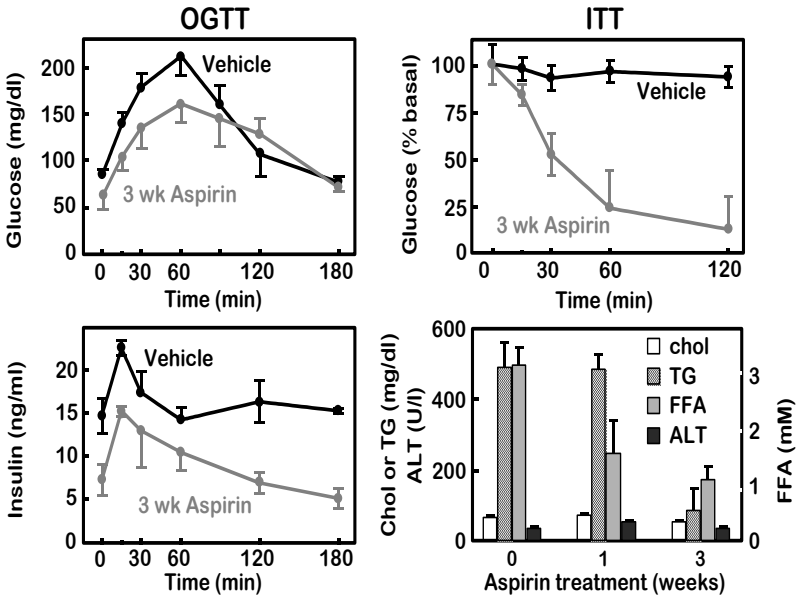


Fig. 6. High-dose aspirin was used to reduce insulin resistance and lipid levels in Zucker fatty rats [11].

salicylates, including aspirin, dramatically improved glycemic control. These results from earlier human studies closely match recent findings in both insulin resistant rodents and patients with T2D, in which salicylates have lowered blood glucose as effectively as troglitazone or metformin. Side effects associated with high-dose aspirin (*e.g.* tinnitus and gastrointestinal distress), the narrow therapeutic window, and positive outcomes obtained with sulfonylureas during the 1950's may have eclipsed the exploitation of aspirin as an oral hyperglycemic agent. But it is not clear why salicylates have been neglected as experimental tools throughout what we consider to be the entire modern era of mechanism-based drug discovery – the interesting old findings simply seem to have been forgotten.

Diet-induced obesity, a problem of epidemic proportion in the United States and worldwide, may impart insulin resistance through activation of IKKβ/NF-κB signaling. Numerous *in vitro* (biochemical, cell biology) and *in vivo* (transgenics, knock-outs, genomic, pharmacologic) studies identify the IKKβ/NF-κB pathway as the molecular target for salicylates, and demonstrate a potential role for salicylates as agents for treating T2D and insulin resistance (Fig. 6 and ***Sections C1 and C2***) [8, 9, 10, 11, 15, 38].

1.3.5 Preliminary Results Using Salsalate

We have conducted three small trials in patients with T2D to test the efficacy of salsalate in reducing glycemia and insulin resistance and to validate IKKβ/NF-κB as a new pharmacologic target for the treatment of insulin resistance. The first two open label studies evaluated two weeks of high dose (4.5 g/d) or standard dose (3.0 g/d) salsalate regimens. The higher dose showed significantly greater efficacy, although at both doses salsalate reduced fasting and post-

intravenous load glucose. Insulin plasma levels were increased with salsalate likely due to an effect on insulin clearance. Increased glucose utilization was noted during euglycemic hyperinsulinemic clamp, being ~50% and ~15% greater at the high and standard dose of salsalate respectively. Dose limiting tinnitus occurred only at the higher dose. In a third, double-masked placebo controlled trial of one-month duration, salsalate at a maximum tolerable dose, but not placebo, improved fasting and post-oral load glucose. In addition, free fatty acids levels were reduced and adiponectin increased in all treated subjects. Additional details of the third trial are summarized below.

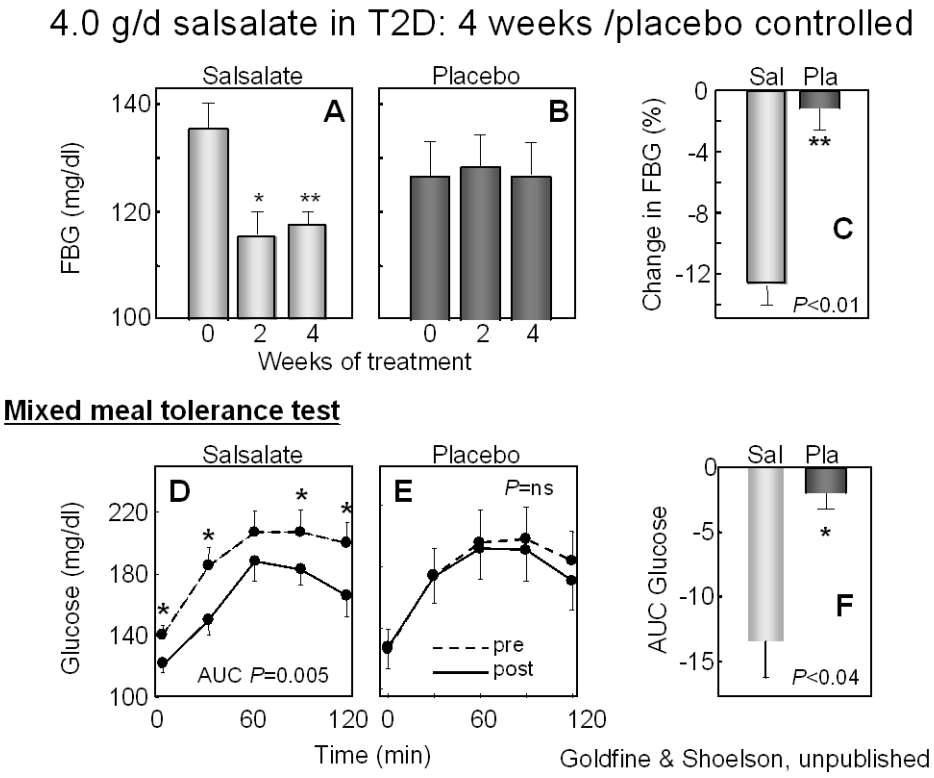


Fig. 7. Fasting blood glucose (A,B) and glucose levels measured during a mixed meal tolerance test (D,E) in patients with T2D treated with salsalate at maximum tolerated dose (A,D) or placebo (B,E). Between group comparisons of change from baseline are at the right (C,F).

Subjects in the active and treated groups were generally similar at baseline, although cholesterol levels were modestly higher in the placebo treated group (142 ± 10 *vs.* 165 ± 8 mg/dl, active *vs.* placebo). Decreases in fasting glucose were seen only in the salsalate group at 2 and 4 weeks of treatment (Fig. 7A and 7B). At 4 weeks the lowering of fasted glucose was a 20 mg/dl (14%); (136 ± 6 *vs.* 116 ± 5 mg/dl, pre *vs.* post, p=0.002). The between group comparison of change from baseline was significant (p=0.003, Fig. 7C). Likewise, salsalate but not placebo reduced the glycemic response to a mixed meal (ΔAUC glucose, p=0.005) (Fig 7D-F).

Salsalate was associated with a 33% reduction in fasting free fatty acid concentration (FFA 0.57 ± 0.07 *vs*. 0.38 ± 0.06, pre *vs* post, p=0.0009) and the effect was sustained following a mixed meal. These metabolic changes were associated with a 66% increase in plasma ΔAUC insulin levels (p=0.004), but a 19% reduction in C-peptide (ΔAUC p=0.04), consistent with reduced insulin clearance as documented previously by Hundal et al [15]. While one month is considered a short time to demonstrate changes in glycohemoglobin, a significant reduction was

demonstrated in the salsalate treated group (HbA1c 7.1 ± 0.4 *vs.* 6.8 ± 0.4, pre vs post, p=0.03) that was not significant in the placebo group (6.7 ± 0.5 *vs.* 6.5 ± 0.5, pre vs post, p=0.2).

While CRP values changed significantly in our previous small trials, this parameter was unchanged in either active or placebo treated groups. However, adiponectin increased 46% following drug (15.5 ± 1.9 *vs* 22.7 ± 2.5 mg/ml, pre *vs* post, p=0.001) with no change following placebo (11.2 ± 2.2 *vs*. 10.6 ± 2.0, pre *vs* post, p=0.3), with a highly significant change from baseline between group comparison (p=0.0003).

Five subjects tolerated salsalate 4.0 g/day dosing and three subjects developed mild tinnitus that resolved with dose reduction to 3.5 g/day. Salsalate levels were 210 ± 4 and 130 ± 3 mg/l, at 2 and 4 weeks respectively, in the treated group. While there was no change in serum creatinine or anion gap, there was an 8% increase in systolic blood pressure (p=0.01) seen only in this cohort. Blood pressure was unchanged in the other small trials using salsalate.[64]

1.3.6 Results from TINSAL-T2D Stage I

The first stage of the TINSAL-T2D study was a multicenter, randomized, placebo-controlled, dose ranging trial designed to evaluate safety and evidence of efficacy. The intent of Stage 1 was to select an optimal dose for the larger Stage 2 trial, a double-masked, placebo-controlled trial of 6 months duration to determine the efficacy and safety of salsalate to improve glycemia in patients with T2D.

A total of 277 participants were screened and 126 (46%) entered the run-in phase of the study. The 54% screen failure rate was close to the predicted screen failure rate of 50%. The principle reason for screen failure was HbA1c (108 of 149 screen fails). Of the 126 entering run-in, 108 were randomized to active or placebo medication, 27 in each group. 4 randomized subjects did not complete the study. Thus, the rate of loss during randomization was less than anticipated. Reasons included move (1 subject in placebo and 1 in 4.0 g/d dosing group), tinnitus (1 in 3.5 g/d dosing group) and loss to follow-up (1 in 4.0 g/d dosing group).

The trial demonstrated clear efficacy towards the primary endpoint of 0.5% HbA1c lowering for all three doses compared to placebo (overall p=0.009). All doses compared to placebo also improved fasting blood glucose by 25-30 mg/dL (p<0.001) and triglycerides by 30-50 mg/dL (p=0.005). Consistent with these data, mild hypoglycemic events were significantly greater in the treatment groups. The three doses were similar with respect to efficacy, with only hints of greater efficacy at that the highest dose.

Salsalate was well tolerated, with >94% compliance by pill count throughout the trial. The side effect of tinnitus, and dose reductions for tinnitus, were less than expected. Additionally, certain quality of life measures from the SF36 also improved, including physical and social functions.

However, there was evidence for potential increases in blood pressure and urinary microalbumin/creatinine ratio (MCR). Changes of 3.5-4.5 mm Hg systolic and 1.6-2.5 mm Hg diastolic were seen, although these were not statistically significant (p=0.40 and p=0.68, respectively). These will warrant confirmation and further evaluation. While fluctuations in MCR levels are common in the clinic, we also note that the MCR values increase in a subset of patients treated with salsalate, with dose-dependent increases in MCR across groups (median increases of 3, 4, 11 and 19 µg/mg for placebo vs. 3.0, 3.5 and 4.0 g doses, respectively, p=0.002). This was not associated with a reduction in renal function, as assessed by eGFR, or by either cystatin C or the cystatin C estimate of glomerular function, both of which showed significant within group improvements from baseline at each dose.

There were also small changes in LDL and HDL cholesterol, with both increasing proportionately such that there was no change in their ratio (p=0.55).

In conclusion, salsalate certainly appears to improve glycemia in the 3-month TINSAL-T2D stage 1 trial. The DSMB convened on July 24th to review the results of the TINSAL-T2D stage 1 trial data. Approval for stage 2 of the trial was provided for any of the three doses studied, at the discretion of the steering committee. The DSMB was unanimous in its support for a longer trial preferably 12 months. Also with increased duration, there might be greater separation in HbA1c between active and placebo due to upward drift in the placebo group over time. Furthermore, it was noted by the steering committee and the DSMB that a dose of 4.0 g/d would allow exploration of the maximum efficacy while gaining a greater understanding of any potential effects on LDL, blood pressure and albumin excretion. However, safety criteria alone would drive the choice of dose, as with most all pharmaceutical products. Additional monitoring of blood pressure and renal function for assessment of the safety-risk profile will be provided in the second stage of the trial. Approval for stage 2 of the trial was provided for any of the three doses studied, at the discretion of the steering committee.

In addition, the stage 1 trial results were discussed with the FDA. Again, approval for stage 2 of the trial was provided for any of the three doses studied, at the discretion of the steering committee.

After careful consideration of the safety and efficacy data from stage 1 of the trial the steering committee recommends further evaluation of the 3.5 g/d dose.

1.3.7 Safety of Salsalate

There is extensive experience with long-term human use of salsalate. The therapeutic to toxic ratio is low as mild toxic manifestations of salicylates may be present when plasma levels are in the therapeutic range of 200-300 mg/l. In a study of patients with rheumatoid arthritis, after 12 weeks of therapy at a tolerable dose, the respective median and mean salsalate dose was 3 and 3.3g/day (range 2-4.5g/day) [65]. When salicylates are given in large amounts, as in the treatment of arthritis, the pharmacokinetics of salicylate are altered, resulting in a prolongation of the half-life. This makes it possible to administer a salicylate on a twice-a-day dosing basis, with doses large enough to sustain mean serum levels of 150-250 mg/l. While the gastric intolerance associated with aspirin, makes it clinically inappropriate to administer that drug in a twice-daily regimen, salsalate, with fewer serious gastrointestinal side effects than aspirin [65], lends itself well to such a twice-daily regimen.

In clinical trials the following reversible adverse experiences were most common: tinnitus, nausea, hearing impairment, rash and vertigo. Tinnitus or reversible hearing impairment are often used to guide therapy. Severe toxicity may result when plasma levels are in excess of 400 mg/l. Several studies note less gastrointestinal bleeding with salsalate than with aspirin.

Salsalate undergoes particulate dispersion in the stomach, but as it is insoluble in acid it passes undissolved into the small intestine, thus sparing the gastric mucosa direct contact with chemically active salicylic acid. On the other hand, cationic salts such as magnesium salicylate preparations are well dissolved in the stomach and are hydrolyzed in residence there, releasing free salicylic, which is more likely to cause gastric distress.

Several studies have demonstrated that salsalate causes no greater intestinal occult blood loss than placebo [66, 67, 68, 69, 70], in contrast to aspirin, which causes up to ten times as much as placebo [666, 71, 72, 69]. Other NSAIDs cause greater occult blood loss than placebo [71, 72].

Salsalate has no suppressive effect on renal prostaglandin production, measured by plasma rennin activity, while aspirin and naproxen showed definite suppression (34% and 49%, respectively) [73].

Clinical efficacy studies show that 7-10% of patients receiving salsalate discontinue therapy because of tinnitus or hearing disorders [65,74]. Cessation of salsalate treatment usually results in prompts recovery of hearing, and disappearance of tinnitus. Some individuals, usually elderly, seem to have an idiosyncratic sensitivity to salicylate wherein the ototoxixcity is expressed following as few as one or two doses of salsalate. Once again, this subjective hearing loss generally reverses promptly following discontinuation.

CHAPTER 2: OUTCOMES AND OBJECTIVES

2.1 PRIMARY OUTCOMES

The primary outcome for the TINSAL-T2D study is change in HbA1c level from baseline to week 48 in the intent-to-treat (ITT) population.

2.2 SECONDARY OUTCOMES AND ANALYSES

Important secondary outcomes include:

- Change from baseline to either 48 weeks or last HbA1c measurement prior to rescue therapy
- Trends in HbA1c over time
- Change from baseline and trends in fasting glucose over time
- Response rates for reduction in fasting glucose of ≥20 mg/dl, a reduction in HbA1c of ≥0.5%, and a reduction in HbA1c of ≥0.8%
- Change in lipids (low-density lipoprotein cholesterol [LDL-C], non-high-density lipoprotein cholesterol [non-HDL-C], triglycerides [TG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL C], TC/HDL-C ratio, and LDL-C/HDL-C ratio)
- Change in insulin sensitivity (insulin, C-peptide, homeostasis model [HOMA] index)
- Response rates for exceeding hyperglycemic targets between active and placebo treated groups
- Need for rescue therapy
- Need for discontinuation of study medication
- Response rates in patients initially treated with lifestyle modification, insulin secretagogue, metformin or combination therapy
- Response rates for a reduction in HbA1c for obese vs non-obese participants
- Response rates by baseline CRP
- Safety and tolerability of salsalate compared to placebo
- Change in body weight
- Changes in WBC and differential, high-sensitivity C reactive protein (hsCRP), other inflammatory markers (IL-6, IL-1β, TNF-α, PAI-1, adiponectin, serum amyloid A, the adhesion molecules ICAM and VCAM), lipoproteins (apolipoprotein A-I [apo A-I] and apolipoprotein B [apo B]), and free fatty acids [FFA] (plasma will be stored for batch measurement of these markers)
- Change in liver function [ALT, AST, GGT], stratified according to baseline liver function, as an indices of NASH and to assess potential improvements or decline.

2.3 ANCILLARY STUDIES

2.3.1 Plasma Storage

Among consenting participants, blood specimens and immortalized lymphocytes will be stored for future genetic (DNA and RNA) studies relevant to inflammatory and metabolic pathways.

2.3.2 Vascular Ultrasonography of the Brachial Artery to Measure Vascular Function (Flow Mediated Vasodilation)

In a subset of about 9 clinics (Columbia University; Emory University School of Medicine; Indiana University; Joslin Diabetes Center; MedStar Research Institute; Tulane University Health Sciences Center; University of Nebraska Medical Center; University of California, San Diego; University of Michigan) vascular ultrasonography of the brachial artery will be used to determine whether (salsalate) targeting inflammation improves endothelium-dependent vasodilation in patients with type 2 diabetes. All subjects in Stage II of the trial at the participating sites will be eligible to participate in this ancillary study. Measurements of flow-mediated, endothelium dependent vasodilation, and endothelial independent vasodilation will be assessed following sublingual administration of nitroglycerin, each determined by ultrasonography of the brachial artery, at baseline, 3-month, and 6-month time points. In addition, change in endothelial function will be assessed as a function of change in glycemia and in circulating inflammatory mediators including CRP, IL-6, TNF-α, CD-40 ligand as well as nitrotyrosine, free fatty acids and adiponectin. For specifics regarding the vascular ultrasonography substudy refer to the FMD protocol or Manual of Procedures (MOP).

CHAPTER 3: RECRUITMENT, SCREENING, ENROLLMENT, AND RUN-IN

3.1 RECRUITMENT

Participants will be recruited over a 1.5 – 2 year period. Recruitment techniques include notices placed on bulletin boards at the medical centers, newspaper and radio advertisements, and referral from colleagues. Prescreening of electronic and clinical medical records is strongly encouraged to reduce available screen failure rates. Selection is based solely on the participant's ability to meet the criteria stated in the protocol and his/her willingness to participate in the study.

Several centers have been specifically selected from geographical areas with higher representation of minority persons in an effort to have adequate minority recruitment in the trial as a whole.

3.1.1 Patient Compensation

Participants are compensated for each visit with a bonus for completion of the final visit, for time lost from work and inconvenience of participation. The maximum individual compensation is $400/participant. The study group may consider compensation as needed for travel and other expenses (such as parking) incurred as a result of participating in the study. Items of nominal value are distributed at visits to thank patients for their participation.

Additional compensation is available for participation in the sub-studies. All plans for compensation are approved by the local IRB.

3.2 ELIGIBILITY CRITERIA

Inclusion:

1. Type 2 diabetes on diet and exercise therapy or monotherapy with metformin, insulin secretagogue (including SFU, non-SFU, and dipeptidyl peptidase IV (DPP-4) inhibitors), alpha-glucosidase inhibitors, or bile acid sequestrants (dosed once per day such that study drug can be administered ≥ 4 hours prior to sequestrant); or a combination of up to three* of these at maximal dose (see Appendix). Dosing must be stable for 8 weeks prior to screening. Participant must have been diagnosed with T2D at least 8 weeks before screening.
2. FPG ≤ 225 mg/dL and HbA1c≥7% and ≤ 9.5% at screening.
3. Age ≥18 and <75
4. Women of childbearing potential agree to use an appropriate contraceptive method (hormonal, IUD, or diaphragm)

*Note: Combination preparations are to be counted by individual agents within the respective combinations.

Exclusion:

1. No prior participation in Stage I of TINSAL-T2D; *exception*: a participant who failed screening for HbA1c in Stage I will be allowed to re-screen for Stage II.
2. Type 1 diabetes and/or history of ketoacidosis determined by medical history
3. History of severe diabetic neuropathy including autonomic neuropathy, gastroporesis or lower limb ulceration or amputation
4. History of long-term therapy with insulin (>30 days) within the last year
5. Therapy with rosiglitazone (Avandia) or pioglitazone (Actos), alone or in combination in the previous 6 months; or extendin-4 (Byetta), alone or in combination in the previous 3 months
6. Pregnancy or lactation

7. Patients requiring oral corticosteroids within 3 months or recurrent continuous oral corticosteroid treatment (more than 2 weeks)
8. Use of weight loss drugs [e.g., Xenical (orlistat), Meridia (sibutramine), Acutrim (phenylpropanol-amine), or similar over-the-counter medications] within 3 months of screening or intentional weight loss of ≥ 10 lbs in the previous 6 months
9. Surgery within 30 days prior to screening
10. 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, eGFR (ml/min/1.73m2)=186 x $(S_{cr})^{-1.154}$ x $(age)^{-0.203}$ x (0.742 if female) x (1.210 if African American)] †
11. History of chronic liver disease including hepatitis B or C
12. History of peptic ulcer or endoscopy demonstrated gastritis
13. History of acquired immune deficiency syndrome or human immunodeficiency virus (HIV)
14. History of malignancy, except participants who have been disease-free for greater than 5 years, or whose only malignancy has been basal or squamous cell skin carcinoma
15. New York Heart Association Class III or IV cardiac status or hospitalization for congestive heart failure
16. History of unstable angina, myocardial infarction, cerebrovascular accident, transient ischemic attack or any revascularization within 6 months
17. Uncontrolled hypertension (defined as systolic blood pressure >150 mmHg or diastolic blood pressure >95 mmHg on three or more assessments on more than one day). If on blood pressure medications, dosing should be stable for 2 weeks prior to randomization.
18. History of drug or alcohol abuse within 5 years, or current weekly alcohol consumption >10 units/week (1 unit = 1 beer, 1 glass of wine, 1 mixed DCCktail containing 1 ounce of alcohol)
19. Hemoglobin <12 g/dL (males), <10 g/dL (females) at screening*
20. Platelets <100,000 cu mm at screening
21. AST (SGOT) >2.50 x ULN or ALT (SGPT) >2.50 x ULN at screening
22. Total Bilirubin >1.50 x ULN at screening
23. Triglycerides (TG) >500 mg/dL at screening
24. Poor mental function or any other reason to expect patient difficulty in complying with the requirements of the study
25. Previous allergy to aspirin
26. Chronic or continuous use (daily for more than 7 days) of nonsteroidal anti-inflammatory drugs within the preceding 2 months
27. Use of warfarin (Coumadin), clopidogrel (Plavix), dipyridamole (Persantine), heparin or other anticoagulants
28. Use of probenecid (Benemid, Probalan), sulfinpyrazone (Anturane) or other uricosuric agents
29. Macroalbuminuria, defined as spot urine protein >300 mcg/mg Cr at screening
30. Pre-existing chronic tinnitus

† In some patients eGFR by the MDRD equation underestimates renal function. If a 24 hour urine creatinine clearance that demonstrates adequate function is available the eGFR value from the creatinine clearance will be used for eligibility. The 24 hour urine for creatinine clearance is more precise then the estimated eGFR by the equation.
*Ethnic differences in normal range hemoglobin may be considered such that persons with levels below 2SD of the normative range are excluded. [75]

3.3 INFORMED CONSENT

Consent is obtained in a quiet setting prior to the initiation of any study procedures. The patient is given opportunity to delay consent until he/she has taken adequate time to read and

understand the written consent and discuss the study with family and/or other physicians outside the clinic setting. Patients are not consented in writing until they demonstrate adequate understanding of all aspects of the study and consent process. Asking the patient to explain the study in his own words establishes his understanding. A copy of the consent form is given to the patient. The signed consent form remains in the patient's study files at the clinical center.

3.4 SCREENING PROCEDURES

The screening visit (Visit 1) occurs at Week -5. After explaining the nature of this study in detail, written informed consent is obtained prior to any study procedure being performed at the participating sites' outpatient facilities. Participants are screened for inclusion and exclusion criteria. The screening visit includes a medical history, and routine lab panel (Chemistry profile including Na, K, Cl, Bicarb, BUN, Cr, ALT, AST, LDH, GGT, Alk phos, Total Bili, Ca, Phos, Uric Acid, Total Pro, and Alb; lipid panel; TSH; glucose; HbA1c; CBC; PT/PTT; UA and β-HCG in females).

3.5 RUN-IN

Eligible patients return 1 week after the screening visit for Visit 2 (Week -4) for physical examination, weight, vital signs, EKG, stool guaiac, and to initiate treatment with single-blind placebo. Patients take placebo tablets three times daily. Participants are instructed to continue taking other medications exactly as they had prior to this study, including sulfonylurea (SFU)/Insulin secretagogue, metformin, or α-glucosidase inhibitor. All participants are counseled to follow an ADA-accepted diet, and to be physically active. At the initiation of the single-mask lead in period, home blood glucose meters and reagent strips (Lifescan, One Touch Ultra) are provided to the patients, and they are instructed in correct use. The patient is asked to test blood glucose using the meter provided by the study (One Touch Ultra 2) while at the clinic to verify knowledge of how to use the meter. Verification of technique is performed by taking a blood glucose level readings by the patient using his or her meter and simultaneously testing the patient's blood with another clinic meter (One Touch Ultra 2, calibrated by the clinical site to standard solutions). A goal of ±10% variability is acceptable between the meters. Participants are instructed to check fasting blood glucose each morning and to call the clinic if the level exceeds 250 mg/dl on 3 occasions during a week.

The 4 week run-in provides a period for adapting diabetes management to the increased scrutiny of a clinical trial. Improvements in glycemic control are expected during the run-in period, likely due in part to increased compliance with ongoing treatment regimens. This single-masked interval also provides an opportunity to begin to assess study drug compliance. Participants with less than 80% compliance by pill count at the completion of the placebo run-in are not eligible for continued participation, to minimize the negative impact that a lack of medication compliance would have towards blunting or skewing the study results. If a participant's adherence rate is less than 80% after four weeks due to a significant life event, run-in may be extended by two weeks, at the discretion of the investigator. In this case, the participant must have 90% adherence during the final two weeks of run-in to be considered eligible.

3.6 RANDOMIZATION AND BASELINE VISIT

Permuted block randomization, stratified by clinical site, is used to allocate patients to achieve comparability among treatment groups with respect to known and unknown prognostic factors. Stratification is only by clinic, and adjustment for any imbalances across groups on other

characteristics is made in the analysis. With permuted blocks, excessive stratification increases the likelihood of unfilled blocks, thus increasing the chance of imbalances. Patients are randomized to either placebo or 3.5 g/d dose of salsalate using a 1:1 randomization scheme for each clinic. Sample sizes across the treatment arms remain relatively equivalent as the trial progresses, but the next treatment assignment cannot be anticipated. The clinical center coordinator uses the study website to randomize the patient and receive a Drug ID for obtaining the appropriate blinded medication dose.

Participants continuing to the baseline visit present to the research facility after an overnight fast. Vital signs and weight are recorded as part of the brief physical exam. Blood pressure is assessed with the participant resting quietly for 5 minutes. History is collected and height is measured (at Visit 3 only). Blood samples are collected for participant safety and outcomes assessment. Blood samples are obtained and stored for potential analysis at a later date for vascular and inflammatory markers (hsCRP, IL-6, IL-1β, TNF-α, PAI-1, resistin, adiponectin, serum amyloid A, the adhesion molecules ICAM and VCAM, serum nitrotyrosine as an assessment of oxidative stress among other biomarkers). The Quality of Life (QOL) survey SF36 and a rheumatological questionnaire is administered. Study drugs are dispensed after the baseline evaluation.

CHAPTER 4: POST-RANDOMIZATION TREATMENT PERIOD

4.1 STUDY DRUG AND DOSE

In Stage II, 282 patients are randomly assigned in a 1:1 ratio for a 48-week treatment period to either placebo or salsalate initiated at 3.0 g/d (2 x 500 mg, po, three times daily) and escalated at week 2 to 3.5 g/d (3 x 500 mg qAM; 2 x 500 mg at lunch and supper), as tolerated. Participants initiate therapy at 6 tablets daily and increase to 7 tablets daily after two weeks, as tolerated. Participants on active or placebo treatment are instructed to take 7 tablets (each tablet is either 500 mg salsalate or placebo) daily, divided, following the regimen: three in morning, two at lunch and two at dinner (7 daily). Participants experiencing minor or expected side effects (specifically tinnitus) are instructed to skip the next dose and then reduce dosing by one tablet. If side effects persist, continue to reduce by one tablet. If side effects subside, one attempt may be made to escalate back to maximal dose. As during the placebo lead-in phase, participants are instructed to continue taking stable doses of other medications, including SFU or metformin, and to continue home glucose monitoring.

Placebo tablets, which look identical to the active tablets, are dosed similarly to the active drug. Caraco Pharmaceutical Laboratories, Detroit, MI, prepares placebo and active tablets. As during the placebo lead-in phase, participants are instructed to continue taking other medications, including SFU/insulin secretagogue, metformin, α-glucosidase inhibitor, or bile acid sequestrant and to continue home glucose monitoring.

Physician and participants are not masked to the number of tablets administered daily, but are masked to active or placebo.

4.2 VISIT SCHEDULE

Participants are seen at weeks 0, 4, 8, 12, 16, 24, 36, 48 to assess safety, compliance and response to treatment. There is a phone follow-up at 2 weeks (Visit 4) for dose adjustment as tolerated. Post treatment follow-up takes place at week 50 (Visit 12) and week 56 (Visit 13). Participants come to the clinic after an overnight fast. Weight and vital signs are recorded. Blood samples are collected for participant safety and outcomes assessment. Home glucose monitoring diaries are reviewed and meter data is downloaded for safety purposes, but the data is not collected by the coordinating center. Participants are asked about adverse events, pills are counted to assess compliance, and additional supplies of study drugs are provided. QOL surveys and the rheumatological questionnaire are repeated at week 24 and 48. Ambulatory blood pressure measurements and 24 hour urine collection are done at selected sites at weeks 0,24, and 48. All participants receive a post treatment follow-up clinic visit which is scheduled 2 weeks after the final visit to obtain their interim medical history, vital signs, chem 7 (electrolytes, bun/cr and glucose), and lipid and urine microalbumin measurements.

4.3 ADJUNCT CARE

Medical management of the patient is the responsibility of the participant's primary care physician (PCP). However, TINSAL-T2D investigators will make recommendations to the PCP regarding dosing of diabetes and concomitant medication, particularly if the participant meets criteria for initiation of rescue therapy (see Section 4.5), or for severe or recurrent mild hypoglycemia.

Participants taking low-dose aspirin (81-325 mg) may continue to do so for antithrombotic prophylaxis (80-100 mg represents only 1.5-2.5% of the study drug dose in terms of molar equivalents of salicylate).

Changes in the dosing of other concomitant medications are avoided as much as possible throughout the trial according to best standards of clinical practice. This is especially important over the first six months of the trial to assess drug effect. The participant and their personal physician are advised that, if at all possible, dosages of diabetes, lipid lowering and blood pressure medications should remain stable for the first 6 months (24 weeks) and then adjusted based on good clinical practice.

4.3.1 Treatment of Dyslipidemia

Use of statins, fibric acid derivatives or other medication to treat dyslipidemia is at the discretion of the managing physician with the intent being that these agents not be initiated or dose adjusted during the first 6 months (24 weeks) unless clinically mandated. At any time in the trial following the 24 week visit, if a patient's LDL exceeds 200 mg/dl or 160 mg/dl with an increase of 40 mg/dl, a letter is sent the site investigator noting the reason for the alert to be shared with the participant and the primary care provider who evaluates and may opt to observe or adjusts medications as warranted.

4.3.2 Treatment of Hypertension

Use of angiotension converting enzyme inhibitors (ACEI) or receptor blockers (ARB), beta blockers, calcium channel blockers and/or diuretics and other medication to treat hypertension will be at the discretion of the managing physician with the intent being that these agents not be initiated or dose adjusted during the first 6 months (24 weeks) of the study period unless clinically mandated. At any time in the trial following the 24 week visit, if a participant's blood pressure exceeds 140 systolic or 90 diastolic on two consecutive visits (including the visit at week 16) or an increase in systolic or diastolic by more than 10 mm Hg s from baseline, or exceeds 170 systolic or 95 diastolic on the average of the multiple readings at one visit, a letter is sent to the participant and the primary care provider who evaluates and may opt to observe or adjusts hypertension medication as needed.

4.4 WITHDRAWAL OF PARTICIPANT CONSENT AND DISCONTINUATION OF STUDY DRUG

Study therapy should be discontinued for any of the following reasons:

a. Subject decision to withdraw consent for study participation
b. Evidence of allergy to administered products
c. Acute change in renal function (eGFR decline >50%, validated)
d. Intolerable adverse event as judged by investigator and participant

If the study drug is discontinued, unless the subject withdraws consent, the subject is followed for the full treatment period (48 weeks) and all data is collected as scheduled. Attempts should be made to schedule an early end of study assessment in the case of study drug discontinuation.

4.5 RESCUE THERAPY

Subjects with signs and symptoms of hyperglycemia (excessive thirst, urination or weight loss) or 3 home glucose levels greater than 250 mg/dl in one week will be instructed to call their investigator. An interim appointment is scheduled (fasting) within one week for additional history, examination, and fasting laboratory assessment. Confirmation of fasting glucose >250 will warrant medication adjustment. For subjects in whom there are no symptoms of hyperglycemia and no fasted home glucose monitoring levels >250, but for whom the fasting glucose on scheduled visit is found to be >250 or HbA1c ≥10.5% will have the laboratory profile repeated within 2 weeks at an interim visit. If similar hyperglycemia is detected on repeat evaluation medication adjustment will be warranted.

At any time in the trial following the 24 week visit, if a patient's fasting blood sugar exceeds 200 mg/dl or HbA1c exceeds 9.5%, a letter is sent to the participant and the primary care provider who evaluates and may opt to observe or adjusts hypertension medication as needed.

If medication adjustment is warranted, the investigator makes the following recommendation to the participant's PCP regarding rescue therapy. For participants not on maximal dose metformin and sulfonylurea, first maximize metformin and sulfonylurea combination therapy as follows: For persons on lifestyle or sulfonylurea therapy, metformin is added. For persons entering the trial on submaximal metformin, metformin dosing is titrated. For persons already receiving maximal dose metformin, glipizide is added. If or when metformin and sulfonylurea combination therapy is maximal and hyperglycemia adjustment is warranted, recommend addition of a third agent, either another oral agent or NPH insulin 10 Units SQ q hs at the discretion of PCP. If three oral agents have been maximized then insulin should be added. Dosage of insulin should be titrated to current practice medical goals by the investigator and /or clinician. Of note, salsalate has not been specifically studied in combination with insulin. In view of the ACCORD trial results and lack of data on interaction of the study drug with insulin, we do not recommend aggressive titration of insulin. Participants continue to be followed through the end of the trial and all medication adjustments are noted (76).

CHAPTER 5: RESEARCH PROCEDURES AND APPROACH

5.1 DATA COLLECTION

The table below lists the schedule of data collection, measurements, and assessments.

Schedule of Assessments

	Screen-ing	Run -in	Randomization								F/U visit		
Visit #	**1**	**2**	**3**	**4**[A]	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Week	**-5**	**-4**	**0**	**2**	**4**	**8**	**12**	**16**	**24**	**36**	**48**	**50**	**56**
Informed Consent	x												
Medical History	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical Exam (full)		x[B]									x		
Physical Exam (brief) [B]			x[B]	x	x	x	x	x	x	x		x	x
EKG		x									x		
Questionnaires			x						x		x		
Con Meds	x[C]	x	x	x	x	x	x	x	x	x	x	x	x
Adverse Events	x[D]	x[D]	x[D]	x	x	x	x	x	x	x	x	x	x
Drug Dispensing - new bottle(s)		x	x			x		x	x[I]	x[I]			
Laboratory Tests													
Chem Panel[E]	x		x				x		x		x		
Chem 7[F]												x	x
Pregnancy Testing	x												
TSH	x										x		
Lipid Panel[G]	x		x				x	x	x	x	x	x	
Glucose	x		x			x	x	x	x	x	x		
HbA1c	x		x			x	x	x	x	x	x		x
Salicylate							x		x	x	x		
CBC	x		x				x		x	x	x		
PT/PTT	x												
UA	x		x				x		x		x		
Microalbumin/Creatinine	x		x				x		x	x	x	x	x
Samples to Joslin											x		
Blood			x				x		x	x	x		x
Urine			x				x		x	x	x		x
Samples to Cedar			x										
24 Hour Urine[H]			x						x		x		
Ambulatory BP Measurement[H]			x						x		x		
Home BG Monitoring Instructions		x											

A. Phone Visit
B. Brief physical exam includes: HR, BP & weight collection (Note: height is only collected at Visit 3)
C. At screening, on diabetes medications and medications excluded for eligibility needed
D. These are technically not adverse events because they are pre-treatment
E. Chemistry panel consists of: NA,K,CL,Bicarb,Bun,Cr, ALT,AST,LDH,GGT,Alk phos.Total Bili,Ca,Phos,Uric Acid,Total Pro, Alb
F. Electrolytes, Bun/Cr, and Glucose
G. Lipid panel consists of: Chol, Trig, HDL-C, Calc LDL-C
H. At selected sites
I. Two bottles are dispensed at these visits

Dark Gray shading indicates a blinded specimen

5.2 CONFIDENTIALITY

All data are labeled with the study ID, including forms and specimens. All data transferred to the coordinating center for accumulation in the central database identify the patient only with the study ID and acrostic (nickname). The coordinating center does not receive any personal identifiers.

Each field center maintains a file on each patient that includes personal identifiers, linking name and contact information to the study ID. These data are not entered into the study data management system. Patient files are kept in secure locations and the clinical center is responsible for taking every other reasonable measure (those set by the state, the site, and the study) to ensure and maintain record confidentiality and patient privacy.

However, subjects are also to be made aware that confidentiality cannot be ensured. Each site adheres as required by law to regulatory oversight by federal and state agencies that have authority over the conduct of clinical research such as the Department of Health and Human Services, the Food and Drug Administration, the National Institutes of Health, the Office of Human Research Protections, the Department of Social Services and the Data Safety Monitoring Board.

CHAPTER 6: SAFETY AND MONITORING

6.1 DATA SAFETY MONITORING BOARD (DSMB)

A Data Safety Monitoring Board consisting of appropriately qualified independent experts is appointed by the NIDDK to provide review of data on patient safety and study progress. The purpose of the DSMB is to assure independent review as to whether study patients are exposed to unreasonable risk because of study participation, and to monitor study progress and integrity. Monitoring did include the review of Steering Committee recommendations regarding dose selection during Stage I of the study. DSMB members are chosen by NIDDK, and the DSMB determines a report format and reporting frequency before the start of data collection. The coordinating center provides reports on adverse events to the DSMB, including summary tabulations and narrative summaries on individual events.

The purpose of safety reports is to present the DSMB with information regarding adverse events experienced by study patients as a result of undergoing the study procedures. Clinical centers report adverse events to the coordinating center in a timely fashion, including a narrative summary of the event as well as indication of the duration, perceived relationship to the study procedures, and resolution. The coordinating center summarizes and reports adverse events to the DSMB. Severe or unexpected adverse events are reported immediately to the NIDDK and DSMB.

A summary of DSMB deliberations is prepared by NIDDK and distributed to the clinical centers to submit to their IRB.

6.2 SAFETY MONITORING AND RISK MANAGEMENT

Participants have a complete medical history at the screening evaluation and interim history and physical examination at visit two (prior to dispensing the drug/placebo), scheduled interim visits and additional visit(s) prior to addition of alternate medication for hyperglycemia. Vital signs are assessed at each visit. Laboratory safety measures (chemistry and hematology) are measured at screening, prior to randomization and every 3 months during drug/placebo administration. Adverse events are assessed at each visit following dispensing of drug/placebo. As described in Section 4.5, participants are assessed for hyperglycemia and medications are adjusted accordingly. End of treatment assessment is performed at week 48 for Stage II. All participants will return for a visit at week 50 which is two weeks after discontinuing study drug to follow the time course of any changes noted during study drug administration. In addition a subset of participants will return for a visit at study week 56 which is eight weeks after discontinuing study drug. Those patients include those who meet any of the following criteria:

1. Have persistent tinnitus at week 50 and did not have tinnitus during screening or the run-in period,
2. Have a MCR value > 30 mcg/mgCr at week 48
3. Have an increase in SBP or DBP of more than 10 mm Hg at both week 48 and week 50 compared with value at baseline.
4. Have BP > 150/ 90 despite treatment

For the follow-up of persistent tinnitus, the visit may be conducted by telephone.
In routine clinical practice, patients are typically seen at ≥3 month intervals, while participants enrolled in the clinical trial have more frequent scheduled evaluations allowing more prompt intervention than might occur in clinical practice.

6.2.1 Risk of Hypoglycemia

In contrast to insulin secretagogues, insulin-sensitizing agents are less likely to induce hypoglycemia. Insulin sensitizers including metformin and TZDs have been safely administered to persons with modest elevations of blood sugar, for example participants with impaired glucose tolerance but no overt diabetes. Salsalate has been administered for almost over 50 years for pain management without notable hypoglycemia, including persons without diabetes. Hypoglycemia was not seen in the preliminary studies of overweight patients without diabetes. However, mild hypoglycemia was more common in the TINSAL-T2D stage 1 studies in the groups using salsalate compared to placebo. Not all cases of mild hypoglycemia were documented by home glucose monitoring. Hypoglycemia, which was documented by home glucose monitoring, occurred primarily in patients also using sulfonylurea based therapy. One episode of severe hypoglycemia occurred in a salsalate treated patient, although this was also associated with a missed meal. Hypoglycemia has been reported with salicylate overdose most notably in certain infirmed pediatric populations [77].

6.2.1.1 HYPOGLYCEMIA MANAGEMENT

Although salsalate has been administered for years for pain management without notable hypoglycemia, concomitant use of salsalate and an insulin secretagogue might result in hypoglycemia. Participants are instructed in the signs, symptoms, and treatment for hypoglycemia, and are told to check blood glucose using the study-provided home glucose meter if hypoglycemia is suspected, and to call and inform the Study Investigator of such symptoms. Participants should attempt to measure and document their blood sugars as close to the time the symptoms occur as possible. Participants should be instructed to notify the study site within 1 working day if any value less than 60 mg/dl is noted. Prompt dose reduction of concurrent diabetes therapy should be initiated according to the algorithms noted below and the patient's care provider is notified. If the participant is not taking SFU or metformin, the dose of salsalate is decreased or discontinued.

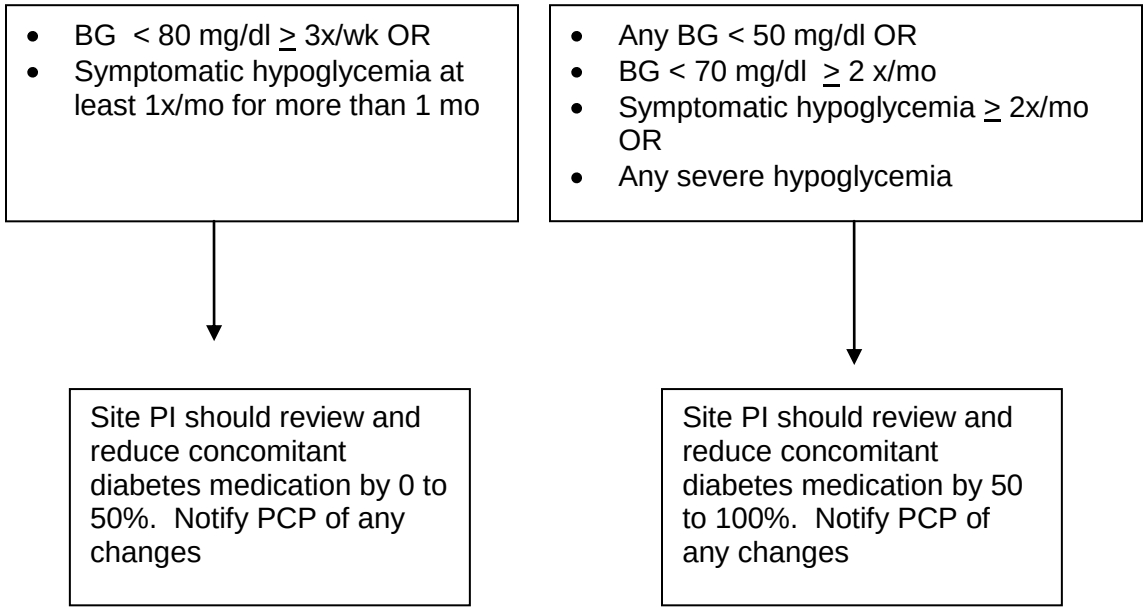
Severe hypoglycemia is defined as any episode of documented or clinically suspected hypoglycemia that:

- Needs to be treated with glucagon;
- needs assistance from a third party to resolve the event;
- and/or results in the loss of consciousness or seizure.

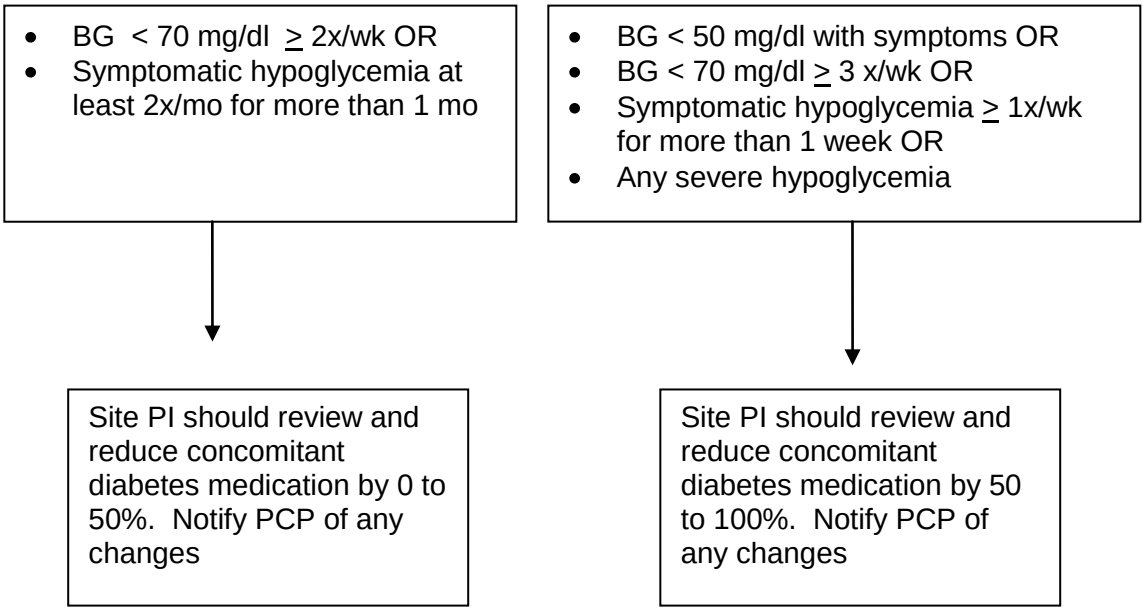
Mild hypoglycemia is defined as self-reported transient symptoms such as lightheadedness, tremor, shaking, sweating, tingling, blurry vision, trouble concentrating etc., or blood sugars less than 70 mg/dl that are self-treated by ingestion of carbohydrates and resolved on their own or within 15 minutes of such self-treatment.

The TINSAL T2D study staff should notify the participant's PCP that they may make changes in concomitant diabetic medications to prevent hypoglycemia when they are enrolled into the trial. Medication reduction for participants will be conducted by TINSAL T2D site investigator medical staff and communicated to both the participant and the primary care provider. The protocol for reductions are described below. Plan A should be used for newly randomized participants for the first 3 months post randomization. Plan B should be used after that point.

Hypoglycemia Management Algorithm Plan A
(Weeks 0 to 24 of randomized period)



Hypoglycemia Management Algorithm Plan B
(Weeks 24 to 56 of randomized period)



BG = blood glucose

PCP = primary care provider

These schemes are intended as a general guide. A TINSAL T2D physician or nurse should use clinical judgment to modify these recommendations for specific circumstances of any participant. Reduction in sulfonylurea is more likely to lead to resolution of hypoglycemia rather than reduction of metformin.

If the reason for hypoglycemia is identified and correctable (eg missed meal) and dose reduction leads to loss of diabetes control, the original dose of concomitant medication may be resumed after a few weeks, with appropriate education of the patient about avoiding similar situations.

6.2.2 Risk of Hyperglycemia

While we anticipate that glycemia will be reduced for subjects treated with salsalate there is a risk that hyperglycemia will worsen over the course of the study. This could occur if dosing were too low, for persons with atypical forms of diabetes (ie mitochondrial diabetes) who may not respond to the medication, or for subjects receiving placebo. Subjects are provided meters and test strips for home glucose monitoring, and instructed in correct use. They are requested to monitor their glucoses fasting, daily. Subjects with signs and symptoms of hyperglycemia or 3 home glucose levels greater than 250 in one week will be instructed to call their investigator. Additional procedures for monitoring and medication adjustment are described in Section 4.5 (Rescue Therapy).

6.2.3 Risk of Gastrointestinal Distress or Bleeding

In contrast to aspirin, salsalate is not soluble in the acid environment of the stomach, but is hydrolyzed into two molecules of salicylic acid and absorbed in the alkaline environment of the small intestine. This reduces the incidence of gastrointestinal irritation and intolerance. Although GI side effects have been reported with salsalate, they tend to occur in people with pre-existing GI disease [78]. Salsalate causes no greater intestinal occult blood than placebo [79, 80] and does not inhibit platelet aggregation [81]. Salsalate neither prolongs prothrombin nor bleeding times [82]. Rectal examination and stool guaiac will be performed prior to study entry. Hemoglobin and hematocrit are monitored throughout the study and drop in levels will lead to prompt evaluation. Dose escalation, rather than starting at the highest dose of salsalate, should reduce side effects. Pill counts and drop out rates are used to assess compliance and tolerability.

6.2.4 Risk of Bronchospasm

Salsalate is less likely than aspirin to induce bronchospasm. 3M, the previous manufacturer of salsalate and Caraco, the current manufacturer, report infrequent occurrence of bronchospasm with salsalate (personal communications). However, persons with aspirin intolerance are excluded from study participation.

6.2.5 Altered Renal, Liver, or Hematological Function

Salsalate has been prescribed extensively without untoward effects in large populations of arthritis and rheumatology patients. There is no known contraindication to use in patients with diabetes. Having been used in hundreds of thousands of patients for years at a time, with at least 10% of these ageing patients with arthritis expected to have diabetes, we can already

conclude that salsalate has been used in large numbers of type 2 diabetic patients without untoward side effects.

Hematology and chemistry profiles will be assessed at baseline and at weeks 24 and 48. Serum creatinine is monitored at weeks 12, 24 and 48, and the study drug discontinued for decreases in calculated creatinine clearance by 20mL/min, even within the normative range. Decrease is confirmed at an interim visit before drug is discontinued. Calculation of eGFR is made using the Modification of Diet in Renal Disease (MDRD) equation: eGFR ($ml/min/1.73m^2$)=186 x $(S_{cr})^{-1.154}$ x $(age)^{-0.203}$ x (0.742 if female) x (1.210 if African American) (conventional units)].

Given the great importance of early detection of declining renal function in patients with type 2 diabetes we also plan to monitor cystatin C, a nonglycosylated protein is produced at a constant rate by nucleated cells [83]. It is freely filtered by the renal glomeruli and catabolized in the tubule such that it is neither secreted nor reabsorbed as an intact molecule. Furthermore, serum cystatin C concentrations are independent of age, gender and muscle mass. For these reasons, cystatin C is thought to be better marker of glomerular filtration rate (GFR) than serum creatinine [84]. Changes in serum cystatin C correlate with iothalamate clearance in patients with diabetes [84] and serum cystatin C levels are stable over multiple freeze thaw cycles [85]. We will measure serum cystatin C at weeks 0, 12, 24,36, and 48 to monitor renal function in addition to following serum creatinine. Cystatin C is measured at Dr. Andrzej Krolewski's laboratory at the Joslin Diabetes Center as described [84, 85]. In brief, cystatin C is assessed by immunoassay using anti-human cystatin C antiserum and a BN Prospec System nephelometer (Dade Behring Inc, Newark, DE). The inter-assay coefficient of variation is 3-4%.

Increased urine microalbumin in salsalate treated patients was demonstrated in the stage 1 trial, with a median increase of 10 µg/ mg of creatinine (and a median increase of 3 in the placebo group). The clinical importance of a change of this magnitude is unclear, and it was not associated with a decline in renal function assessed by eGFR or by cystatin C. We will assess whether this finding is reproducible in the second stage of the study. For safety, if a participant's microalbumin/creatinine ratio increases by 100 µg/mg creatinine or more, a letter is sent to the primary care provider who will assess function and adjusts hypertension medication or other therapies as needed.

Additional renal biomarkers will be assessed including N-acetyl glutamine (NAG) and beta-2-microglobulin at 3 times points (in stored biospecimens, to be analyzed if the albumin to creatinine ratio is confirmed to increase with salsalate therapy) [86].

6.2.6 Tinnitus

Tinnitus is an expected side effect of salsalate. This effect usually subsides as treatment continues. Participants who experience tinnitus are instructed to skip the next dose and then reduce dosing in one-tablet increments until symptoms resolve.

6.2.7 Pharmacokinetics in Patients with Diabetes

Salicylate metabolism has been studied for decades and is known to be complex. Because clearance could be altered in patients with diabetes due to enhanced renal clearance associated either with hyperfiltration or glycosuria, renal tubular acidosis, or partitioning into adipose tissue, salicylate levels will be measured at weeks 12, 24, 36 amd 48. Glycemia and urine pH will be assessed and correlated with drug levels.

6.3 ADVERSE EVENT AND SERIOUS ADVERSE EVENT REPORTING

6.3.1 Purpose of Adverse Event Reporting

The reporting of adverse events experienced by study participants meets three important purposes:

1. It identifies the frequency and severity of known and unanticipated side effects of the study intervention (salsalate) within each study arm of the trial.
2. It provides the mechanism for reporting the occurrence and severity of adverse events to the study group, the NIH, the FDA, and the pharmaceutical company(s) providing the medications.
3. It fulfills the FDA requirements for reporting adverse reactions to medications.

The timely and complete reporting of adverse events is a critical requirement in the conduct of this trial.

6.3.2 Definitions of Adverse Events

- *Adverse Event (AE):* any untoward medical occurrence in a study participant temporally associated with participation in the clinical study or with use of the experimental agent being studied. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.) or any combination of these. AEs are reported only at scheduled study visits unless they meet the criteria for being serious.

- *Serious Adverse Event (SAE):* any adverse event that results in one or more of the following outcomes:

    - Death
    - A life-threatening event
    - Inpatient hospitalization or prolongation of existing hospitalization
    - A persistent or significant disability/incapacity
    - A congenital anomaly or birth defect
    - Important medical event based upon appropriate medical judgment

6.3.3 Non-serious Adverse Events

It is essential that AEs be ascertained in an unbiased manner using standard questions that are identical and identically administered to patients in all treatment arms. Therefore, AEs are reported on a standard form that is completed by the study staff at each regular follow-up visit. AEs are ascertained by asking questions relating to specific events of import in diabetic patients on any of the study treatment arms. AEs also include any significantly abnormal physical finding identified on examination and any significantly abnormal laboratory result obtained on the patient between visits or at the time of the visit. Questions answered YES and any new abnormal physical findings are pursued by the study staff in order to determine the seriousness of the event and the need for further evaluation, follow-up, or referral. Adverse events reported or ascertained between clinic visits are captured and reported at the time of the next scheduled visit.

Pre-existing conditions (that is, conditions present prior to randomization) are not considered or recorded as AEs or SAEs unless the condition worsens in intensity or frequency

after randomization. Likewise, continuing adverse events are not reported as AEs at subsequent visits unless they increase in severity or frequency between the visits, they result in criteria for an SAE, and/or they resolve between visits.

6.3.4 Serious Adverse Events

Study patients are instructed to contact the clinic with any serious adverse event meeting the above criteria. Each SAE is recorded on the study form and sent to the DCC as soon as possible after they occur and preferably within 24 hours of the notification of the clinic staff. This notification should occur even if data are incomplete. Additional data and follow-up information are sent subsequently as an update to the original report. The DCC immediately forwards SAE reports to the study chair and the NIDDK project office. NIDDK forwards the SAE to the DSMB, which convenes expeditiously at the discretion of the chair.

SAEs are also reported to the local IRB and any other institutional monitoring committee, as per local requirements. SAEs are reported to the FDA since the study is operating under an IND.

6.3.5 Tracking of Adverse Events by the Study Group

- *Serious adverse events:* All SAEs are reported to the DCC within 24 hours. The DCC forwards all SAE reports to the study chair and NIDDK who assess each event to determine if immediate action is required by the study group in response to the event. If the chair and NIDDK determine that immediate action should be considered, they consult with the DSMB to recommend a course of action. In addition, any SAE that results in death or permanent or severe disability and any SAE judged by the local PI as PROBABLY or DEFINITELY related to study participation are discussed by the DSMB as soon as feasible. Any actions recommended are communicated to the study chair and NIDDK for consideration of study wide action. If the SAE is not deemed to warrant immediate study wide action, it is discussed at the next scheduled meeting of the DSMB.

- *Non-serious adverse events:* Non-serious adverse events (AEs) are tabulated by the Coordinating Center in the same format as is done for the Data and Safety Monitoring Board (DSMB). Summaries of the AEs, tabulated by clinic, are provided to the Steering Committee and discussed on a monthly basis. The Steering Committee makes decisions on actions needed, if any, for the study group.

CHAPTER 7: DATA PROCESSING AND MANAGEMENT

Data from the clinical centers and central labs are accumulated in a central database at the coordinating center. The coordinating center can accommodate data entered and transferred in a variety of formats. Remote data entry and management systems developed and provided by the coordinating center may be accessed from the study secure website. Data are entered via the web by clinical staff into a central database at the DCC. Double data entry verification is used to ensure accurate data entry.

Quality control procedures are pre-programmed to check skip patterns, range checks, and miscodes for multiple choice items. Database quality control performed at the coordinating center includes range checks, inter-item checks, cross-table checks, and missing, incorrect, or questionable values. The coordinating center generates queries for the clinical center regarding data issues and quality. Query edit reports with the necessary patient identifying information and problem values are sent to the clinical centers for resolution. Corrected values are entered and checked again for consistency with other items. The goals are to make quality control a continuous process, to make the turnaround time between error detection and correction as short as possible, and to document any changes made to the database.

7.1 BACKUP, DATA SECURITY, AND CONFIDENTIALITY

The Biostatistics Center's data backup and security policies ensure the safety and confidentiality of the data. Backup procedures include: twice-weekly system backup, daily incremental backup, and off-site fire proof storage. Security procedures include: logon and link password protection, remote password logon, and for internet access, separate Web servers which use SSL and encryption algorithms. Regularly updated virus scanning software is used routinely to check personal computers for computer viruses. University computing facilities provide support in the event of a disaster.

The coordinating center maintains confidentiality of patient data and emerging results per a confidentiality policy, which every staff member is required to sign annually.

7.2 TRACKING STUDY PROGRESS

The purpose of tracking reports is to keep the collaborative group informed of study progress, and to report special problems and resolutions. Reports are produced regularly by the coordinating center, as directed by the Steering Committee. These reports are distributed to the study group through the study website.

Tracking reports include the following types of information:

- screening and enrollment, by clinical center, gender, and race/ethnicity
- tables describing adherence to the study protocol (attendance at scheduled study visits, study intervention compliance)
- database inventory
- characteristics of the patient population, by clinical center
- progress of analysis and manuscripts

7.3 ARCHIVAL AND STUDY CLOSE-OUT

At the end of the study, after all data have been received and edited, the database is archived in computer readable format, including: readme documentation files, text files of study documents (forms annotated with variable names, protocols, and manuals of procedures), data files in the form of SAS transport files and input statements, data dictionaries, and program code documenting primary derived variables.

After the results have been published, data will be made available to other investigators. Data will be stored at a readily accessible site.

CHAPTER 8: STATISTICAL CONSIDERATIONS

8.1 SAMPLE SIZE FOR STAGE II

The primary outcome for the TINSAL-T2D study is change in HbA1c level from baseline to week 48. Based on previous studies [87, 88, 89] of patients with type-2 diabetes, the standard deviation of the change in HbA1c is estimated to be 1.33. Given that assumption, a sample size of 226 patients (113 patients per treatment arm) is required to have 80% power to detect a difference in change from baseline of 0.5% using a significance level of 0.05 (2-sided). To allow for a 20% dropout rate over the course of the trial, the total number of patients randomized during Stage II is 282.

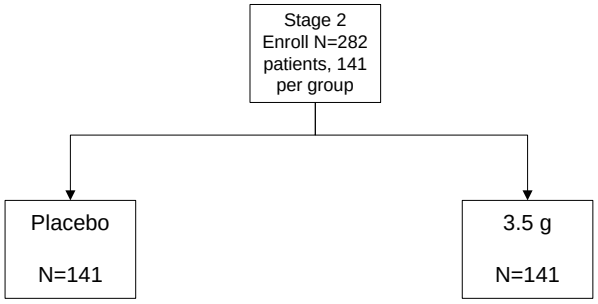


Fig. 8

8.2 DATA ANALYSIS

The principal analyses of primary and secondary outcomes employ the "intent-to-treat" approach [90]. The intent-to-treat analyses include all randomized patients with at least one post-baseline HbA1c measurement. All patients are included in their randomly assigned treatment group; treatment group assignment is not altered based on the patient's adherence to the assigned treatment regimen. Statistical tests are two-sided with the overall significance level of the primary outcome α=0.05. However, because interim analyses are conducted, the significance levels used in the interim and final analyses of the primary outcome are adjusted to account for the multiplicity of interim analyses.

- Baseline characteristics: Comparison of the baseline characteristics between the two treatment groups uses standard parametric and nonparametric statistical techniques, such as the Chi-square test and Fisher's exact test for categorical data and the t-test and the Wilcoxon rank sum test for continuous data.

- Primary outcome: The principal analysis of the TINSAL-T2D study is performed using a general linear model. The model will include change in HbA1c from baseline to 48 weeks as the response variable, and treatment and baseline HbA1c as terms.

- Longitudinal data analysis techniques are used to analyze repeated measures data (e.g., glycemia, fasting lipids, blood pressure, physical activity, quality of life). These include: (1) analyses of the point prevalence of a discrete characteristic (e.g., hypertension) at successive repeated visits over time [91]; (2) multivariate rank analyses of quantitative or ordinal (e.g., the SF36 subscales) measures over successive visits [92]; (3) the parametric linear random effects model of Laird and Ware [93] to compare participant slopes over time (e.g., rate of change in HbA1c or fasting glucose) under linearity and normality assumptions; and (4) techniques developed by Liang and Zeger [94] to compare participant slopes under a generalized linear models framework.

- Interim analysis: The Lan-DeMets [95] spending function approach is used to adjust the probability of a type I error for testing the primary outcome when interim 'looks' of the data are taken by the Data Safety Monitoring Board. The spending function corresponding to an O'Brien and Fleming [96] boundary is used. The Lan-DeMets procedure is flexible, in that the number of looks does not have to be specified in advance and the time interval between looks does not have to be the same. The rate at which the type I error is spent is a function of the fraction of total information available at the time of the interim analysis (i.e., information time) [97]. Prior to taking any 'looks' at the data, the TINSAL-T2D Steering Committee and the DSMB will develop study-specific procedures for interim analyses and stopping rules.

CHAPTER 9: STUDY ADMINISTRATION

9.1 ORGANIZATION

The major organizational components and their responsibilities are described:

- The *TINSAL-T2D Study Group* is composed of investigators and study staff from the 20 clinical sites, the coordinating center, and the NIDDK project office. The study group is responsible for the conduct of the TINSAL-T2D study.
- The *TINSAL-T2D Steering Committee* is the voting body of *TINSAL-T2D Study Group*, and is responsible for the design of the TINSAL-T2D study. Members are the primary authors of the original grant proposal (Steven Shoelson, Allison Goldfine, and Vivian Fonseca, the DCC, and the NIDDK project office).
- The *NIDDK project office* participates in all decision-making activities and selects and oversees the activities of the Data Safety Monitoring Board.
- The *clinical sites* are responsible for recruiting study participants and implementing the protocol.
- The *data coordinating center* is located at the George Washington University Biostatistics Center with responsibility for coordinating all aspects of the study, including production and distribution of materials and documents, development and conduct of study staff training sessions, development and administration of the data management system, maintenance of the central database, data analysis, and report of results in collaboration with the other investigators.
- The *Data Safety Monitoring Board (DSMB)* is composed of outside experts in the design and conduct of clinical trials, and in type 2 diabetes. The board is responsible for reviewing the study documents, monitoring study progress, and monitoring participant safety.
- The *Central Laboratory* is Quest Diagnostics. They are responsible for supplying all materials needed for obtaining, preparing and shipping the blood samples and for all test results.

9.2 CENTRAL LABORATORIES

In collaboration with the coordinating center and study investigators, the central blood laboratory performs the following tasks:

1. Provides procedures and standards for training staff involved in the measurement, collection, preparation, handling, transfer, and all other procedures and processes.
2. Provides training materials for the study manuals of procedures.
3. Provides or facilitates the acquisition of necessary materials, including specifying brands, sizes, and suppliers as applicable.
4. Establishes procedures for data entry and transfer of data to DCC via “care360”.
5. Follows procedures for internal as well as external quality control, and provides periodic reports on quality control surveillance.
6. Provides short-term (21 day) storage of reserve specimens or materials.

9.3 TRAINING AND CERTIFICATION

During the start-up period, the DCC holds a training workshop for study staff. Investigators provide instruction in various aspects of the study. The purpose of the training workshop is to provide training for study staff in order to assure that the study is conducted in a standardized manner across all participating centers. The training, based on the study manual of procedures,

includes the study design, eligibility criteria, conducting patient assessments, patient follow-up schedule, use of the distributed data entry software and electronic forms, transferring data to the DCC, maintaining patient and data confidentiality, and patient treatment guidelines. Throughout the study, new staff are trained by the clinical center principal investigator, study staff and by the DCC.

Prior to being allowed to recruit patients, each clinical center must pass certification criteria, including supplying the coordinating center with the IRB approval letter and stamped informed consent forms, and completion of conflict of interest policy by all investigators.

9.4 SITE VISITS

The two types of site visits are (1) scheduled monitoring and (2) as needed to address specific problems.

The DCC organizes site visits necessary to monitor study procedures and records. The site visit team includes representatives from the DCC, and if possible, investigator(s) or coordinator(s) from other clinical centers, and usually a representative of the NIDDK program office. Each visit follows a predetermined format and site visitors complete a checklist to record findings. The site visit team reviews study procedures and compares data collection records to listings from the central database.

Site visits conducted to address specific problems at the clinical center are attended by the study chair, the NIDDK project office, the DCC, and others as needed.

9.5 STUDY WEBSITE

The DCC maintains the study website, which is a secure site requiring a user ID and password combination for access. The web server utilizes the Secure Socket Layer (SSL) protocol that encrypts all traffic to and from the server. Investigators, coordinators, consultants, and other study staff who would benefit from access to the information on the website are each given a unique user ID and password, which identifies the user to the web server and can be used to restrict access to particular web pages if desired.

The website contains study documents such as the protocol, manual of procedures, and forms, study calendar, directory, meeting and conference call information, links to other sites, tracking reports, minutes, and agendas.

9.6 CONFLICT OF INTEREST POLICY

The TINSAL-T2D investigators have adopted a conflict of interest policy similar to that used by other NIDDK collaborative groups. On an annual basis or whenever there is a significant change in status, TINSAL-T2D collaborators are required to disclose any financial or related interest that could present an actual conflict of interest or be perceived to present a conflict of interest. Disclosure is required to protect each individual's reputation and career from potentially embarrassing or harmful allegations of inappropriate behavior, and to protect the integrity of TINSAL-T2D study research. Forms are kept on file at the DCC.

The TINSAL-T2D Steering Committee determines (1) if the disclosed interests could directly and significantly affect the performance of study responsibilities and (2) the management, reduction, or elimination of the conflict. In addition to complying with the TINSAL-T2D conflict of interest policies, collaborators must certify to the Steering Committee that they have complied with all of their local and institutional requirements regarding conflict of interest and disclosure. This is accomplished by supplying the DCC with copies of the local IRB letter of approval and stamped informed consent form(s).

9.7 PUBLICATIONS AND PRESENTATIONS POLICY

It is anticipated that the research may lead to oral and written presentations including one or more jointly-authored publications and investigators shall have the right to present or publish the results. The party proposing any publication, presentation or grant application shall provide an early draft thereof to the other Steering Committee members for review prior to its first presentation or submission. The contribution of investigators will be acknowledged in accordance with scientific custom, by co-authorship if appropriate, in all published and oral communications concerning the Research or the Results.

9.8 PROTOCOL AMENDMENTS

Adoption of protocol amendments requires three-fifths majority approval by voting members of the Steering Committee. The amended protocol is resubmitted to the IRB.

CHAPTER 10: APPENDIX: RECOMMENDED DOSING OF DIABETIC MEDICATION

Generic Name	Brand Name	Dose Range
INSULIN SECRETAGOGUES (SFU)		
glimepiride	Amaryl	1-8 mg qd
glipizide	Glucotrol	10-40 mg qd
glipizide extended release	Glucotrol XL	XL: 5-20 mg qd
glyburide	Micronase, Diabeta, Glynase PresTab	1.25-20 mg qd
INSULIN SECRETAGOGUES (non-SFU)		
nateglinide	Starlix	60-120 mg tid
repaglinide	Prandin	0.5-16 mg qd
α-GLUCOSIDASE INHIBITORS		
acarbose	Precose	50-100 mg tid
miglitol	Glyset	50-100 mg tid
BIGUANIDES		
metformin	Glucophage	500-2550 mg qd
Liquid metformin	Riomet	500 mg/5 mL qd
metformin extended release	Glucophage XR, Glumetza, Fortamet	500-2000 mg qd (XR and Glumetza) 500-2500 mg qd (Fortamet)
DPP-4 INHIBITOR		
sitagliptin	Januvia	100 mg
saxagliptin	Onglyza	2.5-5.0 mg qd
DOPAMINE RECEPTOR AGONIST		
bromocriptine mesylate	Cycloset	1.6-4.8mg qd
BILE ACID SEQUESTRANT		
colesevelam hydrochloride	Welchol	625 mg, six tablets once a day [twice daily dosing is permitted by the FDA but exclusionary for the study. Study drug must be administered 4 hrs prior to colesevelam dosing]
FIXED COMBINATIONS		
metformin and glipizide	Metaglip	2.5/250 mg qd-10/2000 mg qd
metformin and glyburide	Glucovance	1.25/250 mg qd or bid with meals – 20/2000 mg qd
metformin and sitagliptin	Janumet	50/500 mg bid – 50/1000 mg bid
THIAZOLIDINEDIONES (TZDs)		
pioglitazone	Actos	15-45 mg qd
rosiglitazone	Avandia	4-8 mg qd
FIXED COMBINATIONS		
metformin and pioglitazone	Actoplus Met	15/500 mg qd – 45/2550 mg qd
rosiglitazone and glimepiride	Avandaryl	4/1 mg qd – 8/4 mg qd
rosiglitazone and metformin	Avandamet	1/500 mg qd – 8/2000 mg qd
GLUCAGON-LIKE PEPTIDE 1 (GLP-1) RECEPTOR AGONIST		
exenatide	Byetta	5-10 mcg bid

liraglutide	Victoza	0.6 mg qd for 1 week (Initial dose) 1.2 mg -1.8mg qd
AMYLIN		
pramlintide	Symlin	15-120 mcg tid

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