COMPLETE CLINICAL PROTOCOL

LONG TITLE: Neutrophil Elastase Inhibition as Adjunctive Therapy to Improve Glucometabolic Variables in Overweight and Obese, Insulin-Resistant Type 2 Diabetic Patients (IMPALA)

SHORT TITLE: AZD9668 adjunctive treatment for T2D
VERSION NUMBER: 15
DATE OF LATEST VERSION: June 13, 2017
IRB #: RC-6059
IRB initial date of approval: August 11, 2015/**Full Approval: September 8, 2015**
IND# and initial date assigned by FDA: **127407/Assigned August 4th, 2015**
Clinicaltrials.gov ID: NCT02597101

Study Sponsor/IND Sponsor & Holder: Nick Giannoukakis, Ph.D.
NIH Funding Mechanism: UH3

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Protocol Synopsis

Title	Neutrophil Elastase Inhibition as Adjunctive Therapy to Improve Glucometabolic Variables in Obese, Insulin-Resistant Type 2 Diabetic Patients (IMPALA)
Short Title	AZD9668 adjunctive treatment for T2D
Clinical Phase	***Phase II*** AND ☒ Mechanistic or ☐ Observational
Number of Sites	One: Allegheny Health Network (AHN) with additional procedures being completed at University of Pittsburgh/UPMC.
IND Sponsor/Number	Nick Giannoukakis/ IND # 127407
Investigational Product(s)/Intervention(s)	INVESTIGATIONAL PRODUCTS: i) AZD9668 tablet (orally administered neutrophil elastase inhibitor) ii) Saxagliptin tablet (orally administered DPP-IV inhibitor) iii) Metformin tablet (orally administered insulin sensitizing drug) iv) Placebo tablet for AZD9668 INTERVENTIONS: -oral ingestion of tablets i) venipuncture ii) hyperinsulinemic-euglycemic clamp -oral glucose tolerance test i) collection of peripheral whole blood following venipuncture ii) collection of finger blood droplet after lancet puncture iii) collection of urine
Study Objectives	**Primary Objective:** To assess the efficacy of 60 mg bid AZD9668 PO bid versus placebo as adjunctive treatment to improve insulin sensitivity in obese, insulin-resistant T2D subjects as assessed by the hyperinsulinemic-euglycemic clamp method at baseline and 6 months post randomization. **Secondary Objectives:** i) To assess the safety of AZD9668 vs. placebo (rate and severity of adverse events including hypoglycemia) in obese, insulin-resistant T2D subjects;

	ii) To measure the glycemic and metabolic control variables (change in baseline HbA1c levels, fasting plasma glucose levels, 2h-post-glucose challenge levels, body weight, waist- and hip-circumference, HbA1c trajectory, metformin and/or SAXA dose requirements) in AZD9668or placebo-treated obese, insulin-resistant T2D subjects; iii) To measure changes in glucose, insulin and C-peptide levels during a 3 hour oral glucose tolerance test (OGTT) at 6 months from baseline. From the OGTT, parameters of glucose metabolism will derived: insulinogenic index using C-peptide and insulin [1-5]; glucose area under the curve (AUC), and Matsuda Index of insulin resistance [6-8], and the product of Matsuda index times the insulinogenic index iv) To identify changes in inflammatory variables (concentrations of serum/plasma IL-6, high sensitivity-C-reactive protein, fibrinogen, TNF-alpha, soluble TNF-alpha receptors) in AZD9668 or placebo-treated obese, insulin-resistant T2D subjects. v) Biomarkers to Determine the Expression of Functional Pharmacologic Activity of AZD9668: Desmosine levels in serum and urine. **Exploratory/Pilot and Mechanistic Objectives:** i) To investigate whether or not delayed AZD9668administration (at 6 months) is inferior to the early AZD9668 treatment (at trial start) on IR improvements in insulin sensitivity, metabolic measures, and beta cell function; ii) To assess the effects of AZ9668 and placebo on the frequency and absolute cell numbers of neutrophils, T-cells, B-cells, dendritic cells (DC) as well as other peripheral blood leukocytes (PBL) over the entire study period of both trial stages; iii) To determine the molecular signatures in PBL and purified neutrophils in AZ9668 and placebo recipients over the entire study period of both trial stages including HLA and TCR levels; iv) To determine the changes in neutrophil cell surface phenotype over the duration of the study in placebo and AZD9668-treated subjects; v) To identify cellular and molecular signatures of responder versus non-responder status and/or of effect of therapy in AZD9668 or placebo recipients over the entire study period of both trial stages; vi) To correlate changes in neutrophil absolute numbers with activation of TCR and HLA-specific PBLs over time.

Study Design	This is a phase II trial to be conducted in the USA. The trial consists of a 2-arm, randomized, double-blinded, placebo-controlled, crossover study in 42 T2D subjects between 21-75 years of age, inclusive, with a monitoring period of 12 months following a 1:1 randomisation into AZD9668:placebo arms. Subjects will be randomised following a 3 month run-in period to achieve/maintain an HbA1c level <=8.5 using metformin (MET) with saxagliptin (SAXA). Subjects will then receive 60 mg AZD9668 PO bid (two tablets of 30 mg each, twice a day) or placebo as adjunctive treatment for a 6 month period. Then, the treatment arms will be crossed over to AZD9668 or placebo. Insulin sensitivity, assessed by the hyperinsulinemic-euglycemic clamp method and AE monitoring of all arms will continue to 12 months.
Primary Endpoint(s)	The primary efficacy endpoint is the improvement of insulin sensitivity by 40% or greater at 6 months compared to baseline, assessed by the hyperinsulinemic-euglycemic clamp method.
Secondary Endpoint(s)	**SAFETY** Identification of AEs: i) rate and severity of all adverse events including hypoglycemia ii) rate and severity of known Aes of AZD9668 iii) rate and severity of known Aes of SAXA iv) rate and severity of known Aes of Metformin **EFFICACY:** i) Improvement in glycated HbA1c levels (range=0.2 – 1.5 or better) at 6 months compared to baseline; ii) Improvement in OGTT-derived measurements, namely: glucose tolerance, Matsuda Index of insulin resistance [6-8], insulinogenic index

	using C-peptide [1-5]; and the product of the Matsuda Index times the Insulinogenic Index. iii) Improvement in fasting plasma glucose by 6% or better at 6 months compared to baseline; and iv) Decreased concentrations of serum/plasma IL-6, high sensitivity-C-reactive protein, fibrinogen, TNF-alpha, soluble TNF-alpha receptors at 6 months compared to baseline
Mechanistic Endpoint(s)	i) To assess the effects of AZD9668 and placebo on the frequency and absolute cell numbers of neutrophils, T-cells, B-cells, dendritic cells (DC) as well as other peripheral blood leukocytes (PBL) over the entire study period of both trial stages; ii) To determine the molecular signatures in PBL and purified neutrophils in AZD9668 and placebo recipients over the entire study period of both trial stages including HLA and TCR levels; iii) To determine the changes in neutrophil cell surface phenotype over the duration of the study in placebo and AZD9668-treated subjects; iv) To identify cellular and molecular signatures of responder versus non-responder status and/or of effect of therapy in AZD9668 or placebo recipients over the entire study period of both trial stages; v) To correlate changes in neutrophil absolute numbers with activation of TCR and HLA-specific PBLs over time.
Exploratory/Pilot Endpoint(s)	To investigate whether or not delayed AZD9668 administration (at 6 months) is inferior to the early AZ9668 treatment (at trial start) on IR improvements in insulin sensitivity, metabolic measures, and beta cell function
Accrual Objective	42
Study Duration	The subject's complete participation in this trial will last approximately 15 months (up to 66 weeks).
Treatment Description	Screening: Will include reviewing medical histories from the volunteers (most will have had a diagnosis of insulin-resistant T2D based on clinical criteria), focusing on age, timing of the diagnosis of diabetes, and on medication use. At the initial screening visits, HbA1c, liver function tests (ALT/AST), hematocrit, and blood serum creatinine levels will be measured and pregnancy testing in women with reproductive potential performed in addition to immune competence and

	the other measurements used to determine inclusion/exclusion (see further below). Once eligibility has been confirmed, subjects will proceed to the run-in period. Run-in Period: The run-in period will be used to determine the subject's ability to adhere to the study protocol and to optimise, as much as possible, the background drug dosage and the HbA1c levels. Subjects will adjust MET doses up to 2000 mg daily, as tolerated. Subjects who cannot tolerate at least 1000 mg daily of immediate or extended-release MET by the time of final run-in will be excluded from further participation. Subjects who have been treated with >2000 mg per day will have their dose adjusted to 2000 mg. If a subject is also on a DPPIV inhibitor, a GLP-1 analog, or a sulfonylurea, it will be replaced by 5 mg SAXA qd and the metformin dosage will be adjusted to achieve a glycated HbA1c target of <=8.5 by the time of the final run-in visit. Study subjects who exhibit FPG <=140 and HbA1c <=8.5% following any titrated metformin dose <2000 mg/day will remain on that dose (between 500-2000 mg/day) until such time as further titration is required (not to exceed 2000 mg/day) to maintain this FPG/HbA1c level. Any subject who, despite achieving FPG <=140 and HbA1c <=8.5%, cannot tolerate at minimum 1000 mg/day of metformin during any stage of titration (i.e. exhibits SAEs confirmed to be attributed to metformin) will be discontinued from the study. The run-in period will be no less than 6 weeks and not more than 3 months [usually 8 to 12 weeks will allow equilibration of the HbA1c on the background treatment (MET+SAXA)]. The run-in period will also be used to familiarize the study subjects with the study and the study staff with the study subjects. Randomisation: Eligible study participants will be randomised within one week following the end of the run-in period and will be assigned a study randomisation number to which a treatment group assignment has been made. **Treatment Period A:** i) The AZD9668 arm will remain on the optimized MET+SAXA as established during the run-in period and will be treated with 60 mg AZ9668 bid (two tablets of 30 mg, twice daily) as adjunctive treatment for a 6 month period; ii) The placebo arm will remain on the optimized MET+SAXA as established during the run-in period and will be treated with a color and flavor matched placebo tablet bid as adjunctive treatment for a 6 month period. **Washout Period:** The crossover design obviates the

	requirement of a washout period, since the AZD9668 arm will go on placebo (i.e. the background drugs) and vice versa. **CROSSOVER INTO:** **Treatment Period B:** i) At the start of month 7, subjects originally in the placebo arm will be crossed over into the AZD9668 arm. These subjects will remain on the optimized MET+SAXA as established during the run-in period and will be treated with 60 mg AZD9668 bid (two tablets of 30 mg, twice daily) as adjunctive treatment for a 6 month period; ii) At the start of month 7, subjects originally in the AZD9668 arm will be crossed over into the placebo arm. These subjects will remain on the optimized MET+SAXA as established during the run-in period and will be treated with oral placebo bid that is flavor and color matched to the 30 mg AZD9668 tablets as adjunctive treatment for a 6 month period.
Enrollment Inclusion Criteria	1. Patients 21-75 years of age inclusive who meet the American Diabetes Association standard criteria for type 2 diabetes mellitus (T2D). 2. Subjects are currently on metformin (at least 1000 mg per day) for a minimum of 4 weeks prior to screening visit, alone or in combination with any of the following diabetes medications: a. DPPIV inhibitor (any dose level/frequency) b. Sulfonylurea (any dose level/frequency) c. GLP1 agonist (any dose level/frequency) d. Sulfonylurea (any dose level/frequency) + GLP1 agonist (any dose level/frequency) e. Meglitinide (any dose level/frequency) f. SGLT2 inhibitor (any dose level/frequency)
	3. Patients must have a body-to-mass index (BMI) of greater than or equal to 27 kg/m^2. 4. Patients exhibit glycated HbA1c between 7.3-11 during eligibility screening and then <=8.5 at final run-in visit. 5. Willingness to replace current diabetes therapies (listed in inclusion 2) with metformin and saxagliptin and to adjust metformin dose during run-in period. 6. Subjects present adequate immune competence as assessed by immunoreactivity to viral antigens (CEF Pool Assay) in vitro at the time of screening. 7. Participants of childbearing potential must agree to practice an effective form of birth control which may include any one of the following: barrier method, oral contraception, or

	surgery. These measures must be maintained throughout the study. 8. Subjects must have good peripheral venous access for the hyperinsulinemic-euglycemic clamp and the 3-hr. OGTT procedures. 9. Patients understand the study procedures, alternative treatments available, risks involved in the study, and voluntarily agree to participate by giving informed and signed written consent for screening and enrollment. 10. Participants can be on anti-inflammatory therapies that are not diabetes-focused (e.g. non-salicylate anti-inflammatory therapies, non-salicylate NSAIDs) and/or anti-hypertensive medicaments or statins.
Enrollment Exclusion Criteria	1. Patients with type 1 diabetes mellitus as defined by the American Diabetes Association criteria or a history of ketoacidosis, or the patients are assessed by the study team as possibly having type 1 diabetes mellitus confirmed with the presence of at least one of the typical autoantibodies (insulin, GAD65, IA-2, ZnT8) AND a serum C-peptide level of <0.7 ng/mL. 2. Patients have been treated with any therapies specific for their diabetes other than those listed in the inclusion criteria within 4 weeks of the screening visit. 3. Patients have been treated with insulin within 2 months of the screening visit. 4. Patients are currently participating in or have participated in another study with an investigational compound or device within the prior 12 weeks of signing the informed consent or do not agree to refrain from participating in any other study while participating in this study. 5. Patients have a history of hypersensitivity or any contraindication to DPPIV inhibitors, including saxagliptin (Onglyza), or metformin based upon the labels of the USA. 6. Patients are on a weight loss medication (such as orlistat, phentermine, Qsymia, or Belviq) within the prior 6 weeks. 7. Patients are required by treating physicians to remain on any one of these agents during the trial: macrolide antibiotics, cisapride, anti-arrhythmics, steroids, rifampicin, phenobarbital, phenytoin, secobarbital, carbamazepine, norethindrone, isoniazid. AZD9668 is metabolized by CYP3A4, 3A5, and 2B6. SAXA is metabolized by CYP3A4 and 3A5, potentially leading to drug-drug interactions with hypothetical adverse events in patients on the above agents. Also, AZD9668 causes weak inhibition of CYP2C9 and therefore patients on fluconazole, amiodarone, fenofibrate, fluvoxamine, phenylbutazone, probenecid,

	sertraline, will also be excluded to avoid the hypothetical adverse events due to this effect. 8. Patients have undergone surgery within the prior 6 weeks or have any type or form of major surgery planned during the study (at the discretion of the physician). 9. Patients are on or are likely to require treatment with 14 consecutive days or repeated courses of pharmacologic doses of corticosteroids or any other immunomodulatory agent. For example, patients requiring chronic systemic corticosteroids (does not include topical or inhaled corticosteroids). Exceptions are over the counter non-salicylate NSAIDs. 10. Enrollment or history of enrollment in a drug, or biologic therapy clinical trial that affects the immune system within the past 12 months (e.g., systemic immunosuppressive pharmacologics, immunosuppressive cytokines, therapeutic immunomodulating antibodies, therapeutic immunomodulating fusion proteins and/or cytokine receptor decoys as well as any intervention and/or non-intervention induced immunodeficiencies). 11. Prior history of coronary artery disease (defined as myocardial infarction, angina, bypass surgery, or angioplasty) 12. Prior history of arrhythmia (excludes premature beats) 13. Prior history of heart failure defined as i) symptomatic OR ii) pulmonary edema, leg edema or low ejection fraction (<40%) 14. Evidence of refractory chronic migraine (defined in ICHD-3 and Martelletti et al. [9,10]). 15. History of persistent bradycardia within the last year prior to screening visit (more than three episodes in a calendar year of a heart rate <60 beats per minute that required hospitalization on each of these occasions). 16. Leukopenia (<3000 leukocytes/microliter), neutropenia (<1500 neutrophils/microliter), lymphopenia (<800 lymphocytes/microliter), or thrombocytopenia (<125000 platelets/microliter), any other clinically relevant abnormal hematology value. 17. Positivity for HIV, active CMV, chlamydia, any evidence of serious fungal infection, active HSV1, HSV2, hepatitis B or C, or history of HSV1 or HSV2 at screening. Minor skin fungus, or minor candidiasis is not an enrollment or treatment exclusion criterion. Also, with the exception of HIV history, hepatitis B and C, successfully-treated, disease-free individuals (> 6 months between time of successful treatment confirmation and time at screening) would be eligible for enrollment in this trial. 18. Patients are required by treating physician to remain on any medications listed in inclusion #2 (that directly affect glucose metabolism such as, but not limited to

	thiazolidinediones, pramlintide, or amylin. 19. Vaccination with any form of live vaccine product within the last 3 months prior to initiation of study agent administration. 20. Any chronic disease that in the opinion of the investigators would affect the patient's safety and/or the integrity of the study outcome. This does not include dyslipidemia, patients on statin or anti-hypertension treatment, or patients with well-controlled hypo- or hyperthyroidism. Evidence of liver dysfunction, with ALT or AST> 1.5 times the upper limit of normal. 21. Evidence of renal insufficiency as indicated by blood creatinine of > 2 times the upper limit of normal at baseline screening OR an eGFR < 45 mL/min OR A past history or current clinical evidence of renal failure or low creatinine clearance at screening. 22. Females who are pregnant at the time of screening or unwilling to defer pregnancy during the study period. 23. Lactating women. 24. Poor accessibility to veins for the 3-hour OGTT and hyperinsulinemic-euglycemic clamp procedures. 25. The following therapies cannot be administered while patients are undergoing treatment on this protocol: i) radiation therapy; ii) chemotherapy; iii) corticosteroids (except for very short courses of topical or inhaled); iv) agents used to treat attention deficit and hyperactivity disorder (ADHD); v) rifampicin or phenytoin; vi) other protein, particle or cell vaccine immunomodulation therapies. If these therapies are essential for treatment of other conditions, participation in this study will be terminated. 26. A condition which interferes with the ability to accurately determine glycated HbA1c. Examples include: blood transfusion, Genetic variants (e.g. HbS trait, HbC trait), elevated fetal hemoglobin (HbF) and chemically modified derivatives of hemoglobin (e.g. carbamylated Hb in patients with renal failure); Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia); iron deficiency anemia, iron replacement therapy 27. Subjects who cannot tolerate at least 1000 mg daily of immediate or extended release metformin by the time of final run-in will be excluded from further participation. 28. Subjects who do not exhibit a glycated HbA1c level <=8.5 by the end of the run-in period.

Study Stopping Rules	1. The NCATS, FDA, the IRB or the DSMB requires termination of the study upon review of the safety data. 2. Any death possibly or definitely related to AZD9668. 3. If within the first three months of their first dose, three or more of the first (initial) 6 participants treated with AZD9668 experience a study-related serious adverse event resulting in the permanent discontinuation of the treatment (as defined in Section 4.7.2). 4. Infection that persists for > 2 weeks from expected time of mean clearance (except for skin fungus) 5. Any expected adverse event considered possibly or definitely related to AZD9668 that occurs at a greater severity than previously observed or listed in the study documents and is therefore considered unexpected and classified as an AE of special interest (AESI). The FDA, IRB and DSMB will be immediately notified of any such event. Resumption of enrollment is contingent upon a favorable IRB and DSMB review.

Table of Contents

Glossary of Abbreviations

AE	Adverse Event(s)
AZD9668	Astra-Zeneca Drug 9668
Breg(s)	B-regulatory cells
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DC	Dendritic Cell(s)
DSMB	Data Safety Monitoring Board
EXPT	Experimental
FDA	Food and Drug Administration
FPG	Fasting Plasma Glucose
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HbA1c	Hemoglobin A1c
ICH	International Conference on Harmonization
IEC	Institutional Ethics Committee
IL	Interleukin
IR	Insulin Resistant
IMM	Independent Medical Monitor
IMPALA	IMProving insulin resistance with ALvelestat Adjunctive therapy
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MET	Metformin
MOP	Manual of Procedures
NCATS	National Center for Advancing Translational Sciences (NIH)
NIH	National Institutes of Health
NE	Neutrophil Elastase
NEI	Neutrophil Elastase Inhibitor
OGTT	Oral Glucose Tolerance Test
PC	Protocol Chair
PI	[Site] Principal Investigator

SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event(s)
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SAXA	Saxagliptin
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction
T2D	Type 2 Diabetes Mellitus
TCC	Trial Coordinating Center
Treg(s)	T-regulatory cell(s)
UP	Unanticipated Problem(s)

1.0 PROJECT SUMMARY

1.1 TITLE

Neutrophil Elastase Inhibition as Adjunctive Therapy to Improve Glucometabolic Variables in Overweight and Obese, Insulin-Resistant Type 2 Diabetic Patients

1.2 INDICATION

AZD9668 will be an adjunctive therapy in combination with saxagliptin and metformin to improve insulin sensitivity in overweight and obese, type 2 diabetic (T2D) individuals. Although AZD9668 has been used in previous trials approved by the FDA (e.g. clinicaltrials.gov - NCT01054170, NCT01023516, NCT00769119, NCT00757848), it is considered an investigational new drug (IND) for the purposes of this trial. Saxagliptin is marketed as a treatment option for T2D along with other antidiabetic agents including metformin (an established treatment option on its own for T2D).

1.3 RATIONALE

Overweight, insulin-resistant (IR) T2D individuals manifest a chronic systemic inflammation which impairs beta cells and peripheral insulin sensitivity. This systemic inflammation is associated with an atherogenic lipid profile and predisposes individuals to higher risk for micro- and macro-vasular disease, irrespective of well-controlled glycemia. Although a variety of pharmacologic approaches maintain daily glycemic control, it is becoming evident that there is an urgent need to identify adjunctive therapies to improve, insulin sensitivity, beta cell function, and HbA1c since they begin deteriorating quite substantially by 5 years following initial treatment. Ideally, such adjunctive therapies should be well-tolerated, easy to administer, should not promote hypoglycemia and should also attenuate the systemic inflammation.

The role of neutrophils in T2D and metabolic inflammation represents an important gap in knowledge to better understand inflammation in T2D especially since neutrophils are the most abundant leukocyte population in humans and constitute the bulk of inflammatory leukocytes. Emerging evidence indicates that neutrophils along with neutrophil-derived elastase serve as an important nexus of T2D-associated inflammation. This trial offers a first-in-kind opportunity to better understand the role of neutrophils in T2D diabetics.

1.4 HYPOTHESIS

We hypothesize that inhibition of neutrophil elastase (NE) will attenuate the chronic systemic background inflammation in overweight and obese, IR T2D subjects and that the potential improvement in insulin sensitivity and glucose control could concurrently facilitate functional maintenance and induce the rescue of pancreatic beta cell mass.

1.5 BACKGROUND AND SIGNIFICANCE

AZD9668 is a potent orally-administered competitive inhibitor of neutrophil elastase (NE) with considerable selectivity for human NE over other serine proteases, including human cathepsin G (680-fold), and >1900-fold selectivity over chymotrypsin, pancreatic elastase, trypsin and proteinase-3 (proprietary data from Astra-Zeneca). Obese, insulin-resistant (IR) type 2 diabetic (T2D) patients are characterized by a chronic systemic inflammation which affects insulin sensitivity and pancreatic beta cell function [11,12]. Emerging evidence points to an important role of neutrophils as responders of the obese state, as well as participants in the maintenance and persistence of the systemic inflammation (reviewed in [13-20], and see further below). NE deficiency and NE inhibition has been shown to substantially and significantly improve obesity-induced IR and to suppress adipose and liver inflammation in animal models [21,22]. These, along with other facets of neutrophil biology in systemic and metabolic inflammation, compel a novel use for AZD9668 as an adjunctive therapy in T2D which can short-circuit the systemic inflammation that exacerbates obesity-associated IR. The hypothesis underlying this clinical trial *is that inhibition of NE will attenuate the chronic systemic background inflammation in overweight and/or obese, IR T2D subjects and that the potential improvement in insulin sensitivity and glucose control could concurrently facilitate functional maintenance and induce the rescue of pancreatic beta cell mass (Figure 1)*.

Both IR and beta cell dysfunction are involved in the pathogenesis of T2D [11,12,23] whose prevalence continues to increase around the globe [24,25] and is currently estimated to be at more than 385 million[26]. As many as 1 in 3 people in the United States could have diabetes by the year 2050[27] with significant economic consequences. In 2014, 1 in 5 health care dollars was spent to support the care of patients at a total estimated cost > $245 billion [28] [29,30]. Many of these costs are associated with the long-term complications of the disease (cardiovascular, microvascular, renal, nervous, ophthalmic, wound healing, and infections). Without concerted efforts to address the pathogenesis and treatment of this syndrome, the harmful macrovascular and microvascular outcomes of T2D will remain a major burden for decades to come. Of particular concern and alarm is the growing trend of T2D onset in youth and children[24,31-38], and even higher in Asia and China [24,31-35,39].

1.5.1 Obesity, inflammation, IR and neutrophils in T2D

Obesity, an outcome of the overweight state, is often characterized by systemic inflammation, and preclinical evidence links systemic inflammation to beta cell dysfunction[40,41]. Systemic inflammation is also associated with insulin sensitivity and beta cell function[42,43][44,45]. The expansion of adipose tissue is associated with accumulation of activated macrophages secreting several proinflammatory molecules which locally impair insulin signaling [46,47]. An explosion of studies in the past decade have begun to reveal the contributions of inflammation to the development of IR and subsequent metabolic abnormalities in other tissues, such as liver [48-53] and most recently brain [54]. Adipose tissue macrophages are the main component of adipose tissue immune cells (40–60 % of all adipose tissue immune cells), and their number increases progressively after only 1 week of high-fat diet feeding [55]. In addition to macrophages, additional leukocyte subpopulations have recently been demonstrated to be involved in obesity and IR, such as T cells, B cells, eosinophils, mast cells, natural killer cells***, but most importantly as concerns humans - neutrophils***. *In contrast to rodents, in humans, lymphocytes account for the minority of implicated immune cells; granulocytes appear to be the more relevant immune cell population. In fact, granulocytes comprise 60-79% of human blood leukocytes and more than 90% of the granulocytes are neutrophils thus making up the largest fraction of white blood cells.* Neutrophils directly interact with macrophages, dendritic cells, and lymphocyte subsets and modulate their effector functions, and accumulating evidence implicates them in T2D and T1D (Reviewed in [16,56-59]). For example, activated neutrophils induce Th1 and Th17 cell chemotaxis [60]. In acute inflammation, neutrophils are the leukocyte subpopulation arriving first in the inflamed tissue

and promote the subsequent recruitment of inflammatory monocytes by producing MCP-1 and other chemokines [61]. Neutrophil-derived proteases regulate the biological activity of cytokines in the inflammatory microenvironment. For instance, human and murine NE and cathepsin G cleave full- length IL-33 into mature forms [62]. Neutrophils also participate in inflammation through the process of neutrophil extracellular trap-associated apoptosis ("NET"osis), a form of suicide that consists of a stepwise progression of chromatin decondensation, nuclear swelling, spilling of the nucleoplasm into the cytoplasm, and finally membrane perforation [63,64]. In men, elevated neutrophil markers, especially myeloperoxidase, are present in the plasma of obese subjects [65]. Abnormal expansion of visceral adipose tissue is accompanied by influx of immune cells, including neutrophils. Activated neutrophils infiltrate adipose tissue early during diet-induced obesity in mice in an attempt to limit the local inflammatory process [66]. Moreover, in vitro studies have shown that neutrophils physically bind adipocytes in a CD11b/ICAM-1 interaction and in a manner dependent on their activation state [66]. Neutrophils are rare in adipose in a lean state; however, a high-fat diet causes neutrophils to infiltrate white AT early after nutrient excess, and increased neutrophil recruitment to the obese AT is seemingly sustained over a longer period [67]. Most interestingly, obesity-induced, AT-invading neutrophils (CD11b+ Ly6g+ F4/80□ CD11c□), established local IR by secreting elastase [68].

The deletion of NE in high-fat diet-induced obese mice led to reduced macrophage content, improved glucose tolerance, and improved AT and liver insulin sensitivity, accompanied by a marked reduction in neutrophil recruitment to the white AT [18]. Intriguingly, NE can degrade IRS-1 protein and reduce insulin-induced Akt phosphorylation in adipocytes [18]. This mechanism may be involved in the neutrophil- and NE-dependent effect on IR in diet-induced obesity, as the levels of IRS-1 were higher in the AT and liver of elastase-deficient mice, as compared to elastase-sufficient mice [18]. Similar results were obtained in a very recent study by another group [21,69]. In humans, an increased blood level of myeloperoxidase, a marker of neutrophils, in obese women [65], and increased activity of neutrophils in obese subjects have also been noted [69]. Very recently, myeloperoxidase-deficient mice were shown to exhibit resistance to diet-induced obesity, glucose intolerance and diabetes [70]; the enzyme deficiency was further shown to be neutrophil-relevant. NE can increase the expression of proinflammatory genes in peritoneal macrophages in a TLR4-dependent manner [18]. Consistently, NE-deficient white AT displayed a decrease in expression of proinflammatory markers concomitantly with an increase of anti-inflammatory markers, while reduced numbers of M1-polarised macrophages were observed in the NE-deficient obese AT [18]. Thus, a dual mechanism of action of NE, involving interference with insulin signaling, as well as activation of the TLR4-dependent AT inflammation, could account for its role in promoting development of IR. The profound phenotype of NE deficiency on AT inflammation and IR is intriguing. NE can activate immune cells via protease-activated receptors and by interacting with beta2 integrins, thereby activating leukocyte adhesion [71-73]. Moreover, NE can modify the chemokine and cytokine network by proteolytic activation or inactivation [74].

Non-insulin, anti-inflammatory T2D therapeutics and the potential for neutrophil-targeting drugs: The increasing prevalence of T2D has stimulated development of many approaches to safely treat hyperglycemia. Metformin is generally recommended as the initial drug for patients with T2D, but most patients eventually require a second, third, or even fourth drug to achieve target glycemic control [75-80]. Concerning inflammation, high-dose sodium salicylate or acetylsalicylic acid can diminish glycosuria and IR, improve glycemic control and HbA1c in diabetic patients[81-84]. Randomized clinical trials have shown that the blockade of IL-1b by anakinra or IL-1b antibodies reduced systemic inflammation and improved glycemia of T2D[85-95]. Etanercept, a TNFa antagonist, can block inflammation[96]. Several studies have been conducted to test whether etanercept improves glucose tolerance in T2D patients; however, despite a suppressive effect on systemic inflammation, the attenuation of glucose tolerance or IR has not yet been achieved[96-98]. The major classes of anti-inflammatory agents that target neutrophils and neutrophil function include corticosteroids, macrolides,

cAMP-elevating agents (including cyclooxygenases), 5-lipoxygenase inhibitors and leukotriene receptor antagonists, chemokine receptor antagonists, kinase inhibitors, non-steroidal activators of histone deacetylase-2 (HDAC2), and anti-oxidants[99-119]. Corticosteroids are not specific to neutrophils and as general immunosuppressives they possess the additional adverse effect of inducing glycogenolysis and raising blood glucose levels[120,121]. Large clinical trials with high doses of antioxidants have been disappointing[122]. The use of the other classes of agents is associated with varying adverse events and grades of adverse events >2. **Our novel approach, targeting NE in order to decouple the inflammatory cycle, we believe, represents a first-in-kind adjunctive therapy, realizing the objective of modulating the three pressure points in T2D: IR, insulin sensitivity and beta cell insulin production.** Two NE inhibitors have been developed and tested in human patients; infusion sivelestat sodium hydrate [123-126] considered for acute lung injury associated with sepsis and systemic inflammation and AZD9668, a fully reversible orally-administered inhibitor considered for chronic inflammatory lung disease [127-131]. Given the better known safety data for AZD9668 [127-129,131-133], we believe that it is a candidate better positioned as an adjunctive therapy to attenuate the systemic inflammation in T2D thereby improving metabolic and glucose control. Especially important is the oral route of administration (tablets) which usually facilitates patient compliance to the treatment.

1.6. Study Medication Information & Prior Clinical Research Experience

This section summarises the indications, known mechanisms of action, and Standard-of-Care or investigational use. Detailed information (preclinical/clinical studies prior to marketing [where a study agent is on the market] can be found in the respective product inserts of the marketed agents (saxagliptin and metformin) and in the Investigator's Brochure (AZD9668).

1.6.1 SAXAGLIPTIN

Saxagliptin (Astra-Zeneca) is a white to light yellow or light brown powder. It is soluble in polyethylene glycol 400, acetone, acetonitrile, ethanol, isopropyl alcohol, methanol; sparingly soluble in water and slightly soluble in ethyl acetate. Each film-coated tablet of saxagliptin contains either 2.5 mg or 5 mg of saxagliptin free base (as saxagliptin hydrochloride) and the following inactive ingredients: lactose, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyvinyl alcohol, macrogol 3350, titanium dioxide, talc-purified, iron oxide yellow CI77492 (2.5 mg tablet only), iron oxide red CI77491 (5 mg tablet only) and Opacode Blue (printing ink).

Mechanism of action:
Saxagliptin is an orally-active inhibitor of the dipeptidyl peptidase 4 (DPP-4) enzyme for the treatment of type 2 diabetes mellitus. It is a member of a class of oral anti-hyperglycaemic agents called DPP-4 inhibitors. Saxagliptin is a reversible, competitive, DPP-4 inhibitor with nanomolar potency. Saxagliptin demonstrates selectivity for DPP-4 versus other DPP enzymes, with greater than 75 fold selectivity over DPP-8 and DPP-9. Saxagliptin has extended binding to the DPP-4 active site, prolonging its inhibition of DPP-4. Saxagliptin differs in chemical structure and pharmacological action from GLP-1 analogues, insulin, sulfonylureas or meglitinides, biguanides, peroxisome proliferators-activated receptor gamma agonists, alpha-glucosidase inhibitors, and amylin analogues. It is increasingly being used as the main adjunctive drug to metformin that with diet and exercise together results in better management of type 2 diabetes to maintain normal glycated HbA1c levels as well as to keep blood glucose within a normal range on a daily average basis. Saxagliptin, like other orally-available and active DPP-IV inhibitors does not elicit hypoglycemia. Saxagliptin exerts its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Concentrations of these active intact incretin hormones are increased by saxagliptin, thereby increasing and prolonging the actions of these

hormones. Saxagliptin also inhibits the cleavage of other substrates in vitro, but the relevance or consequences of DPP-4 inhibition for these substrates in patients is unknown.

Incretin hormones are released by the intestine throughout the day and concentrations are increased in response to a meal. These hormones are rapidly inactivated by the enzyme DPP-4. The incretins are part of an endogenous system involved in the physiologic regulation of glucose homeostasis. When blood glucose concentrations are elevated GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells. GLP-1 also lowers glucagon secretion from pancreatic alpha cells, leading to reduced hepatic glucose production.

Concentrations of GLP-1 are reduced in patients with type 2 diabetes, but saxagliptin increases active GLP-1 and GIP, potentiating these mechanisms. By increasing and prolonging active incretin concentrations, saxagliptin increases insulin release and decreases glucagon concentrations in the circulation in a glucose-dependent manner.

Saxagliptin improves glycaemic control by reducing fasting and postprandial glucose concentrations in patients with type 2 diabetes through improvements in alpha and beta cell function as reflected by the actions described below.

Fasting glucose-dependent insulin secretion: Saxagliptin increases pancreatic beta-cell responsiveness to glucose in the fasting state and leads to enhanced insulin secretion and glucose disposal in the presence of elevated glucose concentrations.

Postprandial glucose-dependent insulin secretion: Saxagliptin increases pancreatic beta-cell responsiveness to glucose in the postprandial state and leads to enhanced postprandial insulin secretion and glucose disposal.

Postprandial glucagon secretion: In type 2 diabetes, paradoxical increases in glucagon secretion from alpha cells following meals stimulate hepatic glucose production and contribute to glycaemic dysregulation. Saxagliptin moderates glucagon secretion and lowers postprandial glucagon concentrations.

Standard-of-Care Use

Add-on combination

Dual Oral Combination Therapy
Saxagliptin is indicated in patients with type 2 diabetes mellitus, to improve glycemic control, in combination with metformin, a sulfonylurea, or a thiazolidinedione, as an adjunct to diet and exercise, when the single agent alone does not provide adequate glycaemic control.

Triple Oral Combination Therapy
Saxagliptin is indicated in patients with type 2 diabetes mellitus to improve glycaemic control in triple combination with metformin plus a sulfonylurea (SU), when the two agents, with diet and exercise, do not provide adequate glycaemic control.

Combination Therapy with Insulin
Saxagliptin is indicated in patients with type 2 diabetes mellitus to improve glycaemic control as add-on therapy to premixed or basal insulin (with or without metformin) when premixed or basal insulin (with or without metformin) used with diet and exercise, do not provide adequate glycaemic control. Saxagliptin

has not been studied in a regimen combining intermediate or long-acting insulin with mealtime bolus doses of short-acting insulin (basal:bolus regimens) and its efficacy in this context has not been established.

Initial combination
Saxagliptin is indicated for use as initial combination therapy with metformin, in patients with type 2 diabetes mellitus, to improve glycaemic control as an adjunct to diet and exercise, when dual saxagliptin and metformin therapy is appropriate. (i.e. high initial HbA1c levels and poor prospects for response to monotherapy).

1.6.2 METFORMIN

Metformin HCl will be the generic brand supplied by the clinical trial pharmacy at AHN.

Mechanism of action:
Metformin is an orally-active biguanide anti-diabetic agent in common use as an adjunct to diet and exercise for the management of type 2 diabetes to maintain normal glycated HbA1c levels as well as to keep blood glucose within a normal range on a daily average basis. Metformin, generally, is the preferred initial oral antidiabetic agent for patients with type 2 diabetes mellitus.

Metformin lowers blood glucose concentrations in patients with type 2 diabetes mellitus without increasing insulin secretion from pancreatic beta cells. Ineffective in the absence of some endogenous or exogenous insulin. Usually does not lower glucose concentrations below euglycemia, but hypoglycemia occasionally may occur with overdosage. Lowers both basal (fasting) and postprandial glucose concentrations in patients with type 2 diabetes mellitus. Improves insulin sensitivity by decreasing hepatic glucose production and enhancing insulin-stimulated uptake and utilization of glucose by peripheral tissues (e.g., skeletal muscle, adipocytes). Insulin secretion usually remains unchanged.

Standard-of-Care Use
Metformin HCl generally is the preferred initial oral antidiabetic agent for patients with type 2 diabetes mellitus. Potential advantages of metformin compared with sulfonylureas or insulin include minimal risk of hypoglycemia, more favorable effects on serum lipids, reduction of hyperinsulinemia, and weight loss or lack of weight gain.

Used as an adjunct to diet and exercise for the management of type 2 diabetes mellitus.
May be used in combination with a sulfonylurea, repaglinide, DPPIV inhibitor or thiazolidinedione antidiabetic agent for the management of type 2 diabetes mellitus in patients who do not achieve adequate glycemic control on monotherapy with metformin or any of these drugs. Also used concomitantly with nateglinide. May be used with insulin to improve glycemic control and/or decrease the required dosage of insulin.

Monotherapy

Oral

Conventional tablets or oral solution: Initially, 500 mg twice daily or 850 mg once daily with meals. Increase daily dosage by 500 mg at weekly intervals or by 850 mg at biweekly (every 2 week) intervals up to a maximum of 2 g daily given in divided doses. May increase dosage from 500 mg twice daily to

850 mg twice daily after 2 weeks. Clinically important responses generally not observed at dosages <1.5 g daily.

Initial dosage of 500 mg once daily suggested by some experts. For additional glycemic control, increase dosage (as conventional tablets or oral solution) up to a maximum daily dosage of 2.55 g given in divided doses.

Extended-release tablets (Glucophage XR) in patients ≥17 years of age: Initially, 500 mg once daily with the evening meal. Increase daily dosage by 500 mg at weekly intervals to a maximum of 2 g daily. If glycemic control is not achieved with 2 g once daily, consider administering 1 g twice daily. If >2 g daily is required, switch to conventional tablet formulation and increase dosage up to 2.55 g daily in divided doses (preferably 3 doses per day for daily dosages >2 g).

Extended-release tablets (Fortamet) in patients ≥17 years of age: Initially, 1 g once daily with the evening meal; 500 mg once daily may be used when clinically appropriate. Increase daily dosage by 500 mg at weekly intervals to a maximum of 2.5 g daily with the evening meal.

Metformin Hydrochloride in Fixed Combination with Sitagliptin (Janumet)

Oral

Individualize dosage based on the patient□s current regimen, effectiveness, and tolerability while not exceeding the maximum recommended daily dosage of 2 g of metformin hydrochloride and 100 mg of sitagliptin.

Patients currently receiving metformin: Initially, 500 mg of metformin hydrochloride and 50 mg of sitagliptin twice daily; gradually increase dosage as needed.

Patients currently receiving metformin: Initial dosage should provide 50 mg of sitagliptin twice daily and the dosage of metformin hydrochloride currently being taken. Patients currently taking metformin hydrochloride 850 mg twice daily should receive an initial fixed-combination dosage of 50 mg of sitagliptin and 1 g of metformin hydrochloride twice daily.

Safety and efficacy of transferring to Janumet from therapy with other oral antidiabetic agents not established; undertake any such change with caution and appropriate monitoring.

1.6.3 AZD9668

AZD9668 is a potent and selective inhibitor of human neutrophil elastase (NE); a serine protease implicated in cigarette smoke induced inflammation, lung injury and emphysema. AZD9668 has been evaluated in clinical studies of up to 12 week duration by Astra Zeneca as a potential novel oral treatment to control the symptoms and exacerbations of chronic obstructive pulmonary disease (COPD), and reduce the progression and severity of the disease.

Mechanism of action

In vitro potency determinations for purified human NE and a number of other serine proteases were performed by measuring the cleavage of fluorogenic or chromogenic peptide substrates to fluorescent or coloured products. Potency (pIC50) was defined as the negative logarithm of the molar concentration of AZD9668 required to inhibit the formation of product by 50%.

AZD9668 was a potent inhibitor of human NE with IC50 and pIC50 values of 0.012 microM (12 nM) and 7.9 respectively. Lineweaver-Burk analysis of the effect of substrate concentration on the Michaelis-Menten constant (Km) and maximum rate of metabolism (Vmax) values in the presence of AZD9668 indicated competitive inhibition and simultaneous non-linear curve fitting of data generated a pKi of 7.7 (19 nM).

AZD9668 showed considerable selectivity for human NE over other serine proteases, including human cathepsin G (680-fold), and >1900-fold selectivity over chymotrypsin, pancreatic elastase, trypsin and proteinase-3.

The binding of AZD9668 to human NE was reversible, with a fast rate of formation of enzyme inhibitor complex (Kon) of 2.5 x 10 6 M-1s-1 and rate of dissociation of enzyme inhibitor complex (Koff) of 1.2 x10-2 s-1. The off rate t1⁄2 was 1.1 minutes and the calculated dissociation constant (KD) was 0.005 microM (5 nM).

Compared to its potent inhibitory activity on human NE (IC50/pIC50 0.012 microM/7.9), AZD9668 inhibited NE from other species with only moderate potency; values for mouse, rat and dog were 0.29 microM/6.5, 0.22 microM/6.7 and 0.39 microM/6.4 respectively)

Instillation of NE (2 mg/kg) into the trachea of mice induces vascular injury, haemorrhage, and degradation of the lung structural proteins, elastin and collagen. AZD9668, given orally, dose dependently inhibited haemorrhage induced by instillation of human NE; interpolated ED50 and ED80 values were 0.85 mg/kg and 2.25 mg/kg (1.6 micromol/kg and 4.1 micromol/kg), respectively. The interpolated total blood concentration of AZD9668 at ED80 was 0.046 microM (46 nM).

Clinical studies/Investigational Data

AZD9668 was being developed by AstraZeneca as a novel oral treatment to control the symptoms and exacerbations of chronic obstructive pulmonary disease (COPD), and reduce the progression and severity of the disease. However, the development in COPD was stopped following the completion of 2 Phase IIb/II studies (BREEZE/MISTRAL).

In these two large studies evaluating the efficacy of AZD9668 on background therapies of tiotropium and budesonide/formoterol, respectively, there was no evidence of a beneficial effect across a range of lung function and symptom variables measured at clinic and at home during 12 weeks of treatment with AZD9668 compared to placebo.

An additional exploratory Phase IIb study, MASCOT, evaluated MSCT in COPD patients treated with AZD9668 compared to placebo on tiotropium background therapy. All patients in MASCOT have completed and the study is under analysis. Initial results indicate no differences between treatment groups for the primary variable of airway wall thickness or for clinic based spirometry measures. Improvements in some secondary measures were observed in this small exploratory study but were not confirmed by the larger BREEZE study.

No treatment-emergent deaths were reported in patients receiving AZD9668 in the 12 completed studies. There were no treatment-emergent SAEs reported during the Phase I study programme. In the Phase II/IIb studies in COPD, the most common SAE in the BREEZE and MISTRAL was exacerbation

of COPD; other SAEs were typically reported at single incidences. In the MASCOT study (all patients have completed and the study is under analysis), there were no SAEs reported in patients receiving AZD9968.

In Phase I studies, in both Caucasian and Japanese healthy subjects, single oral doses of AZD9668 up to 120 mg once daily and multiple oral doses of AZD9668 up to 70 mg orally bid were found to be safe and generally well-tolerated. In Phase II studies AZD9668 (60 mg bid) was well tolerated in patients with COPD, cystic fibrosis and bronchiectasis.

The AE most frequently reported in the Phase I studies and the studies in patients with cystic fibrosis or bronchiectasis was headache, reported by more subjects on AZD9668 than on placebo. In patients with COPD (Study D0520C00002), the AE reported by the greatest number of patients was also headache, reported by fewer patients on AZD9668 than on placebo.

In the Phase II/IIb studies in COPD, the most commonly reported AE was nasopharyngitis (BREEZE and MASCOT), headache (MISTRAL).

In one cystic fibrosis patient treated with AZD9668, creatinine phosphokinase, ALT, AST and LDH increased during treatment. An initial peak was temporally associated with unaccustomed heavy exercise; however there was insufficient information for a second peak in values to attribute the changes to exercise and therefore a relationship to study drug could not be excluded. In one bronchiectasis patient with raised ALT and AST, transaminases increased during treatment with AZD9668, started to decline while the patient continued treatment and returned to baseline levels after the end of the study. There were no associated symptoms; bilirubin and other liver function tests remained normal. A relationship to study drug of the raised transaminases in this patient cannot be excluded.

Elevations in liver transaminases were observed during the Phase II/IIb programme in COPD:

-BREEZE - the proportion of patients with elevation of ALT to 3 x upper limit of normal (ULN) was highest in the AZD9668 5 mg and 60 mg groups, both with 4 (2%) patients vs. 1 (<1%) in both the placebo and AZD9668 20 mg groups. No patients reported elevation of total bilirubin to 2 x ULN in the placebo group with 1 (<1%) in each of the AZD9668 groups. One patient (E2201001), in the AZD9668 5 mg group reported an elevation of ALT to >3 x ULN and total bilirubin to >2 x ULN on Day 97, 27 days after stopping study treatment. This patient was later diagnosed to have a cholangiocarcinoma of the bile duct.

-Although definite evidence of dose relationship was lacking, there was a suggestion of an increased incidence of liver transaminase elevations in patients on AZD9668 for which there were no definite alternative explanations. The highest transaminase elevations occurred in the highest AZD9668 dose (60 mg).

-MISTRAL - there were a few isolated cases of elevated hepatic biochemistry measurements but overall the number of patients with hepatic biochemistry above 1.5 x ULN was small. A higher proportion of patients in the AZD9668 60 mg group had raised transaminases (ALT and AST) above the AstraZeneca ULN (between 1 x ULN and 3 x ULN) compared with the placebo group.

MASCOT - no patients developed high transaminase values (ALT or AST to ¸3 x ULN) in the AZD9668 60 mg dose group during this study.

There were no other clinically relevant changes in clinical chemistry data in the completed studies with AZD9668. No clinically relevant changes in urinalysis were observed in the 12 completed studies with AZD9668. In the completed studies there were no changes in ECG or vital signs data that indicated a clear treatment effect of AZD9668.

1.6.4 AZD9668 Placebo:

Tablet identical in color and shape but without the API.

1.7 STUDY MEDICATION RISK INFORMATION FROM PREVIOUS TRIALS

1.7.1 Metformin:

Hypoglycemia: Although uncommon in patients receiving metformin as monotherapy, debilitated, malnourished, or geriatric patients and patients with renal or hepatic impairment or adrenal or pituitary insufficiency may be particularly susceptible. Strenuous exercise, alcohol ingestion, insufficient caloric intake, or use in combination with other antidiabetic agents may increase risk. Hypoglycemia may be difficult to recognize in geriatric patients or in those receiving beta-adrenergic blocking agents.

Hematologic Effects: Decreased serum vitamin B12 concentrations, with or without clinical manifestations (e.g., anemia). Symptoms rapidly reversible following discontinuation of metformin or supplementation with vitamin B12.

Common Adverse Effects: Diarrhea, nausea, vomiting, abdominal bloating, abdominal cramping or pain, flatulence, and anorexia have all been reported as common AEs.

Drugs That May Antagonize Hypoglycemic Effects: Calcium-channel blocking agents, corticosteroids, thiazide diuretics, estrogens and progestins (e.g., oral contraceptives), isoniazid, niacin, phenothiazines, sympathomimetic agents (e.g., albuterol, epinephrine, terbutaline).

1.7.2 Saxagliptin:

Hypersensitivity Reactions: During postmarketing experience the following adverse reactions have been reported with use of saxagliptin: serious hypersensitivity reactions, including anaphylaxis and angioedema. Because these reactions are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency

Pancreatitis: During postmarketing experience, there have been spontaneously reported adverse reactions of acute pancreatitis. Patients should be informed of the characteristic symptom of acute pancreatitis: persistent, severe abdominal pain.

Use in Patients with Renal Impairment: Assessment of renal function is recommended prior to initiation of saxagliptin and periodically thereafter. No dosage adjustment is required in patients with mild renal impairment (CrCL >50 to ≤80 mL/min). A single dosage adjustment is recommended in patients with moderate (CrCL 30 to ≤50 mL/min) or severe (CrCL <30mL/min) renal impairment. The experience in patients with severe renal impairment is very limited.

Skin disorders: Ulcerative and necrotic skin lesions have been reported in extremities of monkeys in non-clinical toxicology studies. Although skin lesions were not observed at an increased incidence in

clinical trials, there is limited experience in patients with diabetic skin complications. Postmarketing reports of rash have been described in the DPP-IV inhibitor class. Rash is also noted as an adverse event for saxagliptin. Therefore, in keeping with routine care of the diabetic patient, monitoring for skin disorders, such as blistering, ulceration or rash, is recommended.

Immunocompromised patients: Pathology-related, immunocompromised patients, as well as patients with non-intervention-induced immunodeficiency have not been studied in the saxagliptin clinical program. Therefore, the efficacy and safety profile of saxagliptin in these patients has not been established.

In a 24-week, active-controlled study of initial therapy of ONGLYZA in combination with metformin, the adverse reactions reported (regardless of investigator assessment of causality) in ≥5% of patients are: headache (7.5% combination 5 mg saxagliptin + metformin vs. 6.3% saxagliptin 10 mg alone vs. 5.2% metformin alone), and nasopharyngitis (6.9% combination 5 mg saxagliptin + metformin vs. 4.2. saxagliptin 10 mg alone vs. 4.0% metformin alone). In this trial, the less common adverse events when saxagliptin was offered in combination with metformin as initial therapy, reported in => 2% of patients, included:: bronchitis, dyspepsia, back pain, nasopharyngitis and headache.

Hypoglycemia: When saxagliptin is added to metformin as initial therapy, the incidence of hypoglycaemia was 3.4% in patients given saxagliptin 5 mg plus metformin, 1.5% in patients given saxagliptin 10 mg alone, and 4.0% in patients given metformin alone. Under longer term use, the incidence of hypoglycaemia was 4.4% in patients given saxagliptin 5 mg plus metformin, 1.8% in patients given saxagliptin 10 mg alone, and 5.2% in patients given metformin alone.

Laboratory Tests: Across clinical studies, the incidence of laboratory adverse events was similar in patients treated with saxagliptin 5 mg alone or in combination compared to patients treated with placebo. A small decrease in absolute lymphocyte count was observed. From a baseline mean absolute lymphocyte count of approximately 2.2 x 10^9 c/L, a mean decrease of approximately 0.1 x 10^9 c/L relative to placebo was observed in a pooled analysis of five placebo-controlled clinical studies. Mean absolute lymphocyte counts remained stable and within the normal limits with daily dosing up to 102 weeks in duration. In the short term period, the proportion of patients who were reported to have a lymphocyte count ≤750 cells/microL was 1.5% and 0.4% in the saxagliptin 5 mg and placebo groups, respectively. In the short-term combined with long-term extension period of the pooled studies, the proportion of patients who were reported to have a lymphocyte count ≤750 cells/microL was 1.6% and 1.0% in the saxagliptin 5 mg and placebo groups, respectively. The decreases in lymphocyte count were not associated with clinically relevant adverse reactions. The clinical significance of this decrease in lymphocyte count relative to placebo is not known.

Postmarketing experience: During postmarketing experience the following adverse reactions have been reported with use of saxagliptin: acute pancreatitis and hypersensitivity reactions, including anaphylaxis, angioedema, rash, and urticaria. Because these reactions are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency.

Postmarketing reports of severe and disabling arthralgia: The FDA, by searching the FDA Adverse Event Reporting System (FAERS) Database as well as the medical literature [134-137], identified cases of severe joint pain associated with the use of DPP-IV inhibitors as a class of drugs. 33 cases of severe arthralgia reported with the use of DPP-IV inhibitors from October 16, 2006, approval date of the first DPP-IV inhibitor, through December 31, 2013. Each case involved the use of one or more DPP-IV inhibitor. Sitagliptin (n=28) was the most frequently reported, followed by saxagliptin (n=5), linagliptin (n=2), alogliptin (n=1), and vildagliptin (n=2; although it is not marketed in the United States). In five cases, the patient experienced severe arthralgia with two different DPP-IV inhibitors. All 33 patients

experienced arthralgia that resulted in a substantial reduction in their prior level of activity, including 10 patients who were hospitalized due to disabling joint pain. In 22 cases, symptoms appeared within 1 month of initiation of treatment with a DPP-IV inhibitor. In 20 of the 33 cases, the DPP-IV inhibitor was suspected as a possible cause of arthralgia and was discontinued within a month following the onset of symptoms. However, 8 of the remaining 13 cases reported a period of 44 days to 1 year between the onset of symptoms and discontinuation of the DPP-IV inhibitor. In 23 of the 33 cases, symptoms resolved less than 1 month after discontinuation of the drug. Reports of eight of the 33 cases documented a positive rechallenge. In these eight cases, individuals discontinued treatment, experienced a resolution of symptoms, restarted therapy with a DPP-IV inhibitor (a different member of the class in six of the eight cases), experienced the reappearance of the arthralgia, and subsequently, experienced resolution of the symptoms when DPP-IV inhibitor therapy was again discontinued. Twenty-one of the 33 patients were treated for arthritis with drug therapies that included corticosteroids, nonsteroidal anti-inflammatory drugs, methotrexate, and immune-modulating drugs. The FDA reviewed the clinical details in the FAERS cases to determine whether the severe joint pain could have been caused by an autoimmune condition rather than the DPP-IV inhibitors. Ten of the 33 cases reported fever and chills, rash, and swelling, which are suggestive of an immunological reaction. Of the 13 cases with available results of laboratory assays for systemic autoimmune disorders, 8 reported a negative or normal test result. Five cases reported positive test results: antinuclear antibody (n=2), erythrocyte sedimentation rate (n=1), C-reactive protein (n=1), and antinuclear cytoplasmic antibody (n=1). However, none of these tests are specific for a particular autoimmune condition that can cause severe joint pain.

The FDA also searched the medical literature and identified seven case reports [134-137] two of which were also identified in the FAERS database [134,137]. All seven reports described patients who developed arthralgia after starting therapy with either sitagliptin (n=6) or vildagliptin (n=1). In six cases, patients had partial or complete resolution of symptoms within 6 weeks of discontinuing the drug. Only one case reported the pain to be disabling, and none reported the need for hospitalization.

1.7.3 AZD9668:

Emerging safety profile

The following events have been identified as adverse drug reactions associated with AZD9668: Headache. Alanine aminotransferase increased, and Aspartate aminotransferase increased

Headache was the most frequently reported AE among 8/12 studies completed to date. In Study D0520C00001, it was reported by 13/36 (36%) subjects who received single doses of AZD9668, and 7/18 (39%) of those who received multiple doses, and was seen on both active and placebo. There seemed to be a dose effect in the SAD part, which was not apparent in the MAD part of the study. Most of the headaches were mild in intensity, although 1 subject had severe headache. In Study D0520C00004 the headaches generally occurred in a higher proportion of subjects on AZD9668 than on placebo. There was no evidence of a dose-related increase in the incidence of headache in either Japanese or Caucasian subjects. In the first study in patients with COPD (D0520C00002) headache was reported by fewer patients on AZD9668 (1/12 patients) than on placebo (4/6 patients). In the study in patients with cystic fibrosis (D0520C00009) it was reported by 5/29 (17%) patients on placebo and by 7/26 (27%) patients on AZD9668, and in the bronchiectasis study (D0520C00010) it was reported by 2/16 (13%) patients on placebo and by 7/22 (32%) patients on AZD9668. In the BREEZE (D0520C00012) and MISTRAL (D0520C00020) COPD studies, headache remained one of the most common AEs reported and was the most commonly reported treatment-related AEs (3 patients in the AZD9668 60 mg group) in the MISTRAL study.

Liver function In the Phase IIb COPD program there were 14 instances where patients experienced raised ALT or AST to =>3 x ULN across the studied doses (AZD9668 5, 20 and 60 mg bid); there were no cases of Hy's law during the treatment period. Although definite evidence of dose relationship was lacking, there was a suggestion of an increased incidence of liver transaminase elevations in patients on AZD9668 for which there were no definite alternative explanations. The highest transaminase elevations occurred in the highest AZD9668 dose (60 mg). Elevations in hepatic biochemistry assessments (transaminases) observed in patients in the AZD9668 studies were not always consistent with alcohol use and suggest alcohol use was unlikely to be a potential confounding factor in most cases.

A total of 57 treatment-emergent non-serious adverse events (AEs) were reported by 27 subjects. Most of the AEs were mild; 2 AEs (in the same subject) were reported as severe: syncope associated with a bradycardia, 4 h 36 minutes after AZD9668 2 mg. Both these events resolved with supportive care within 3 minutes. Six AEs were reported as moderate in intensity, including headache (1 subject on AZD9668 30 mg and 1 subject on 150 mg), migraine (1 subject on placebo and 1 subject on AZD9668 120 mg), influenza (1 subject on AZD9668 30 mg) and dizziness (1 subject on AZD9668 120 mg).

The most frequently reported AE in the SAD part of the study was headache, mostly mild in intensity. Treatment-emergent headache was reported by 11/36 subjects on AZD9668, including 5 of the 6 subjects on AZD9668 150 mg, compared with 3/12 on placebo; a further 2 subjects on AZD9668 had headaches that started before treatment and continued after dosing. In the subject who developed moderate headache on the 150 mg dose, the investigator considered this to be causally related to the study drug. The 2 events of blurred vision (subjects on AZD9668 60 mg) were mild and were considered unlikely to be related to study treatment. Two subjects on AZD9668 2 mg had vasovagal syncope (in 1 case associated with a bradycardia).

One subject on AZD9668 120 mg developed a non-sustained broad complex monomorphic tachycardia (5 beats, 2 seconds) 37 minutes after dosing. This subject was noted to have frequent supra-ventricular extrasystoles prior to dosing. The investigator considered this event not related to the study drug.

Adverse events following multiple doses of AZD9668: Multiple doses of AZD9668 in healthy subjects (Study D0520C00001): There were no deaths, other SAE, or Other Significant Adverse Events. Two subjects discontinued study drug and were prematurely withdrawn from the study because of AEs. One subject on AZD9668 70 mg was withdrawn from the study after 2 doses, because of an AE of a run of premature atrial contractions on Day 1 of the study, 1 h 25 min post-dose;
AE was not considered to be drug-related. A subject on AZD9668 120 mg was withdrawn after a single dose because of AEs of severe headache, followed by nausea and vomiting (onset 1 to 2 h post-dose), which were considered to be drug-related.

Most AEs were mild (2 were moderate in intensity and 1 was severe). The AEs recorded as moderate in intensity were headache (on AZD9668 30 mg) and dyspepsia (on placebo). The severe AE was headache (on AZD9668 120 mg), which led to withdrawal from the study.

As in the SAD part of the study, the most frequently reported AE in the MAD part was headache (2/9 subjects on placebo, 3/6 subjects on AZD9668 30 mg, 1/6 subjects on AZD9668 70 mg and 3/6 subjects on AZD9668 120 mg

Hematology and clinical chemistry (Single and multiple doses of AZD9668 in healthy subjects):
Study D0520C00001: There were no clinically relevant AZD9668 dose-related changes or trends in any

clinical laboratory parameters in Study D0520C00001.

Study D0520C00004: In Study D0520C00004, raised blood creatine phosphokinase was reported in 3 subjects; in 2 cases (both Caucasians) the finding was reported as a severe AE (1 subject who received a single dose of 30 mg AZD9668, and 1 subject who received placebo). A third subject, on placebo, also had raised creatine phosphokinase. There were no other clinically relevant AZD9668 dose-related changes or trends in any clinical laboratory parameters.

Studies D0520C00007, D0520C00016, D0520C00017 and D0520C00021 There were no clinically relevant changes in safety laboratory variables.

Vital signs and ECG (Single and multiple doses of AZD9668 in healthy subjects):
Study D0520C00001: there were no changes in vital signs judged to be of clinical significance in any of the studies. Evaluation of the digital ECG measurements in the SAD part of Study D0520C00001 indicated that there were no observations of QTcF interval (QT interval corrected using Fridericia's method) >450 ms; 3 subjects had a change from baseline QTcF >30 ms (all <36 ms). In the MAD part of the study, 1 subject had a QTcF measurement >450 ms (467 ms), and 4 subjects had a change from baseline greater than 30 ms, but less than 60 ms. None of the changes was considered to be of clinical significance.

No clinically relevant changes in vital signs or ECG were observed in Studies D0520C00004, D0520C00007, D0520C00016, D0520C00017 and D0520C00021.

Cystic fibrosis (Study D0520C00009): No clinically relevant changes in vital signs or ECG were observed in Study D0520C00009.

Bronchiectasis (Study D0520C00010): In Study D0520C00010, abnormal ECGs were observed in 15 patients (7/16 on placebo and 8/22 on AZD9668), but none was considered to be of clinical significance.

COPD Study D0520C00002: In Study D0520C00002, no patient had a QTcF >450 ms, or prolongation from baseline of QTcF >60 ms. One out of the 12 patients on AZD9668 had an increase in QTcF >30 ms (37 ms), but overall the ECG data indicated no evidence of clinically relevant changes related to study treatment.

Study D0520C00012 BREEZE: Across the AZD9668 treatment groups, there was no evidence of any dose-dependent changes in vital signs and ECG and no apparent difference in each AZD9668 treatment group compared to placebo.

Study D0520C00020 MISTRAL: There were no clinically relevant findings in vital signs or electrocardiograms during the study.

Study D0520C00014 MASCOT (draft data): The results for vital signs and ECG were unremarkable.

1.8 STUDY OBJECTIVES AND ENDPOINTS

The primary objective of this trial is to assess the efficacy of 60 mg bid AZD9668 PO bid versus placebo as adjunctive treatment to improve insulin sensitivity in obese, insulin-resistant T2D subjects as assessed by the hyperinsulinemic-euglycemic clamp method at baseline and 6 months post randomization..

Secondary objectives include:

i) To assess the safety of AZD9668 vs. placebo (rate and severity of adverse events including hypoglycemia) in obese, insulin-resistant T2D subjects;
ii) To measure the glycemic and metabolic control variables (change in baseline HbA1c levels, fasting plasma glucose levels, 2h-post-glucose challenge levels, body weight, waist- and hip-circumference, HbA1c trajectory, metformin and/or SAXA dose requirements) in AZD9668or placebo-treated obese, insulin-resistant T2D subjects;
iii) To measure changes in glucose, insulin and C-peptide levels during a 3 hour oral glucose tolerance test (OGTT) at 6 months from baseline. From the OGTT, parameters of glucose metabolism will derived: insulinogenic index using C-peptide and insulin [1-5]; glucose area under the curve (AUC), and Matsuda Index of insulin resistance [6-8], and the product of Matsuda index times the insulinogenic index
iv) To identify changes in inflammatory variables (concentrations of serum/plasma IL-6, high sensitivity-C-reactive protein, fibrinogen, TNF-alpha, soluble TNF-alpha receptors) in AZD9668 or placebo-treated obese, insulin-resistant T2D subjects.
v) Assessment of biomarkers to Determine the Expression of Functional Pharmacologic Activity of AZD9668: Desmosine levels in serum and urine.
    - Desmosine is released during matrix degradation by NE and cleared by the kidney, although it can also be detected in serum. Results from animal models and human disease studies have indicated that NE activity in vivo is associated with the release of desmosine, resulting in increased serum and urinary desmosine [127,133,147,151-153]. Thus, desmosine levels in serum and urine are relevant indirect biomarkers of the efficacy of AZD9668 on NE activity, and they will be measured in urine of the study subjects at the specified visits of the study.

Exploratory measures will include:
i) To investigate whether or not delayed AZD9668administration (at 6 months) is inferior to the early AZD9668 treatment (at trial start) on IR improvements in insulin sensitivity, metabolic measures, and beta cell function;
ii) To assess the effects of AZ9668 and placebo on the frequency and absolute cell numbers of neutrophils, T-cells, B-cells, dendritic cells (DC) as well as other peripheral blood leukocytes (PBL) over the entire study period of both trial stages;
iii) To determine the molecular signatures in PBL and purified neutrophils in AZ9668 and placebo recipients over the entire study period of both trial stages including HLA and TCR levels;
iv) To determine the changes in neutrophil cell surface phenotype over the duration of the study in placebo and AZD9668-treated subjects;
v) To identify cellular and molecular signatures of responder versus non-responder status and/or of effect of therapy in AZD9668 or placebo recipients over the entire study period of both trial stages;
vi) To correlate changes in neutrophil absolute numbers with activation of TCR and HLA-specific PBLs over time.

1.8.1 STUDY ENDPOINTS

1.8.1.1 Primary Endpoint: Improvement of insulin sensitivity by 40% or greater at 6 months compared to baseline, assessed by the 80 mU/m2 min-1 hyperinsulinemic-euglycemic clamp method.

1.8.1.2 Secondary Endpoints: We are basing these endpoints on the minimum statistically-meaningful improvements of either MET alone or MET+a DPPIV inhibitor [154-162]:

i) Improvement in glycated HbA1c levels (range=0.2 – 1.5 or better) at 6 months compared to baseline;
ii) Improvement OGTT-derived measurements, namely: glucose tolerance, glucose AUC, Matsuda Index of insulin resistance [6-8], insulinogenic index using C-peptide [1-5], and the product of Matsuda index times the insulinogenic index; bone density/total fat mass corrected insulin sensitivity indices as well.
iii) Improvement in fasting plasma glucose by 6% or better at 6 months compared to baseline;
iv) decreased concentrations of serum/plasma IL-6, high sensitivity-C-reactive protein, fibrinogen, TNF-alpha, soluble TNF-alpha receptors at 6 months compared to baseline.

1.8.1.3 Primary Efficacy Endpoint: Improvement of insulin sensitivity by 40% or greater at 6 months compared to baseline, assessed by the 80 mU/m2 min-1 hyperinsulinemic-euglycemic clamp method.

1.8.1.4 Secondary Efficacy Endpoints:
i) Improvement in glycated HbA1c levels (range=0.2 – 1.5 or better) at 6 months compared to baseline;
ii) Improvement OGTT-derived measurements, namely: glucose tolerance, Matsuda Index of insulin resistance [6-8], insulinogenic index using C-peptide [1-5]; bone density/total fat mass corrected insulin sensitivity indices as well.
iii) Improvement in fasting plasma glucose by 6% or better at 6 months compared to baseline;

1.8.1.5 Safety Endpoints: Identification of AEs:
i) rate and severity of all adverse events including hypoglycemia
ii) rate and severity of known AEs of AZD9668
iii) rate and severity of known AEs of SAXA
iv) rate and severity of known AEs of Metformin

1.8.1.6 Exploratory Endpoints: To investigate whether or not delayed AZ9668 administration (at 6 months) is inferior to the early AZ9668 treatment (at trial start) on IR improvements in insulin sensitivity, metabolic measures, and beta cell function

1.8.1.7 Mechanistic Endpoints
i) To assess the effects of AZ9668 and placebo on the frequency and absolute cell numbers of neutrophils, T-cells, B-cells, dendritic cells (DC) as well as other peripheral blood leukocytes (PBL) over the entire study period of both trial stages;
ii) To determine the molecular signatures in PBL and purified neutrophils in AZ9668 and placebo recipients over the entire study period of both trial stages including HLA and TCR levels;
iii) To determine the changes in neutrophil cell surface phenotype over the duration of the study in placebo and AZD9668-treated subjects;
iv) To identify cellular and molecular signatures of responder versus non-responder status and/or of effect of therapy in AZ9668 or placebo recipients over the entire study period of both trial stages;
v) To correlate changes in neutrophil absolute numbers with activation of TCR and HLA-specific PBLs over time.

2.0 RESEARCH DESIGN

2.1 STUDY AGENTS

1) Saxagliptin (saxagliptin HCl) 5 mg
2) Metformin (metformin HCl) 500-2000 mg

Saxagliptin and metformin will comprise the basal drug combination to which AZD9668 or AZD9668 placebo will be added.

3) AZD9668 60 mg (achieved by two tablets each of 30 mg)
4) AZD9668 placebo

2.2 STUDY AGENT DOSAGE/DOSAGE FORM, ROUTE, AND DOSE REGIMEN

Metformin will be administered as oral tablets at a daily dose of no more than 2000 mg; all patients will continue on their stable dose of metformin throughout the course of the study. Saxagliptin will be administered as oral tablets at a dose of 5 mg qd. AZD9668 will be administered as oral tablets at a dose of two tablets of 30 mg bid. AZD9668 placebo will be administered as oral tablets bid. Refer to Sections 4.5 for detailed information regarding the run-in and in-study periods.

2.2.1 Labeling, Packaging, Storage, Dispensing, and Return of Clinical Supplies

2.2.2 Product Descriptions

Investigational materials (excluding metformin) will be provided by Astra Zeneca.

Saxagliptin will be provided in tablet form at 5 mg active pharmaceutical ingredient per tablet.

AZD9668 will be provided in tablet form at 30 mg active pharmaceutical ingredient per tablet.

AZD9668 placebo will be provided in tablet form.

Metformin HCl (generic) will be sourced from the designated trial dispensing pharmacy at AHN.

The pharmacist and/or study coordinator will record the lot number, expiration date, and drug dispensed.

2.2.3 Packaging Information

All study subjects will receive drug based upon the dispensing schedule described in the Visit Schematic (Figure 4, Section 4.5). Supplies will be affixed with a clinical label in accordance with regulatory requirements.

2.2.4 Clinical Supplies Disclosure

The investigator's site personnel are not blinded to the basal drug treatment (saxagliptin+metformin) as all study subjects will take these agents. Drug identity (name, strength) is included in the label text; disclosure envelopes are not provided. However, the investigator's site personnel will be blinded to the study agents (AZD9668 or placebo). Only the dispensing pharmacy will know the identity of the study agent and will maintain a detailed record of the date of dispensing, the lot numbers, any control

numbers and identifiers as the pharmacy will transmit these data to the AHN research staff (data entry, storage, and management center as well as the liaison between the PI and the trial study coordinator as well as the study physicians).

2.2.5 Storage and Handling Requirements

The storage conditions for the saxagliptin 5 mg and the metformin 500 mg tablets will be indicated on the product label. The study agents AZD9668 and placebo will be stored at 15-25 degrees Celcius.. Upon receipt at the investigational site, the study agents should be removed from the outer secondary shipping box and stored immediately.

The clinical supplies storage area at the site must be monitored by the site staff for temperature consistency with the acceptable storage temperature range specified in this protocol or in the product label attached to the protocol. Documentation of temperature monitoring should be maintained. Supplies should be stored in the original nested box with the lid closed to minimize exposure to light. The study agents must NOT be frozen.

A temperature log must be maintained at the site. All subjects will receive storage instructions for the study drugs

2.3 Standard Policies/Return of Clinical Supplies

Investigational clinical supplies must be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the participating research pharmacist, the investigator and designated assistants have access. Study drugs are to be dispensed by the research pharmacist only in accordance with the protocol. Non-drug clinical supplies, like the glucose monitor and the glucose monitor test strips can be dispensed by the study physicians and/or the study coordinator. The research pharmacist and the site clinical investigators are responsible for keeping accurate records of the clinical supplies received from Astra Zeneca, the amount dispensed to and returned by the patients, and the amount remaining at the conclusion of the study. In accordance with Good Pharmacy Practices, gloves should always be worn by study personnel if directly handling tablets or capsules that are returned (i.e., when counting returns). At the end of the study, all clinical supplies including partial and empty containers must be returned to the pharmacy who will store the study agents returned according to the study subject identifier until the end of the trial. The site clinical investigators or designated assistant(s) should not open individual clinical supply containers and count tablets/capsules, etc., before dispensing to the patients. Any deviation from this must be discussed with the PI and Astra Zeneca.

2.4 Distributing to Site and Dispensing to Patients

The designated research staff will allocate patients based on the randomization outcome, will assign study agents to the patients, will coordinate with Astra Zeneca and the pharmacy to ensure timely reception of the study agents.

2.5 Known and Potential Risks of Study Medications

DRUG	COMMON RISKS	LESS COMMON RISKS	RARE RISKS
Metformin	Abdominal or stomach discomfort,	Anxiety, blurred vision, chest discomfort, cold sweats, coma, confusion, cool, pale skin,	Behavior change similar to being drunk, difficulty with concentrating,

	decreased appetite, diarrhea,	depression, difficult or labored breathing, dizziness, fast/irregular, pounding or racing heartbeat or pulse, feeling of warmth, headache, increased hunger, increased sweating, nausea, nervousness, nightmares, redness of the face, neck, arms, and occasionally, upper chest, seizures, shakiness, shortness of breath, slurred speech, tightness in the chest, unusual tiredness or weakness, wheezing cough or hoarseness. fast or shallow breathing, fever or chills, general feeling of discomfort, lower back or side pain, muscle pain or cramping, painful or difficult urination, sleepiness	drowsiness, lack or loss of strength, restless sleep, unusual sleepiness
Saxagliptin	headache, nasal congestion, runny nose, sneezing, sore throat,	Diarrhea, joint pain, pain or tenderness around the eyes and cheekbones, redness of the skin, weakness, welts Anxiety, bladder pain, bloating or swelling of the face, arms, hands, lower legs, or feet, bloody or cloudy urine, blurred vision, body aches or pain, chills, cold sweats, coma, confusion, cool, pale skin, cough, depression, difficult, burning, or painful urination, difficulty with breathing, dizziness, ear congestion, fast heartbeat, fever, frequent urge to urinate, increased hunger, loss of voice, lower back or side pain, nausea, nervousness, nightmares, rapid weight gain, seizures, shakiness, slurred speech, tingling of the hands or feet, unusual tiredness or weakness, unusual weight gain or loss	Constipation, darkened urine, difficulty with swallowing, flaking or peeling of the skin, hives, indigestion, large, hive-like swelling on the face, eyelids, lips, tongue, throat, hands, legs, feet, or sex organs, loss of appetite, pains in the stomach, side, or abdomen, possibly radiating to the back, puffiness or swelling of the eyelids or around the eyes, face, lips, or tongue, shortness of breath, skin rash, tightness in the chest, vomiting, wheezing, yellow eyes or skin

AZD9668		Headache, dyspepsia	Raised liver enzymes, slow heart rate,
AZD9668 placebo		Headache	Dyspepsia, dysgeusia, pneumonia, pulmonary exacerbation, non-cardiac chest pain

2.6 Risks of study procedures

2.6.1 Oral glucose tolerance test (OGTT):
There are minimal risks associated with this procedure. The oral glucose drink may cause the following rare but known adverse reactions: nausea, vomiting, abdominal bloating, and potentially headache. In addition, there is a rare incidence of hypoglycemia. Other risks include pain and phlebitis associated with venipuncture and hyperglycemia, which is transient and self-limited.

2.6.2 Hyperinsulinemic-euglycemic clamp (Clamp):
The clamp infusion uses regular insulin (Humulin-R) and therefore there is a theoretical risk of hypoglycemia. This is extremely unlikely to occur since plasma glucose concentration is monitored every 5-10 mins and fixed at approximately 5 mmol/l by a variable infusion of glucose 20%. Another potential risk is self-limited pain and phlebitis associated with venipuncture and peripheral catheter placement.

2.6.3 Venipuncture:
At the OGTT and the Clamp tests as well as during collection of peripheral blood-Common risk - include pain, bruising, inflammation or excessive bleeding at the venipuncture site and faintness/lightheadedness. Also, multiple punctures might be required to identify the veins. Rare risks include- hematoma, infection.

2.6.4 DEXA total body scan:
The low doses of ionizing radiation encountered in a standard scan are considered insignificant when compared to natural background radiation that one encounters everyday (approximately 7 microsieverts per day). DEXA scans result in radiation of the order of 0.1 – 0.4 microsieverts. This dose for total body scan is lower than that of lumbar spine and proximal femur scans as total body scans use a lower x-ray current setting, the transverse passes of the scan arm do not overlap, and the scan arm moves more quickly down the length of the scan table.

3.0 STUDY POPULATION

Forty two (42) type 2 diabetic subjects will be recruited, see Section 6.5 for details regarding sample size.

3.1. INCLUSION CRITERIA

1. Patients 21-75 years of age inclusive who meet the American Diabetes Association standard criteria for type 2 diabetes mellitus (T2D).
2. Subjects are currently on metformin (at least 1000 mg per day) for a minimum period of 4 weeks prior to screening visit alone, or in combination with any of the following diabetes medications or combinations:
    a. DPPIV inhibitor (any dose level/frequency)
    b. Sulfonylurea (any dose level/frequency)
    c. GLP1 agonist (any dose level/frequency)

d. Sulfonylurea (any dose level/frequency) + GLP1 agonist (any dose level/frequency)
e. Meglitinide (any dose level/frequency)
f. SGLT2 inhibitor (any dose level/frequency)

3. Patients must have a body-to-mass index (BMI) of greater than or equal to 27 kg/m^2.
4. Patients exhibit glycated HbA1c between 7.3-11.0 during eligibility screening and then <=8.5 at final run-in visit.
5. Willingness to replace current diabetes therapies (listed in inclusion 2) with metformin and saxagliptin and to adjust metformin dose during run-in period.
6. Subjects present adequate immune competence as assessed by immunoreactivity to viral antigens (CEF Pool Assay) in vitro at the time of screening.
7. Participants of childbearing potential must agree to practice an effective form of birth control which may include any one of the following: barrier method, oral contraception, or surgery. These measures must be maintained throughout the study.
8. Subjects must have good peripheral venous access for the hyperinsulinemic-euglycemic clamp and the 3-hr. OGTT procedures.
9. Patients understand the study procedures, alternative treatments available, risks involved in the study, and voluntarily agree to participate by giving informed and signed written consent for screening and enrollment.
10. Participants can be on anti-inflammatory therapies that are not diabetes-focused (e.g. non-salicylate anti-inflammatory therapies, non-salicylate NSAIDs) and/or anti-hypertensive medicaments or statins.

3.2 EXCLUSION CRITERIA

1. Patients with type 1 diabetes mellitus as defined by the American Diabetes Association criteria or a history of ketoacidosis, or the patients are assessed by the study team as possibly having type 1 diabetes mellitus confirmed with the presence of at least one of the typical autoantibodies (insulin, GAD65, IA-2, ZnT8) AND a serum C-peptide level of <0.7 ng/mL.
2. Patients have been treated with any therapies specific for their diabetes (other than those listed in the inclusion criteria) within 4 weeks of the screening visit.
3. Patients have been treated with insulin within 2 months of the screening visit.
4. Patients are currently participating in or have participated in another study with an investigational compound or device within the prior 12 weeks of signing the informed consent or do not agree to refrain from participating in any other study while participating in this study.
5. Patients have a history of hypersensitivity or any contraindication to DPPIV inhibitors, including saxagliptin (Onglyza), or metformin based upon the labels of the USA.
6. Patients are on a weight loss medication (such as orlistat, phentermine, Qsymia, or Belviq) within the prior 6 weeks.
7. Patients are required by treating physicians to remain on any one of these agents during the trial: macrolide antibiotics, cisapride, anti-arrhythmics, steroids, rifampicin, phenobarbital, phenytoin, secobarbital, carbamazepine, norethindrone, isoniazid. AZD9668 is metabolized by CYP3A4, 3A5, and 2B6. SAXA is metabolized by CYP3A4 and 3A5, potentially leading to drug-drug interactions with hypothetical adverse events in patients on the above agents. Also, AZD9668 causes weak inhibition of CYP2C9 and therefore patients on fluconazole, amiodarone, fenofibrate, fluvoxamine, phenylbutazone, probenecid, sertraline, will also be excluded to avoid the hypothetical adverse events due to this effect.
8. Patients have undergone major surgery within the 6 weeks prior to signing consent or have any type or form of major surgery planned during the study (at the discretion of the physician).
9. Patients are on or are likely to require treatment with 14 consecutive days or repeated courses of pharmacologic doses of corticosteroids or any other immunomodulatory agent. For example,

patients requiring chronic systemic corticosteroids (does not include topical or inhaled corticosteroids). Exceptions are over the counter non-salicylate NSAIDs.

10. Enrollment or history of enrollment in a drug, or biologic therapy clinical trial that affects the immune system within the past 12 months (e.g., systemic immunosuppressive pharmacologics, immunosuppressive cytokines, therapeutic immunomodulating antibodies, therapeutic immunomodulating fusion proteins and/or cytokine receptor decoys as well as any intervention and/or non-intervention induced immunodeficiencies).
11. Prior history of coronary artery disease (defined as myocardial infarction, angina, bypass surgery, or angioplasty)
12. Prior history of arrhythmia (excludes premature beats)
13. Prior history of heart failure defined as i) symptomatic OR ii) pulmonary edema, leg edema or low ejection fraction (<40%)
14. Evidence of refractory chronic migraine (defined in ICHD-3 and Martelletti et al. [9,10]).
15. History of persistent bradycardia within the last year prior screening visit (more than three episodes in a calendar year of a heart rate <60 beats per minute that required hospitalization on each of these occasions).
16. Leukopenia (<3000 leukocytes/microliter), neutropenia (1500 neutrophils/microliter), lymphopenia (<800 lymphocytes/microliter), or thrombocytopenia (<125000 platelets/microliter),. any other clinically relevant abnormal hematology value.
17. Positivity for HIV, active CMV, chlamydia, any evidence of serious fungal infection, active HSV1, HSV2, hepatitis B or C, or history of HSV1 or HSV2 at screening. Minor skin fungus, or minor candidiasis is not an enrollment or treatment exclusion criterion. Also, with the exception of HIV history, hepatitis B and C, successfully-treated, disease-free individuals (> 6 months between time of successful treatment confirmation and time at screening) would be eligible for enrollment in this trial.
18. Patients are required by treating physician to remain on any medications listed in inclusion #2 that directly affect glucose metabolism such as, but not limited to thiazolidinediones, pramlintide, or amylin.
19. Vaccination with any form of live vaccine product within the last 3 months prior to initiation of study agent administration.
20. Any chronic disease that in the opinion of the investigators would affect the patient's safety and/or the integrity of the study outcome. This does not include dyslipidemia, patients on statin or anti-hypertension treatment, or patients with well-controlled hypo- or hyperthyroidism
21. Any other disease or disorder requiring chronic drug therapy except for treated hypothyroidism (T4 and TSH should be within the normal reference range adjusted for age), celiac disease, or statin-maintained, uncomplicated lipidemia.
22. Evidence of liver dysfunction, with ALT or AST> 1.5 times the upper limit of normal.
23. Evidence of renal insufficiency as indicated by blood creatinine of > 2 times the upper limit of normal at baseline screening OR an eGFR < 45 mL/min. OR A past history or current clinical evidence of renal failure or low creatinine clearance at screening.
24. Females who are pregnant at the time of screening or unwilling to defer pregnancy during the study period.
25. Lactating women.
26. Poor accessibility to veins for the 3-hour OGTT and hyperinsulinemic-euglycemic clamp procedures.
27. The following therapies cannot be administered while patients are undergoing treatment on this protocol: i) radiation therapy; ii) chemotherapy; iii) corticosteroids (except for very short courses of topical or inhaled); iv) agents used to treat attention deficit and hyperactivity disorder (ADHD); v) rifampicin or phenytoin; vi) other protein, particle or cell vaccine immunomodulation therapies. If these therapies are essential for treatment of other conditions, participation in this study will be terminated.

28. A condition which interferes with the ability to accurately determine glycated HbA1c. Examples include: Genetic variants (e.g. HbS trait, HbC trait), elevated fetal hemoglobin (HbF) and chemically modified derivatives of hemoglobin (e.g. carbamylated Hb in patients with renal failure); Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia); Iron deficiency anemia, iron replacement therapy
29. Subjects who cannot tolerate at least 1000 mg daily of immediate or extended release metformin by the time of final run-in will be excluded from further participation.
30. Subjects who do not exhibit a glycated HbA1c level <=8.5 by the end of the run-in period.

3.3 STOPPING RULES

The trial will:

a) **stop enrolling study subjects; AND**
b) **request the study subjects in-trial to stop study agent administration (AZD9668 or placebo);**

IF ANY OF THE FOLLOWING OCCUR:

1. The FDA, the IRB, NCATS or the DSMB requires termination of the study upon review of the safety data.
2. Any death occurs that is possibly or definitely related to AZD9668
3. If within the first three months of their first dose, three or more of the first (initial) 6 participants treated with AZD9668 experience a study-related adverse event resulting in the permanent discontinuation of the treatment (as defined in Section 4.7.2).
4. Infection that persists for > 2 weeks from expected time of mean clearance (clear of symptomatic evidence of disease) in the general population. Fingernail mycosis, athlete's foot, minor candidiasis, limited cutaneous warts and clinically insignificant, minor infections of this type and category allowable.
5. Any expected adverse event considered possibly or definitely related to AZD9668 that occurs at a greater severity than previously observed or listed in the study documents and is therefore considered unexpected and classified as an AE of special interest (AESI).

The DSMB will be notified of any of the above events within 5 days of study team becoming aware. In addition, any events that meet FDA or IRB reporting requirements will be reported within the respective required timeframes.
In the event of trial stop or termination, all study subjects will return to their standard-of-care diabetes management plan that was in place prior to trial enrollment which will include pre-trial medication.

Resumption of enrollment and study agent (AZD9668 or placebo) treatment is contingent upon a favorable IRB and DSMB safety review.

3.4 STUDY TIMELINE AND MILESTONE PLAN

The below Go/No Go points will be reviewed by the PI, study DSMB and Medical Monitor as they evaluate study progress. Our primary milestones are primarily based on detecting AEs and then making Go/No Go decisions based on those along the statistical parameters established by Simon, Gonin and Lazaridis [163-167].

3.4.1 Go/No Go Points: (See Figure 1)– It is important to note that the Go/No Go decisions will be based on an evaluation of AEs/SAEs and these decisions will be carefully made taking into account chiefly what the DSMB believes to be due to the therapeutic intervention. We will evaluate these points at the following timeframes following the start of treatment with study drug:

i) Month 1 after start of AZD9668 treatment: if no unexpected AE considered related to the study treatment or procedures of grade >2 (as defined by the NCI Common Terminology Criteria for Adverse Events version 4.0 [http://ctep.cancer.gov/reporting/ctc.html]) is seen in 3 of the first 6 study subjects enrolled we will continue enrollment

ii) Month 3 after start of AZD9668 treatment: Again, if no unexpected AE considered related to the study treatment or procedures of grade >2 are seen in 6 of the first 12 study subjects enrolled we will continue enrollment

iii) Month 6 after start of AZD9668 treatment: If, again, no unexpected AE considered related to the study treatment or procedures of grade >2 are seen in 9 of the first 18 study subjects enrolled we will continue enrollment as necessary.

The secondary milestones are based on enrollment rate; A Go/No Go point will be evaluated if <25% of the total study subject population is enrolled by 6 months of the start of the trial. A second Go/No Go point will be evaluated if <50% of the study population is enrolled by 1 year following the start of the trial. Given the safety record of AZD9668, we do not anticipate any particular regulatory impediments.

3.4.2 Procedure and Pace of Enrollment and Study Duration

We plan to enroll 42 subjects in this trial. The subject's complete participation in this trial will last approximately 15 months (up to 66 weeks).

3.4.3 Milestone Grid (Figure 1) – REMOVED DUE TO MODIFIED SCHEDULE

4.0 COMPLETE PROTOCOL

4.1 Overview

This is a trial to be conducted in the USA. Potential study subjects must be either on MET monotherapy, GLP-1 analog monotherapy, or a combination of MET+(either a DPPIV inhibitor, OR a sulfonylurea OR a GLP-1 analog) prior to enrollment. These drugs will be substituted by the combination of MET+SAXA as background drugs (also describing the "placebo" arm) for the purpose of the trial. The trial consists of a 2-arm, randomized, double-blinded, placebo-controlled, crossover study in 42 T2D subjects (n=38 + 4 to account for a possible 10% drop-off; refer to power analyses in Section 6.5; between 21-75 years of age, inclusive, with a monitoring period of 12 months following a 1:1 randomization into AZD9668:placebo arms.

A 3-month run-in period will precede baseline assessments. With the inclusion/exclusion criteria proposed herein and based on past experience in T2D clinical trials, we estimate that we can enroll one subject for every 4 screened and every 2 that enter the run-in period. Randomization will take place after baseline assessments. The run-in period is aimed at achieving/maintaining an HbA1c level <= 8.5 using an optimized regimen of MET+SAXA. During the run-in, MET dosing will be according to the MET titration information in Section 4.6.2 and Figure 6.

SAXA will replace the DPPIV inhibitor in subjects on another DPPIV inhibitor and will be administered at a dose of 5 mg qd, which is the most common SAXA regimen in published clinical trials[138-146] for the run-in and study period. Subjects will then receive AZ9668 or placebo as adjunctive treatment to the MET+SAXA for a 6 month period. AZD9668 will be administered bid at two tablets of 30 mg bid for 6 months, in line with other AZD9668 clinical trials where the agent was well-tolerated [127-129,131,132,147]. After the 6 month evaluation point of insulin sensitivity, assessed by the 80 mU/m^2 min^{-1} hyperinsulinemic-euglycemic clamp method [148-150], those on placebo will be crossed over and assigned to AZ9668 and those on AZD9668 will be crossed over into the placebo arm (MET+SAXA+"placebo pill") for comparison of efficacy. All study subjects will remain on MET+SAXA throughout the entire 15 months.

There is no need for a washout period due to the crossover design of the trial.

Insulin sensitivity and AE monitoring of all arms will continue to 12 months. The hyperinsulinemic-euglycemic clamp technique is the gold standard for assessing insulin sensitivity in humans [149,150]. The choice in this trial is for a dose of insulin of 80 mU/m^2/min which will provide sufficient stimulation of glucose uptake by skeletal muscle and completely suppress endogenous glucose production. In addition, this study will also conduct glucose tolerance tests to assess the effect of AZD9668 on glucose tolerance and secondary parameters of glucose metabolism such as insulin secretory response using the 3-hr. OGTT. The OGTT and clamp procedures will be separated by at least a week in the 6- and 12 month assessment points. The subject's complete participation in this trial will last approximately 15 months (up to 66 weeks).

The trial will be conducted under an intent-to-treat design such that all randomized subjects will continue follow-up and complete all outcome assessments until they complete participation in the trial. Otherwise, analyses of all outcomes would be susceptible to a healthy survivor effect where the only subjects evaluated at out years would be those who have not yet experienced primary failure of the assigned regimen.

Metformin has been selected as the foundation therapy based on the same rationale and characteristics as were used in the recently developed consensus algorithm (S41-Pharmacological Therapy for Type 2 Diabetes, in: http://professional.diabetes.org/admin/UserFiles/0%20-%20Sean/Documents/January%20Supplement%20Combined_Final.pdf): namely, its long-term clinical experience; effectiveness in lowering glycemia over a wide range of HbA1c levels without causing hypoglycemia; its weight-neutral or weight-loss effect; probable cardiovascular risk reduction (10, 11, 23); safety profile; side-effect profile; high level of patient tolerance; and its low cost. Recent studies have shown that a large majority of patients with recent-onset T2DM are treated with metformin, making this design both practical and clinically relevant.

4.2 STUDY PROTOCOL DIAGRAM/FLOW CHART

Figure 2

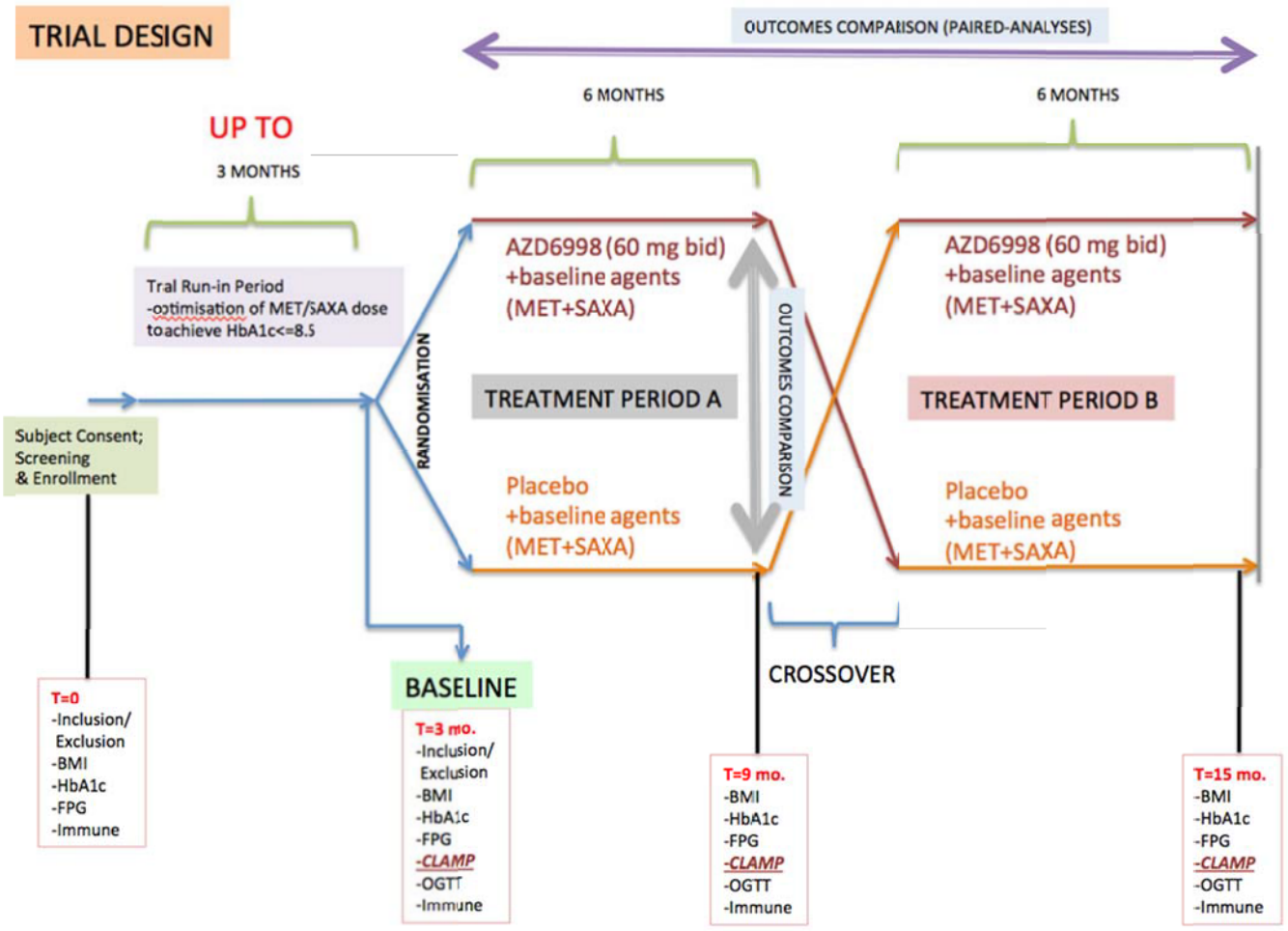
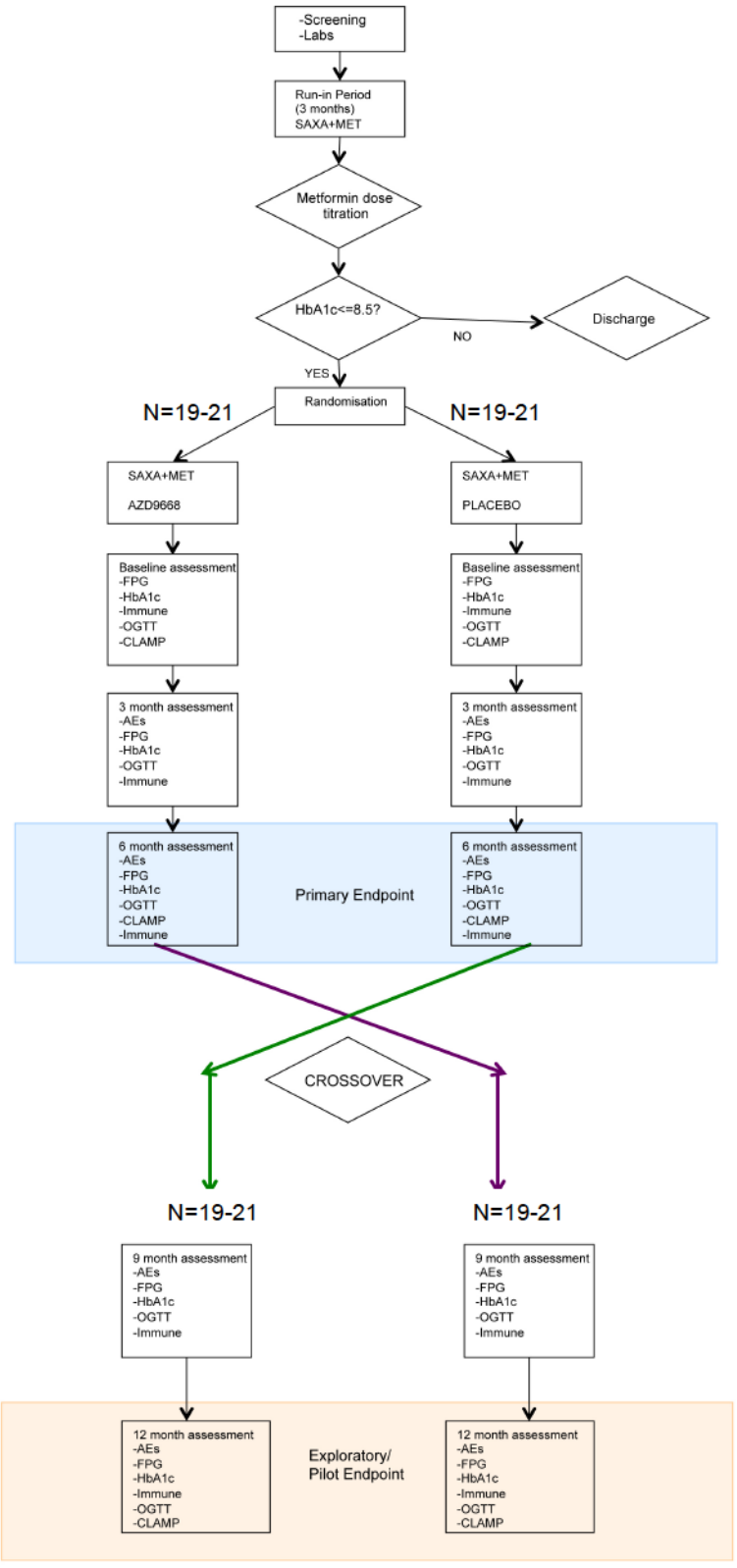


Figure 3


-Screening
-Labs
Run-in Period
(3 months)
SAXA+MET
Metformin dose titration
HbA1c<=8.5?
NO
Discharge
YES
Randomisation
N=19-21
N=19-21
SAXA+MET
AZD9668
SAXA+MET
PLACEBO
Baseline assessment
-FPG
-HbA1c
-Immune
-OGTT
-CLAMP
Baseline assessment
-FPG
-HbA1c
-Immune
-OGTT
-CLAMP
3 month assessment
-AEs
-FPG
-HbA1c
-OGTT
-Immune
3 month assessment
-AEs
-FPG
-HbA1c
-OGTT
-Immune
6 month assessment
-AEs
-FPG
-HbA1c
-OGTT
-CLAMP
-Immune
Primary Endpoint
6 month assessment
-AEs
-FPG
-HbA1c
-OGTT
-CLAMP
-Immune
CROSSOVER
N=19-21
N=19-21
9 month assessment
-AEs
-FPG
-HbA1c
-OGTT
-Immune
9 month assessment
-AEs
-FPG
-HbA1c
-OGTT
-Immune
12 month assessment
-AEs
-FPG
-HbA1c
-Immune
-OGTT
-CLAMP
Exploratory/
Pilot Endpoint
12 month assessment
-AEs
-FPG
-HbA1c
-Immune
-OGTT
-CLAMP

4.3 RECRUITMENT

Potential subjects will be recruited after protocol approval by local IRBs. The combined diabetes clinics of the Allegheny Health Network [AHN] and AHN/Joslin Diabetes Clinics as well as the University of Pittsburgh Medical Center [UPMC] currently follow more than 2000 unique T2D subjects. Also, the two clinics combined diagnose approximately 500 new cases annually. Industry-sponsored and federal government-supported clinical trials are ongoing in all clinics with dedicated resources and personnel. Based on a very recent search of the proposed trial site patient databases (AHN/Joslin and UPMC), there are currently >2000 patients who entered the clinics as of the 1st of January 2011 for treatment of T2D. Potential recruitment approaches include:

i) Trial brochures/flyers in the clinics
ii) Web-site/Facebook/Twitter
iii) Advertising on local radio and public transportation
iv) Advertising and advocacy through the local American Diabetes Association Chapters

We will aim to recruit as much representation as possible from racial and ethnic minority groups that are disproportionately affected by type 2 diabetes, encouraging the clinical centers to pay particular attention to these populations during recruitment. Clinical centers were selected to ensure an adequate distribution by race/ethnicity and age.

4.4 INFORMED CONSENT

Before study initiation, the investigator must have written and dated approval from the IRB for the protocol, consent form, and any other materials to be provided to subjects. All participants capable of providing informed consent must read, sign, and date the IRB-approved consent form and HIPAA authorization before entering the study, receiving study agent, or undergoing any study-specific procedures. Individuals interested in participating in the study will be offered information as well as the study consent forms. There are two consent forms; one for the procedures being conducted at AHN and the other for the procedures conducted at UPMC.

The clinical study investigators will review the consent form and answer questions and the subject will be given ample time to review and decide whether or not to participate. The prospective study subject will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. The investigator must obtain documented consent from each potential patient prior to participating in a clinical trial. Consent must be documented by the patient's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the patient before participating in the trial.

The initial informed consent form and any subsequent revised written informed consent form and any written information provided to the patient must receive IRB approval in advance of use. The patient should be informed in a timely manner if new information becomes available that may be relevant to the patient's willingness to continue participation in the study. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the patient's dated signature.

4.4.1 Consent and Collection of Specimens for Genetic and Other Biomedical Research

In the informed consent, it will be made clear that one of the tests in the peripheral blood leukocytes will involve transcriptomic/epigenotype analyses, and even though these tests and their objective is not intended for "genetic analysis" in the strict definition of the term, the outcome data could in theory be extrapolated to deduce potential genetic risks of disease. The consent form will be clear that such extrapolations are not the objective of the trial, nor will they be pursued. Given the mandatory nature of this testing, if the study subject does not consent, they cannot be enrolled in the trial.

In the informed consent, the study subject will also be made aware that any left-over blood samples are subject for storage and for future research on T2D (de-identified samples). This is contingent upon their consent explicit for this storage purpose. Only those subjects who have consented to allow storage of left-over samples will have those samples stored. The investigator or designate is responsible for explaining the optional nature of the storage of biosamples and that participation in the associated clinical study is not dependent upon giving consent for such storage or any future research use of these samples.

4.5 STUDY APPROACH

All subjects will follow the study procedures outlined in the Visit Schematic (Figure 4, Section 4.5). Unless otherwise noted, all study visits will take place within facilities in the Allegheny Health Network and all procedures and tests will be conducted as approved by AHN IRB.

Every effort should be made to adhere to the visit schedule. Visits should be scheduled ± 7 days of the designated time-point and phone calls within ± 4 days. If unavoidable, a visit may be scheduled at a time outside of this recommended range, but the schedule for subsequent visits must be maintained so that the total duration of the treatment period is as close as possible to 12 months. If a visit is scheduled at a time other than the protocol designated time, careful consideration must be given to the amount of study medication the subject has available.

Patients should be counseled to fast (no food or drink except water and non-study medications as prescribed) for at least 8 hours prior to all study visits where blood and/or urine will be collected or for the OGTT and clamp procedures.

4.5.1 SCREENING

The information collected during the screening visit will allow us to assess eligibility inclusion/exclusion criteria and identify the study subjects to be further considered. Once eligibility has been confirmed, subjects will proceed to the run-in period to determine suitability for randomization. No more than two weeks will elapse between Visit 1 and the Run-in Visit (Visit 2).

FIGURE 4 – VISIT SCHEMATIC

VISIT #	1	2	TC[6]	3	4	5	5p	6	6p	7	8	8p	9	9p	10	11	11p
WEEK #	**-2**	**0**	**1-11**	**10**	**12**	**14**	**18**	**24**	**30**	**36**	**38**	**42**	**48**	**54**	**60**	**62**	**64**
Consent	x																
Diet/exercise		x			x					x					x		
Complete physical exam[1]	x				x	x		x		x	x		x		x	x	
Medical history	x																
Review adverse events		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
Review medications		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
Health status update[5]				x			x		x			x		x			x
Collection of glucose meter data					x			x		x			x		x		
Dispense MET		x			x			x		x			x				
Dispense SAXA		x			x			x		x			x				
MET titration (review & adjust MET dosing)		x	x	x													
Dispense study agent					x			x		x			x				
Assessment of drug compliance					x			x		x			x		x		
Randomization					x												
Crossover										x							
Return of study drug & equipment															x		
3hr OGTT and C-peptide					x					x					x		
Euglycemic clamp[3]						x					x					x	
DEXA[3]						x					x					x	
Virology & Pregnancy	x																
Hematology &	x				x			x		x			x		x		
Chemistry	x				x			x		x			x		x		
HgbA1c[2]	x			x	x			x		x			x		x		
C-peptide[2]					x			x		x			x		x		
Urine	x				x			x		x			x		x		
Immunology flow cytom	x				x			x		x			x		x		
Immunology luminex	x				x			x		x			x		x		
Immunology neutrophils					x			x		x			x		x		
Genomics					x										x		
Immunology Urine					x			x		x			x		x		

p-indicates phone call
1 – includes height (first visit only), weight and vital signs,
2 – all blood samples collected will be fasting samples
3- occurring at Univ of Pittsburgh site
4- total blood draw volume per visit will not exceed 6 tab (with the exception of clamp visits)
5- Health status update includes discussion of doctor visits/hospitalizations, recent glucose readings, current medication doses, pregnancy status, diet, and exercise
6-TC defined as titration contact to be completed weekly (via phone or email) and will include assessment of subject glucose levels and MET dose as described in Section 4.6.2

4.5.2 RUN-IN

All subjects who meet eligibility criteria following the screening visit will enter a run-in period (minimum approximately 6 weeks, maximum approximately 12 weeks, depending on metformin exposure). Potential subjects who are at the study-specified maximum dose (2000 mg per day) of metformin can complete the run-in period in less time (as little as 6 weeks), whereas potential subjects who need to adjust metformin to achieve the study-specified dose can complete the run-in in as little as 8 weeks and a maximum target of 12 weeks.

The goals of the run-in period include the following: adjustment of metformin dose to a goal of no higher than 2000 mg daily (minimum 1000 mg daily); achievement of a glycated HbA1c target of <=8.5 by the time of the final run-in visit; and demonstration of adherence to study procedures (visit attendance, medication taking) and of willingness to perform self-monitoring of blood glucose.. During run-in, Subjects will adjust MET according to the MET titration information in Section 4.6.2.

During the run-in period, the diabetes medications of all participants will be changed to study supplied metformin and saxagliptin (5 mg qd)

Study subjects must also not have at any time during run-in, reported instances (verified by the study team) of persistent arthralgia (i.e. at least twice in 7 days, for at least two weeks, of intense pain at any of the body joints, along with sudden joint swelling or an inability to move the joint(s)). As noted in Section 1.7.2, verified persistent arthralgia deemed by the medical monitor and the DSMB to be saxagliptin-related, in a study subject during the run-in phase at any time, will be grounds for their withdrawal and discontinuation in the trial (Refer to Section 4.7.2 for Discontinuation).

4.5.3 RANDOMISATION

At the end of run-in, if fasting plasma glucose is >140 mg/dL AND if the glycated HbA1c level is >8.5 the study subject will not be eligible to continue in the trial.

If fasting plasma glucose is <=140 mg/dL AND if the glycated HbA1c level is <=8.5 the study subject will be requested to maintain the current dose and dose frequency of metformin and will be eligible to proceed in the trial.

Eligible study participants will be randomized following the end of the run-in period and will be assigned to a randomization code and will be thus randomized to receive either AZD9668 (60 mg bid) or AZD9668 placebo starting at Study Visit 9.

It is formally possible that some study subjects could meet the run-in glycemia targets by 8, or 10, or 12 weeks into the run-in period. If so, they will be randomized without requiring addition time in the run-in period.

Randomization will be conducted via a central web-based system. The participant, the clinical investigator, and clinical personnel will be masked to the treatment assignment. Laboratories performing assays for this protocol will be masked to the treatment assignment and the identity of each participant whose biological material is to be studied. The AHN research staff will have the only access to the identity of the study subject and the treatment arm they are randomized into.

Research staff authorized to conduct randomization as specified in the site delegation log will be assigned an individual unique identifier code. They must use only their assigned identifier code to access the patient information system and must not share their assigned identifier code with anyone.

Randomization of allocation numbers will be performed via a computer-generated allocation schedule. Subject treatment assignment will follow the entry of a unique subject identifier in order to be randomized. The unique subject identifier will preclude a single patient/subject assigned more than 1 randomization number.

4.5.4 TREATMENT PERIOD A

i) The AZD9668 arm will remain on the optimized MET+SAXA as established during the run-in period and will be treated with two tablets of 30 mg AZ9668 bid as adjunctive treatment for a 6 month period;
ii) The placebo arm will remain on the optimized MET+SAXA as established during the run-in period and will be treated with a color- and size- matched placebo tablet bid as adjunctive treatment for a 6 month period.

4.5.5 WASHOUT

The crossover design obviates the requirement of a washout period, since the AZD9668 arm will go on placebo (i.e. the background drugs) and vice versa.

4.5.6 TREATMENT PERIOD B

i) At the start of month 7, subjects originally in the placebo arm will be crossed over into the AZD9668 arm. These subjects will remain on the optimized MET+SAXA as established during the run-in period and will be treated with two tablets of 30 mg AZD9668 bid as adjunctive treatment for a 6 month period;
ii) At the start of month 7, subjects originally in the AZD9668 arm will be crossed over into the placebo arm. These subjects will remain on the optimized MET+SAXA as established during the run-in period and will be treated with oral placebo (two tablets bid) that is size- and color- matched to the 30 mg AZD9668 as adjunctive treatment for a 6 month period.

The first crossover dose of AZD9668 (two tablets of 30 mg) or placebo will be administered in the clinic as a witnessed dose.

4.5.7 UNSCHEDULED VISITS

In addition to the scheduled visits, it is possible that subjects may need to complete an unscheduled visit if they experience an event that the clinical PI believes to warrant follow-up. The unscheduled visit will include a full physical examination and blood sample collection (less than 3 tab) based upon the event.

4.5.8 END OF STUDY PROCEDURES

After study treatment ends, questions concerning the next steps in the management of diabetes will be answered and the subject will then be discharged from the trial. At this point, the study subject will be asked to return all the trial study medication (MET, SAXA, AZD9668, and placebo) to the nurse/study coordinator and will be transferred to their normal diabetes management physician provider for their diabetes treatment and care.

Poststudy Telephone Follow-Up

Approximately 28 days following Visit 10 or two weeks following a trial discontinuation visit, subjects will be contacted by telephone to assess for any AEs that occurred after the administration of the last dose of study medication (see Visit 11p as described in Figure 4.5).

4.6 STUDY PROCEDURES

4.6.1 LABORATORY MONITORING

All laboratory tests will be performed according to the Visit Schematic (Figure 4) either by the central laboratory of the Allegheny Health Network or the PI laboratory within the AHN Institute for Cellular Therapeutics. The specific tests to be completed are detailed in Appendices A and B.

Fasting Prior to Scheduled Study Visits: Subjects should be counseled to fast (no food or drink except water and non-study medications as prescribed) for at least 8 hours prior to all study visits where blood and/or urine will be collected or for the OGTT and clamp procedures.

No laboratory test results will be masked to the research staff for this study. Subjects with elevations greater than 3-times upper limit of normal (ULN) in liver transaminases (i.e., ALT and AST) will be flagged by the central laboratory and subjects will be retested.

NOTE ON SHARING OF LAB RESULTS WITH STUDY SUBJECTS: With the exception of the test results for HIV, CMV, HSV, hepatitis B or C, none of the other lab results will be available for the study subject. Release of lab result data to the study subject will be made only if the study physicians and/or the medical monitor in consultation with the DSMB and the IRB deem there is a medically-necessary reason to do so.

The clinically relevant measurements such as fasting glucose, HbA1c, blood pressure, and lipid levels, may be shared with the subjects' health care provider.

4.6.2 METFORMIN TITRATION AND DOSING

Subjects' diabetes medication will be replaced with metformin 500 mg and will be dispensed from the pharmacy. Metformin will be adjusted as follows: The study subjects will be requested to not take their diabetes medications on the day of Visit 2, in order to transition all participants to study supplied metformin. If already taking 2000 mg per day (1000 twice per day) of metformin and tolerating that dose, no further changes will be made. If taking more than 2000 mg per day of metformin, their dose will be reduced to 1000 mg twice per day. If taking less than 2000 mg per day, the potential participants will increase the dose approximately weekly by 500 mg, with doses taken with meals (usually breakfast and dinner) to a target dose of 2000 mg daily, as tolerated. If potential participants develop gastrointestinal intolerance during metformin titration, metformin XR can be used to try to get them to 2000 mg daily or to a minimum of 1000 mg daily. Potential participants who do not tolerate at least 1000 mg per day of metformin will not be eligible for randomization. Metformin or Metformin XR will be provided free-of-charge to study participants. Subjects who enter run-in but are not randomized will be provided with study metformin as they transition their diabetes care back to their primary care provider and the pre-run-in diabetes medication. The first dose of metformin 500 mg will be administered in the clinic as a witnessed dose. At drug dispensing visits, subjects will return the MET to the pharmacy and procure a new supply until the next scheduled drug dispensing.

METFORMIN TITRATION DURING RUN-IN (to be co-ordinated between study subject and trial study staff: Prior to up-titrating metformin, subjects must call or email study staff requesting up-titration as per schedule shown immediately below. Study staff must confirm the up-titration in response and record the new dose level). Also described in **FIGURE 6**

1. Begin with low-dose metformin (500 mg) taken once per day with meals (breakfast and/or dinner).
2. After 5-7 days, if fasting blood glucose levels are <= 140 mg/dL, maintain the metformin dose until the next week.
3. If fasting blood glucose is >140 mg/dL, increase the metformin dose by 500 mg per day taken once per day with meals (breakfast and/or dinner).

4. If gastrointestinal side effects appear (stomach cramps, abdominal or stomach discomfort, stomach upset, stomach acidity, heartburn, indigestion, nausea, diarrhea), decrease metformin to previous lower dose.
5. 5-7 days later, measure fasting blood glucose. If >140 mg/dL increase the metformin dose by 500 mg per day taken once per day with meals (breakfast and/or dinner). If fasting blood glucose levels are <= 140 mg/dL, maintain the metformin dose until the next week.
6. If gastrointestinal side effects appear (see above) as doses advanced, decrease to previous lower dose.
7. 5-7 days later, measure fasting blood glucose. If >140 mg/dL increase the metformin dose by 500 mg per day taken once per day with meals (breakfast and/or dinner). If fasting blood glucose levels are <= 140 mg/dL, maintain the metformin dose until the next week.
8. If gastrointestinal side effects appear as doses advanced, decrease to previous lower dose.
9. 5-7 days later, measure fasting blood glucose. If >140 mg/dL increase the metformin dose by 500 mg per day taken once per day with meals (breakfast and/or dinner). If fasting blood glucose levels are <= 140 mg/dL, maintain the metformin dose until the next week (Run-in Study Visit 2).
10. If gastrointestinal side effects appear as doses advanced, decrease to previous lower dose.

Do not go over 2000 mg metformin per day.

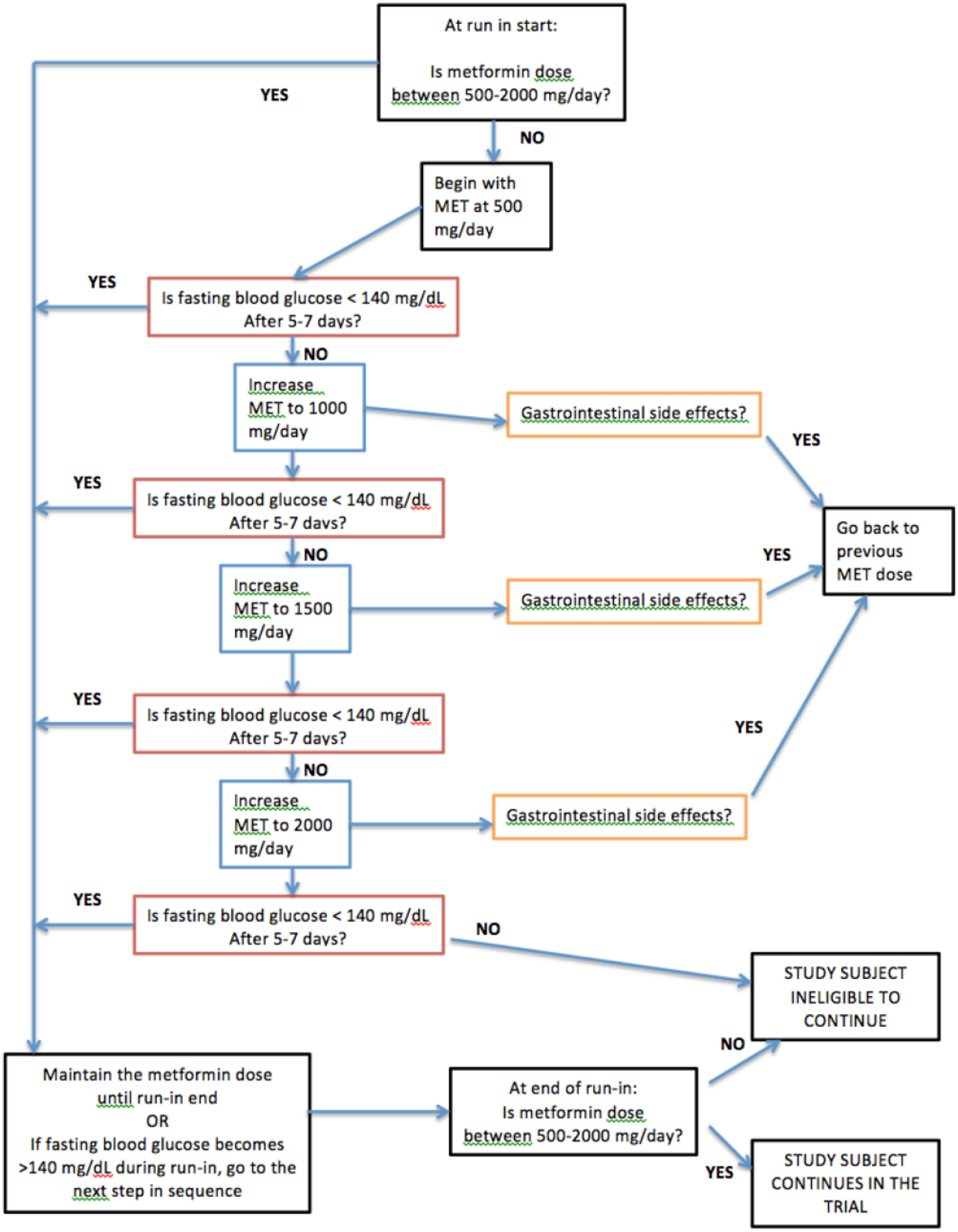
If fasting plasma glucose is >140 mg/dL AND if the glycated HbA1c level is >8.5 the study subject will be instructed to titrate the metformin dose upward by 500 mg per day to no more than 2000 mg per day.

If fasting plasma glucose is <=140 mg/dL AND if the glycated HbA1c level is <=8.5 the study subject will be requested to maintain the current dose and dose frequency of metformin.

If fasting plasma glucose is >140 mg/dL AND if the glycated HbA1c level is <=8.5 the study subject will be instructed to titrate the metformin dose upward by 500 mg per day to no more than 2000 mg per day.

Subjects who meet their glycemic and HbA1c targets at Visit 3 will proceed to Visit 4 to be randomized. If glycemia targets have not been met by visit 3, we will reassess at Visit 4 and determine if subject is able to randomized at that point.

FIGURE 6 METFORMIN TITRATION



4.6.3 SAXAGLIPTIN DOSING

Subjects' diabetes medication will be replaced with saxagliptin 5 mg qd and will be dispensed from the pharmacy. The study subjects will be requested to not take their diabetes medications on the day of Visit 2, in order to transition all participants to study supplied saxagliptin. Saxagliptin 5 mg will be administered qd and maintained at that dose level throughout the trial study. Given the warnings concerning the possibility of arthralgia, noted for the DPP-IV inhibitors as a class, saxagliptin included (see Section 1.7.2, earlier), especially in study subjects without a history or recent evidence of persistent arthralgia (Grade 3 per NCI-CTCAE criteria), the study team will ask study subjects to report all instances of arthralgia. If arthralgia becomes persistent, the guidelines for reporting and discontinuation in Section 4.7.2 and 5.0 will be followed. The first dose of saxagliptin 5 mg will be administered in the clinic as a witnessed dose. At drug dispensing visits, subjects will return the SAXA to the pharmacy and procure a new supply until the next scheduled drug dispensing.

4.6.4 AZD9668 OR PLACEBO DOSING

Subjects should be instructed to withhold their morning doses of AZD9668 or placebo on the days of the study visits. The last dose of study medications should be administered on the day before Visit 10 (final end-point-related study visit). No study medications will be administered on the day of Visit 10. The first dose of AZD9668 (two tablets of 30 mg) or placebo will be administered in the clinic as a witnessed dose. The subjects will be instructed to take two tablets of 30 mg AZD9668 or the placebo (twice a day) until the crossover and to maintain their metformin and saxagliptin for the duration of the study.

4.6.5 STANDARD DIABETES EDUCATION

All participants will be provided standard diabetes education during the run-in period and at some of the scheduled visits thereafter, which will provide basic knowledge about T2D, including skills and behaviors that are important for successful management such as medication taking, healthy eating and weight loss, increased physical activity, and smoking cessation. Standardized printed materials on diabetes management (diet, exercise) will be made available to patients who can discuss them in more depth with the study physicians. This printed information will include information on the pathophysiology of diabetes, prevention of complications, reduction of cardiovascular risk factors, diet/nutrition, exercise goals, and self-care such as foot care and medication taking. The importance of eating a healthy diet, losing weight if overweight or obese, and being physically active will be stressed. These materials will be consistent with the aims of the Look AHEAD (Action for Health in Diabetes) program (full list of bibliography in: https://www.lookaheadtrial.org/public/Bibliography.pdf).

The patient will receive printed information on a weight-maintaining diet and exercise approaches consistent with American Diabetes Association (ADA) recommendations from the study physician or other qualified health professional.

The recommended diet, consistent with ADA and other standard diabetes guideline recommendations should contain approximately 45 to 65% carbohydrate, 10 to 35% protein, and 20 to 35% fat

Patients will receive information (printed) on how to maintain a medically appropriate, routine exercise program; consistency in physical activity levels will be encouraged throughout the study.

4.6.6 GLUCOSE MONITORING

Glucose treatment decisions will be driven by protocol and controlled solely by the study staff. Medications that are titrated in usual care and consistent with their labeling will be titrated to achieve fasting glucose levels 70-140 mg/dl based on self-monitoring of blood glucose without symptomatic hypoglycemia or to the maximum tolerated dose, whichever dose is lower.

Study staff will communicate directly with participants' primary care providers (PCPs) so that it is clear that glycemic management will be assumed by the study staff through the entire course of the study. Similar strategies in the Diabetes Control and Complications Trial (DCCT), United Kingdom Prospective Diabetes Study (UKPDS) and in the ongoing Look AHEAD study resulted in almost no interference with the study implemented interventions and have promoted good retention. Subjects will be scheduled to be seen as shown in the Visit Schematic (Figure 4, Section 4.5).

Glycemic management for participants will follow an established titration protocol based on self-monitoring of blood glucose, aiming for fasting glucose between 70 and 140 mg/dl and a glycated HbA1c levels of <=8.5 without symptomatic hypoglycemia..

Although consideration was given to masking HbA1c values, in order to limit potential bias in the application of the medications and treatment strategies in this unmasked trial, we chose to share HbA1c values with the participants as it more closely resembles usual care. Moreover, although glucose treatment decisions will be driven by protocol and controlled solely by the study staff, patients are likely to have HbA1c levels drawn outside of the study, so that masking of levels would be impractical.

Self-monitoring of blood glucose (SMBG) will be performed for safety (to prevent hypoglycemia) according to usual care recommendations. Metformin will be titrated between visits based on results of SMBG as is common in the course of usual care. SMBG will also be performed for safety reasons by all participants in the presence of symptoms that suggest hypoglycemia, hyperglycemia, or during inter-current illness likely to affect glucose control substantially.

All participants will be followed until the end of their participation in this study.

All participants, including those who have reached the primary and/or secondary outcomes, will continue to be followed until their participation in the study concludes, under the intention-to-treat principle. After the primary outcome has been reached, randomly assigned study drugs and metformin+saxagliptin will be continued and supplied free-of-charge until the subject's participation in the trial ends.

Subjects will: 1) be provided with a glucose meter and receive training in performing SBGM measurements; and 2) receive instructions on hypoglycemia symptoms and hypoglycemia management.

Electronic glucose meters will be supplied to all patients at Visit 2 in order to standardize the procedure for blood glucose measurements. Patients will be instructed on the procedure to perform fingerstick glucose measurements. Following alcohol swab sterilization of a finger, a lancet will be used to pierce the skin in order to draw a single drop of blood onto a glucose. Patients will monitor their fingerstick glucose concentrations with a frequency determined appropriate by the investigator (based upon his/her assessment of the patient's risk of increasing glucose concentrations) with a minimum of 1 fasting determinationper two days. Throughout the duration of the entire trial, patients will be instructed to contact the site if the fingerstick glucose values are 70 mg/dL or less. Furthermore, in order to assess for exclusion/discontinuation criteria, patients should be instructed to contact the study coordinator if the fingerstick glucose values are >140 mg/dL at any time during the trial.

4.6.6.1 ASSESSMENT AND MANAGEMENT OF HYPOGLYCEMIA

At **Visit 2**, the site will review the symptoms and management of hypoglycemia with the subject. The site will counsel the subject to immediately perform a fingerstick glucose measurement if any symptoms occur that may be related to hypoglycemia (e.g., weakness, dizziness, shakiness, increased sweating, palpitations, or confusion), but also to avoid delay in treating these symptoms. Subjects should be instructed to contact the investigational site to report: any episode of possible hypoglycemia resulting in symptoms, any episode of hypoglycemia for which assistance was required (i.e., severe hypoglycemia), any episode of fingerstick glucose less than 70 mg/dL with or without symptoms.

The subject will be instructed to complete the Hypoglycemia Assessment Log (HAL) for any symptomatic episodes he or she believes may represent hypoglycemia. In addition, subjects will be instructed to record in the HAL any fingerstick glucose values less than 70 mg/dL regardless of the presence of clinical symptoms.

Each episode should be evaluated by the investigator and recorded on the Hypoglycemia Assessment (HA) case report form (CRF). The investigator will determine if the event should be considered an adverse event. For episodes determined to be hypoglycemia (symptomatic or asymptomatic), and for all glucose values less than 70 mg/dL, regardless of whether they are considered an adverse event, the HA CRF must also be completed.

4.6.6.2 Management of procedure-associated in-clinic hypoglycemia

In subjects with diabetes, hypoglycemia is defined as all episodes of an abnormally low plasma glucose concentration (with or without symptoms) that expose the individual to harm. Hypoglycemia is also defined as a self-monitored blood glucose (SMBG) level ≤70 mg/dL. While that value is higher than the value used to diagnose hypoglycemia in people without diabetes, it approximates the lower limit of the physiological fasting nondiabetic range, the normal glycemic threshold for glucose counterregulatory hormone secretion, and the highest antecedent low glucose level reported to reduce sympathoadrenal responses to subsequent hypoglycemia.

Common symptoms: dizziness, weakness, delirium, confusion, tremors, and sweating.

4.6.6.3 Treatment of hypoglycemia

In order to treat early symptoms of hypoglycemia, subjects should be certain that fast-acting carbohydrate (such as glucose tablets, hard candy, or sweetened fruit juice) is available at all times. Fifteen to 20 grams is usually sufficient to raise the blood glucose into a safe range without inducing hyperglycemia. This can be followed by long-acting carbohydrate to prevent recurrent symptoms. Alternatively, 25 g of 50 percent glucose (dextrose) intravenously can be given for rapid treatment. A subsequent glucose infusion (or food, if subject is able to eat) is often needed, depending upon the cause of the hypoglycemia.

Unconscious subjects: A subcutaneous or intramuscular injection of 0.5 to 1.0 mg of glucagon will usually lead to recovery of consciousness within 10 to 15 minutes, although it may be followed by marked nausea or even vomiting.

Glucose gel (eg, Insta-Glucose) or cake frosting in the space between the teeth and buccal mucosa, keeping the subject's head tilted slightly to the side.

4.6.7 COMPLETE PHYSICAL EXAM

The complete physical exam will include demographics, smoking status, height, weight, BMI, waist circumference, physical examination, auscultation, eye, ear, nose, throat, blood pressure, temperature, other vital signs.

4.6.8 MEDICAL HISTORY

Medical history assessment will include review of pre-existing conditions, treatments, prior./concomitant medications check.

4.6.9 DEXA SCAN

Study subjects will remove all objects from clothing (wallet, phones, jewelry, underwire bras) and will be asked to lie supine on the scan table. The scanner operator will ensure that the subject is lying straight by looking at the body alignment. A radiolucent pillow will be placed under the study subject's head and under the paper on the table. A large square cushion will then be placed under the lower legs with the thighs as close to a 90 degree angle to the body as possible. The study subject should rest their arms comfortably at their sides. The operator will then begin the scan process on the computer system. The whole scan is on average between 15-30 minutes depending on the height of the subject.

Once the DEXA scan is complete, after a 30 minute rest period, the subject will begin the clamp test in another clinical suite. NOTE: Even though every effort will be made to conduct the clamp and DEXA scan on the same day, scheduling reasons might require the two procedures to be conducted on separate days (separated by no more than 7 days).

4.6.10 80 mU/m^2 min^{-1} HYPERINSULINEMIC- EUGLYCEMIC CLAMP TEST

Subjects will be studied in the supine position. During the procedure, the participants are only allowed to drink water. An intravenous catheter will be placed in an antecubital vein for infusion of insulin (Humulin-R) and dextrose. A second catheter will be placed retrograde in a dorsal vein of the contralateral hand for blood draws. The hand will be gently warmed with a heating pad that has a timer). The other catheter is used for infusion of dextrose 20% (prepared by the hospital's pharmacy) and continuous insulin at 80 mU/m^2 min^{-1}.

After fasting baseline blood samples are collected, the hyperinsulinemic clamp will start with a primed-continuous infusion of insulin (Humulin-R) at a rate of 80 mU/m^2 body surface area per minute for 4 hr. Plasma glucose is measured every 5-10 min to ensure glucose is maintained at a target range of 90-100 mg/dl with a variable 20% dextrose infusion controlled by the investigator.

During the last 30 min of the clamp (steady-state), blood samples are collected for determination of glucoregulatory factors (e.g. insulin, C-peptide, FFA). The glucose disposal rate is calculated based on the mean glucose infusion rate at the steady-state phase. Approximately 250 mL of blood per clamp will be drawn.

Thereafter insulin infusion is discontinued and participants will be offered a carbohydrate-rich meal. The dextrose infusion is gradually tapered during the course of the meal. Bedside glucose monitoring will occur before determination that the participant is safe to be discharged. The subject will remain in the

investigator's watch until ready for discharge to ensure no procedure-associated emergent AE.

4.6.11 3-hour MULTI-POINT ORAL GLUCOSE TOLERANCE TEST (OGTT)

The study subject will will have the OGTT panel (see Appendix A) samples collected as follows:

- 0 minutes: baseline blood draw
  - Subject will then consume 100mg of a oral glucose containing drink within 5 minutes
- 30 minutes: blood draw
- 60 minutes: blood draw
- 120 minutes: blood draw
- 180 minutes: blood draw

.

Each sample will be collected as a separate needle stick within ± 5 minutes of the scheduled time point. The study subject will be monitored for an additional hour for signs of hypoglycemia and then discharged. The OGTT will be stopped if the study subject exhibits signs and symptoms of hypoglycemic attack; hypoglycemia will be treated according to Section 4.6.6.

4.6.12 SAFETY ASSESSMENTS

Subjects will be interviewed regarding adverse events throughout the course of the study according to the Visit Schematic (Figure 4, Section 4.5). We will monitor hematologic parameters (e.g., hemoglobin, serum vitamin B12 concentrations) throughout the trial duration.

In particular, subjects will also be asked if they have recent experience of any recurring arthralgia (given the DPP-IV-related warnings as described in the background, earlier). This will be relevant in determining if any instance of in-trial arthralgia could be due to an underlying chronic condition that is not an exclusion criterion, or saxagliptin-related.

In order to detect concurrent illness, we will evaluate applicable blood markers for evidence of ketoacidosis or lactic acidosis. Temporary withdrawal of metformin therapy and administration of insulin may be required to maintain glycemic control during periods of stress. We will observe patient closely for evidence of altered glycemic control when such drugs are added to or withdrawn from therapy.

4.6.13 CONCOMITANT MEDICATIONS/TREATMENTS

Subjects will be interviewed regarding any concomitant medications taken during the course of the study. If necessary, concurrent lipid lowering, antihypertensive, and thyroid hormone medications are permitted. It is preferable that doses of these medications remain stable after the run-in period and then for the duration of the trial.

If necessary, hormone replacement therapy and birth control medications are allowed, but patients should be on stable regimens, and are expected to remain on that stable regimen during the run-in and the duration of the trial. At no time during trial should any over-the-counter salicylates, NSAIDs or prescription salicylates or NSAIDs be used (e.g. no salicylic acid or derivatives, or COX-2 inhibitors).

4.6.13.1 PROHIBITED MEDICATIONS

1. Other Anti-hyperglycemic Medications: Except for medication given as part of the study protocol, no other anti-hyperglycemic medication (such as GLP-1 analogues, sulfonylureas, glitazones, glimepiride, meglitinides, other biguanides, glucosidase inhibitors, PPAR< agonists, insulin) may be taken during the study.
2. Antiretroviral Drugs: The use of antiretroviral drugs (e.g., protease inhibitors, reverse transcriptase inhibitors) is prohibited.
3. Corticosteroids: The use of 14 consecutive days of systemic corticosteroids (oral, injectable/parenteral) is prohibited. However, oral corticosteroids used for physiologic replacement therapy (i.e., in patients with adrenal insufficiency) and inhaled, nasal, and topical corticosteroids are allowed.
4. ***At no time during trial should any over-the-counter salicylates,NSAIDs or prescription salicylates or NSAIDs be used (e.g. no salicylic acid or derivatives, or COX-2 inhibitors).***
5. Macrolide antibiotics
6. Cisapride
7. Anti-arrhythmics
8. Rifampicin
9. Phenobarbital
10. Phenytoin
11. Secobarbital
12. Carbamazepine
13. Norethindrone
14. Isoniazid
15. Fluconazole
16. Amiodarone
17. Fenofibrate
18. Fluvoxamine
19. Phenylbutazone
20. Probenecid
21. Sertraline
22. Amphetamines, dextroamphetamines, lisdexamfetamine, methylphenidate
23. Atomoxetine, Clonidine, Guanfacine

Use or need for use of excluded medications will require consultation with the study investigator.

4.7 INTERRUPTION OF STUDY MEDICATION OR DISCONTINUATION/WITHDRAWAL FROM STUDY

Randomized subjects who discontinue due to an adverse event or for any other reason will have a discontinuation visit (mirroring the procedures listed in Visit 10, Figure 4) scheduled.

Subjects may withdraw at any time or be dropped from the study at the discretion of the investigator should any untoward effects occur. In addition, a subject may be withdrawn by the investigator or the if he/she violates the study plan or for administrative and/or other safety reasons. The investigator or study coordinator will follow local applicable IRB reporting guidelines for subjects being discontinued/ withdrawn due to a serious adverse event. When a subject discontinues/withdraws prior to study completion, all applicable activities scheduled for the final study visit should be performed at the time of discontinuation. Any adverse experiences which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 5.0.

Subjects may withdraw, at any time, consent for participation in this research study without penalty or loss of benefits to which they are otherwise entitled. Subjects who optionally consent to having left-over blood samples stored for future research on T2D may also request that their specimen(s) be removed from storage and destroyed in accordance with the terms outlined in the associated consent form. Requests for withdrawal and destruction of the optional stored samples should be made in writing to the principal investigator. The principal investigator will be informed when the associated specimens are destroyed and should notify the subject that specimen destruction is complete.

The Medical Monitor and the IRB will be notified according to local IRB reporting requirements regarding subject discontinuation or interruption in study medication due to an AE/SAE or laboratory safety test abnormality. Given the warnings concerning the possibility of arthralgia, noted for the DPP-IV inhibitors as a class, saxagliptin included (see Section 2.6.1.2.9, earlier), the study team will closely document and monitor all instances of arthralgia. If arthralgia becomes a Grade 3 persistent arthralgia per NCI-CTCAE criteria (i.e. at least twice in 7 days, for at least two weeks, of intense pain at any of the body joints, along with sudden joint swelling or an inability to move the joint(s)), the medical monitor and DSMB will be notified within 5 days and can recommend to withdraw the study subject from saxagliptin (and therefore from the trial). The study subject will be requested to withdraw from the study and discontinue saxagliptin if the medical monitor and DSMB concur that the arthralgia is saxagliptin-related. If required, these events will also be reported to the FDA and or IRB based upon their reporting guidelines.

4.7.1 Metformin and/or saxagliptin interruption

Since all subjects will be receiving treatment with metformin and saxagliptin, if a subject undergoes an imaging study requiring the use of radiocontrast dye (for example, an intravenous pyelogram or computerized tomography study with contrast), treatment with metformin should be temporarily discontinued for the time of the radiocontrast dye study. The subject's renal function should be reassessed 48 hours after the procedure, and metformin should be reinstituted (at the same dose prior to interruption) only after renal function has been evaluated and found not to have been reduced by the dye study. In a subject requiring an imaging study, if considered clinically appropriate, studies not using radiocontrast dye (e.g., ultrasound-based studies, MRI with gadolinium contrast, or non- contrast CT studies) should be performed instead of radiocontrast dye studies, so as to avoid the interruption of metformin.

4.7.2 Reasons for protocol-specified discontinuation from the study

All subjects will be followed until resolution (i.e., return to baseline values or diagnosis determined or new stable state established, based upon investigator and study sponsor assessment) for any laboratory safety test abnormality resulting in discontinuation.

1. Informed consent withdrawn or subject requests discontinuation from the study.
2. Hypoglycemia: Repeated (2 or more episodes) FPG or fingerstick glucose <50 mg/dL with or without symptoms of hypoglycemia or FPG or fingerstick glucose less than 70 mg/dL with symptoms of hypoglycemia and without a reasonable explanation (such as increased physical activity and/or skipped meal) occurring after a subject has had their metformin/saxagliptin interrupted. Note: Subjects who experience hypoglycemia should have their dose of metformin down-titrated to avoid further hypoglycemia. If hypoglycemia persists despite down-titration of metformin to zero mg (i.e., until dosing of metformin interrupted), then the subject should be discontinued.
3. Hyperglycemia. criteria are as follows:

i) -FPG (with value repeated and confirmed within 7 days from initial report):

ii) >270 mg/dL after Visit 2 through Visit 4. FPG (with value repeated and confirmed within 7 days from initial report)
iii) >240 mg/dL after Visit 4 through Visit 6. FPG (with value repeated and confirmed within 7 days from initial report)
iv) >200 mg/dL at any other period after Visit 6, through the remainder of the study.

4. Elevation in ALT and/or AST (3X).
5. Blood serum creatinine is consistently 1.5 mg/dL (133 mmol/L) in men and 1.4 mg/dL (124 mmol/L) in women. Note: Serum creatinine values meeting discontinuation criteria must be repeated and confirmed within 7 to 10 days of initial measurement.
6. Requirement for one of the excluded medications listed in Section 4.6.13.1.
7. Run-in period or in-trial persistent arthralgia as described in Section 4.6.12.
8. Pregnancy. Note: If the subject reports pregnancy after randomization and has a positive serum pregnancy test, study medication will be immediately interrupted Subject must be permanently discontinued and followed per Section 4.12.
9. Any medical condition or personal circumstance which, in the opinion of the investigator, exposes the subject to risk by continuing in the study or does not allow the subject to adhere to the requirements of the protocol.

4.8 MEDICATION COMPLIANCE

Adherence to treatment will be assessed by subject report which may be facilitated by tablet counts for saxagliptin, metformin, AZD9668 or AZD9668 placebo remaining in the specific bottles. Every effort will be made to maintain adherence as close to 100% as possible. If a subject is found to have reduced compliance (<75%), site personnel should begin frequent contacts with the subject to remind the subject to take the study medication.

Overdosages will be managed as described in Section 5.2. In the event that the study agents are depleted prior to the next scheduled pharmacy visit, the research staff will communicate with the subject and coordinate re-supply.

4.9 UNBLINDING

The study code should only be broken for valid medical or safety reasons, e.g. in the case of a severe adverse event where it is necessary for the study team or treating health care professional to know which treatment the subject is receiving before the participant can be treated. This decision should made in consultation with the clinical PI and Medical Monitor, and reported to the DSMB.

4.10 EFFICACY MEASUREMENTS

Efficacy measurements include laboratory assessment of insulin sensitivity as measured by the hypersinsulinemic-euglycemic clamp, beta cell function as assessed by the oral glucose tolerance test, glycemic control as assessed by the glycated HbA1c level, fasting plasma glucose, and lipid panel.

4.11 SAFETY MEASUREMENTS

Safety assessments will include collection of AEs, physical examination, vital signs, body weight, and laboratory safety studies. For further details refer to Section 5.0.

4.12 Pregnancy Testing and Contraception

Pregnancy Testing

All pre-menopausal women who are not surgically sterilized participating in the study will have a blood (serum) pregnancy test at the screening visit (Figure 4). A positive serum pregnancy test prior to randomization requires exclusion. If the subject reports pregnancy after randomization and has a positive pregnancy test study medication will be immediately interrupted and the subject must be permanently discontinued and followed (see Section 4.7.2).

Contraception

Non-pregnant, non-breast-feeding women may be enrolled if they are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, 2) postmenopausal, 3) not heterosexually active for the duration of this study, or 4) heterosexually active and willing to use a birth control method. The birth control method can be either a barrier method or a hormonal method to prevent pregnancy, used throughout the study starting with **Visit 2** through to 14 days after the last dose of study medication.

The following are considered adequate barrier methods of contraception: diaphragm, condom, copper IUD, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents). Postmenopausal is defined as 6 months of spontaneous amenorrhea with serum follicle stimulating hormone (FSH) levels in postmenopausal range as determined by the laboratory, or 12 months of spontaneous amenorrhea in women >45 years of age.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study and agree that in order to participate in the study they **must adhere to the contraception requirement (described above) for the duration of the study.** If there is any question that a subject will not reliably comply with the requirements for contraception, she should not be entered into the study.

Contraception does not apply to male study subjects; the short half life of AZD9668 is incompatible with accumulation of the molecule in the seminal fluid, or the germ line, to a concentration level that could be functionally relevant.

4.12.1 Reporting of Pregnancy to study team

Although not considered an adverse experience, it is the responsibility of investigators or their designees to report any pregnancy in a subject (as reported by the subject to them) which occurs during the study or within 14 days of completing the study. All subjects who become pregnant must be followed to the completion/termination of the pregnancy. If the pregnancy continues to term, the outcome (health of infant) must also be reported to one of the individuals listed on the study team Contact Information page.

4.13 Immune/immunomodulation assays

4.13.1 Overview

The scheduled immunological monitoring along with the mechanistic studies are aimed at ascertaining how neutrophil elastase inhibition might change the natural progression of T2D by alterations in peripheral blood cells including but not limited to neutrophils, regulatory T-cells, B-cells, M1 and M2 macrophages, dendritic cells and signaling pathways in these cell populations, modulation of neutrophil

bioactivity, NET activity, and changes in the balance between TH1 and TH2 immunity. Also, markers of inflammation (e.g. hs-CRP, cytokines, adipokines) in serum can be correlated to changes in the cell populations and signaling in the specific cells.

Upon consent to permit storage of left-over blood, this blood could be used in future studies and assays to re-evaluate and further explore biologic responses in the constituent leukocytes and serum as knowledge accumulates over time.

Also, baseline, and in-trial gene expression profiling analysis will also be conducted using RNA isolated from PBL and purified neutrophils using microarrays and then high-throughput real-time quantitative polymerase chain reaction (RT-qPCR) for verification. Changes in gene expression may provide a signature of responder versus non-responder status and/or of effect of therapy.

Of particular interest, and a novel research direction based on the proposed mechanistic studies, is the accumulating evidence supporting the existence of distinct neutrophil subsets that have diverse roles in infection, inflammation and cancer immunology [168-174]. For example, Kamp and colleagues, Fridlender et al. and Christoffersson et al. have described distinct immunostimulatory as well as putative immunosuppressive neutrophils [169,170,175]. Even though there are still questions about whether these cells represent truly distinct lineages or instead develop from a single plastic neutrophil precursor, it is formally possible that a dynamic ratio of “pro- inflammatory” and “anti-inflammatory” neutrophils might shape the course of the T2D chronic inflammation which might modulate insulin sensitivity and beta cell function, directly, or indirectly by other cells (e.g. affecting M1 and M2 macrophages)[176]. Thus, among the PBL subset frequency and absolute numbers we intend to measure, we include neutrophils characterized by the surface expression of CD11b+ Gr-1+ CXCR4low (“pro-inflammatory”) and CD16bright CD62Ldim (“anti-inflammatory”) [170,175].

4.13.2 Peripheral blood leukocyte cell multi-parameter/multi-color flow cytometry

The effect of the study agents on the frequency and absolute cell numbers of T-cell, B-cell, dendritic cell, neutrophil, and macrophage cell populations relevant to the study (refer to Appendix B for details) will be assessed over time using flow cytometry.

4.13.3 Peripheral blood transcriptome studies

There are observations indicating that marked changes in the gene expression of peripheral blood cells from pre-diabetic vs. recent-onset diabetic subjects can predict onset of complications based on immune activation [177,178]. Changes in gene expression of whole blood may provide a signature of treatment-responder versus non-responder status and/or of effect of therapy as has been demonstrated in other studies [179-184].

4.13.4 PBMC T-cell response viral CEF pool

The effect of the study agents on general immune competence will be measured in standard PBMC response to viral CEF pool (IFNgamma or TNFalpha or GranzymeB ELISPOT).

4.14.5 Serum cytokine panel

Serum will be collected at each of the visits designated for labs and monitoring. Levels of various immune cytokines will be measured to determine if cytokine levels correlate with clinical AEs and potential efficacy.

4.13.6 Frozen peripheral blook leukocytes or PBL-derived cell assays

Peripheral blood leukocytes and/or PBMC not used for monitoring from study subjects will be cryopreserved for assays that could identify progressors to complications as well as responders vs. non-responders to treatment.

5.0 ADVERSE EVENTS REPORTING

5.1 SAFETY MONITORING

5.1.1 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) that will include NIH NCATS and Astra-Zeneca representatives as non-voting members who attend open session in addition to at least two clinical experts in type 2 diabetes will monitor the study and will have the authority to suspend the trial if it determines that the risks to individuals exceed the originally described risks and/or that modifications to the protocol are needed to minimize the risks. The DSMB will receive and review safety data during their regular reviews as described in Sections 3.3 and 5.1.3.

5.1.2 Definitions

5.1.2.1 Adverse event

An AE is any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07); http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2).

5.1.2.2 Serious adverse event

An AE or SAR is considered "serious" if, in the view of either the investigator, the DSMB, the FDA, IRB, NIH NCATS or Astra-Zeneca, it results in any of the following outcomes (21 CFR 312.32(a)):

i) Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment-related or not.
ii) A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DSMB, the FDA, IRB, NIH NCATS or Astra-Zeneca, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
iii) Inpatient hospitalization or prolongation of existing hospitalization.
iv) Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
v) An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
vi) Congenital anomaly or birth defect.
vii) Other conditions specified in the protocol.

Regardless of the relationship of the adverse event to study drug, the event must be reported as an SAE if it meets any of the above definitions.

5.1.2.3 Unexpected adverse event

A SAR is considered "unexpected" if it is not listed in the available risk information on the article or test article. For example, in the investigator's brochure, the product insert, or peer reviewed publications.

In addition, if the specificity or severity has been previously observed, this meets the criteria for "unexpected". (21 CFR 312.32(a)).

5.1.2.4 Adverse reaction and suspected adverse reaction

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)). Adverse events and treatment-emergent laboratory abnormalities, such as aminotransferase elevations, rises in BUN and/or creatinine, and abnormalities in hemoglobin, WBC count and differential, and/or platelets will be followed until they resolve or stabilize.

5.1.2.4.1 Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- -Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- -Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- -Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

5.1.2.5 Collecting and reporting procedures

All AEs will be recorded. Additionally, AEs that are classified as serious according to the definition set forth by the health authorities must be reported promptly to the IRBs, health authorities, investigators, and the DSMB according to their reporting requirements and timeframes. The next sections define the types of adverse events and outline the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events version 4.0 (http://ctep.cancer.gov/reporting/ctc.html).

Adverse events will be collected from the time the study subject signs the informed consent until the time an event is resolved or until 30 days after the participant completes study treatment, whichever comes first.

Adverse events may be discovered through any of these methods:

i) Observing the participant.

ii) Questioning the participant in an objective manner.

iii) Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., biochemistry, hematology, metabolic, immune testing) can also indicate an adverse event. If this is the case, then the evaluation that produced the value or result should be repeated until the value or result returns to normal or can be explained and the participant's safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be recorded as an adverse event on the appropriate laboratory evaluation form(s).

Unanticipated problems will be recorded in the data collection system throughout the study.

The PI will record all AEs with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

5.1.2.6 Recording procedure

Throughout the study the investigator will record AEs on the appropriate adverse event CRF regardless of their severity or relation to study medication or study procedure. All AEs will be recorded on the adverse event CRFs except those that are expected following treatment. The investigator will treat participants experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilises. Further, all AEs attributable to the study agent reported in the AZD9668 Investigator's Brochure will be recorded. For Saxagliptin and/or Metformin, AEs attributable to those agents can be recorded and designated as such (attributable to saxagliptin and/or metformin).

5.1.2.7 Grading and attribution of all adverse events

5.1.2.7.1 Grading criteria

The study site will grade the severity of AEs experienced by study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf). These criteria provide a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs which will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = Mild adverse event. no intervention required; no impact on activities of daily living (ADL)

Grade 2 = Moderate adverse event. minimal, local, or non-invasive intervention indicated; moderate impact on ADL

Grade 3 = Severe and undesirable adverse event. significant symptoms requiring invasive intervention; subject seeks medical attention, needs major assistance with ADL

Grade 4 = Life threatening or disabling adverse event.

Grade 5 = Death.

All AEs will be reported and graded whether they are or are not related to disease progression or treatment.

5.1.2.8 Definition of attribution

Adverse events will be categorized for their relation to placebo, metformin, saxagliptin and/or AZD9668. The principal investigator will do the initial determination of the relation, or attribution, of an AE to study participation and will record the initial determination on the appropriate CRF and/or SAE reporting form. The relation of an AE to study participation will be determined using definitions in Table 2, below. Final determination of attribution for safety reporting will be decided by the DSMB.

Table 2: Definition of Attribution

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study treatment)
UNRELATED CATEGORIES		
1	Unrelated	The AE is clearly unrelated; Alternative etiology compelling
2	Unlikely	The AE is unlikely related; Most of evidence supports an alternative etiology.
RELATED CATEGORIES		
3	Possible	The AE has a reasonable possibility to be related;
4	Probable	The AE is likely related;There is evidence to suggest a causal relationship
5	Definite	The AE is clearly related; As demonstrated by dose-titre effect, rechallenge,compelling etiologic mechanism, etc.

5.1.3 Event reporting procedures

Adverse events, unanticipated problems (UP) and non-compliance will be collected from the time the study subject begins study treatment until 30 days after he or she completes study treatment or until 30 days after he or she prematurely withdraws from the study. The following procedures for reporting ensure study compliance with 21CFR312 and ICH guidelines.

All AEs/SAEs and Unanticipated Problems (UP) will be recorded in the subject study records and reviewed at minimum at quarterly study DSMB meetings and at the time of IRB annual review. These will also be forwarded to the Medical Monitor at the time of the above-mentioned reviews.

The IND holder will submit any applicable events to the FDA according to the following reporting requirements:

i) Standard reporting (to be reported in the IND annual report). This option applies if the event is classified as one of the following:

a) Expected SAE
b) SAE unlikely to be or not related to study drug

ii) Expedited Safety Reporting. The following events will be reported on the standard FDA reporting form according to the FDA timeframe requirements (15 calendar days, or 7 days for death of life-threatening events):

a. SAE that is unexpected and possibly or definitely related to study drug
b. Aggregate analysis of SAEs when at least 5 SAEs suggest a causal relationship to the study drug
c. Any findings from clinical or epidemiological studies, analysis of data pooled across multiple studies, published or unpublished scientific papers, or from animal or in vitro testing that would result in a safety-related change in the protocol, informed consent, investigator brochure or other aspects of the overall conduct of the trial will be reported;
d. Any Unanticipated Problem or Non-compliance that suggests a significant risk of subjects

Events that meet the FDA expedited reporting requirements will also be reported concurrently to the IRB (within 5 or 10 days based upon the event), Medical Monitor, and study DSMB. In cases of various reporting timeframes, the shortest timeframe will be followed.

5.2 Definition of an Overdose for This Protocol

An overdose must be reported if any of the following occur during the conduct of the study:

1. Dosing with more than 1 tablets per day of saxagliptin (>5 mg).
2. Dosing with more than 4 tablets per day of metformin (>2000 mg) for more than 5 consecutive days.
3. Dosing with more than 4 tablets per day of AZD9668 (>120 mg)

For recommended management of acute overdose, please refer to the Investigator's Brochure or the product insert/label for each of the above study agents.

5.2.1 Reporting of Overdose

If a dose of test drug meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI).

All reports of overdose with and without an adverse experience must be reported to the Medical Monitor. If the event meets IRB or FDA reporting requirements, it will be reported per the timeframes described in Section 5.1, otherwise they will be reported within 15 calendar days.

5.3 Guidance on Adverse Events Related to Glycemia

5.3.1 Guidance on Adverse Events Related to Hyperglycemia: An adverse event of hyperglycemia requires that a subject have one or more symptoms (e.g., increased thirst, polyuria) typically associated with an increased glucose level. At the discretion of the investigator, this may be captured as an adverse event of "hyperglycemia." This diagnosis may be supported by, but does not require, results from a glucose meter or the study central laboratory. Further, at the discretion of the investigator, an elevated blood glucose value without associated symptoms that is considered to be an adverse event may be reported as an adverse event of "increased blood glucose. General guidance regarding the determination as to whether an event is considered to be an adverse event should be followed (see Section 5.1.2).

5.3.2 Guidance on Adverse Events Related to Hypoglycemia

Documentation
Regardless of whether an episode is considered an adverse event, the Hypoglycemia CRF must be completed for the following:

-all episodes determined by the investigator to be hypoglycemia (symptomatic or asymptomatic)

-all glucose values less than 70 mg/dL

Guidance: All episodes considered as likely to represent symptomatic hypoglycemia by the investigator should be captured as an adverse event of symptomatic hypoglycemia. This diagnosis may be supported by, but does not require, confirmatory blood glucose results (such as those measured using a fingerstick or from a clinical laboratory sample). Further, at the discretion of the investigator, an asymptomatic blood glucose value less than 70 mg/dL may be reported as an adverse event of symptomatic hypoglycemia. General guidance regarding the determination as to whether an event is considered to be an adverse event should be followed (see Section 5.1.2).

5.4 Guidance on Adverse Events Related to Arthralgia

Given the warnings concerning the possibility of arthralgia, noted for the DPP-IV inhibitors as a class, saxagliptin included (see Section 1.7.2, earlier), the study team will ask study subjects to report all instances of arthralgia. If arthralgia becomes persistent the guidelines for reporting and discontinuation in Section 4.7.2 will be followed.

5.5 Assessment of compliance with study drugs

For the summary of subject compliance, a day within the post-randomisation Treatment Period will be considered a compliant day if the subject was compliant on that day with study medication, defined as follows:

-For saxagliptin: a subject will be compliant if the subject took exactly 1 tablet.

-For metformin: a subject will be compliant if the subject took at least 1 tablet, and no more than a total dose of 2000 mg;
or
3

0 tablets with an accompanying indication on the CRF that this occurred due to the Investigator's decision to interrupt metformin.

-For AZD9668 or placebo: a subject will be compliant if he/she took two tablets, but not less than one tablet.

If the study medication CRF indicates general compliance problems with any study medication, the subject will be considered non-compliant for that day regardless of the number of tablets reported.

For a subject who is followed for the entire study period, the "Number of Days in Treatment Period" is the total number of days from the post-randomisation first dose of study medication to the last scheduled day for treatment administration for that subject. For a subject who discontinues from the study prematurely, the "Number of Days in Treatment Period" is the total number of days from the post-randomisation first dose of study medication to the date of the last dose of study medication.

For each subject, percent compliance will then be calculated using the following formula:

Compliance = (Number of Compliant Days/Number of Days in Treatment Period) x 100%

5.6 Protocol Non-Compliance

Defined as any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of non-compliance, corrective actions are to be developed by the study staff and implemented promptly.

These practices are consistent with investigator and sponsor obligations in ICH E6.

All instances of protocol non-compliance will be documented in the study records. In cases where protocol non-compliance is considered to cause increased risk to study subjects, the event will be reported to the IRB and the study DSMB within 10 days and to the FDA within their Expedited Reporting timeframe. All other instances of protocol non-compliance will be reported to the IRB at continuing review and to the study DSMB at their quarterly meetings.

5.6.1 Protocol Violations

Protocol violations are any deviations from the protocol. The following rules define protocol violators during the study period of interest and will be used to identify such subjects prior to database lock. Subjects identified as protocol violators will be excluded from all time points in all analyses.

5.6.1.1 Drug compliance <75%: Non-compliance will be assessed by prime therapy records as provided by the investigator in the CRF. Assessment will be based on a subject's prime therapy records.

-If a subject has a compliance rate < 75% during the study this will be considered protocol non-compliance and reported as detailed in Section 5.6. Compliance rate is defined in Section 5.5.

5.6.1.2 Use of prohibited medications: The list of prohibited medications can be found in Section 4.6.13.1.

-If a subject takes any prohibited/anti-hyperglycemic medications after randomization (**Visit 5 and onwards**) for a total of 14 days or 7 consecutive days, he/she will be identified as a protocol violator.

-A subject with pharmacological dose (as defined in protocol) corticosteroid use 14 days during the last 90 days of the study period of interest is considered a protocol violator.

5.6.1.3
-If a subject receives incorrect study medication for a total of 14 days during the last 90 days of the study period of interest, then he/she will be identified as a protocol violator.

The final determination on protocol violations, and thereby the composition of the study population, will be made prior to database lock and will be documented in a separate communication to the study team.

5.7 Updating source documentation

Documents describing the safety profile of a study agent, such as the investigator's brochure, will be amended as needed to ensure that the description of safety information adequately reflects any new clinical findings. Until these documents are updated, expedited reporting will be required for additional occurrences of a reaction.

6.0 STATISTICAL ANALYSIS PLANS

6.1 General Analysis Strategies

6.1.1 General Statistical Analysis: Descriptive data will be presented as mean +/- SD. Independent sample t tests and Mann-Whitney U tests will test group differences for continuous variables. GDR determined from the hyperinsulinemic-euglycemic clamp will be also adjusted for weight, BSA, FFM, or FFM+17.7 to determine insulin sensitivity. For the OGTT data, the statistical significance of differences between the same parameters calculated from different sampling schedules will be calculated using the Wilcoxon's signed-rank test. Linear regression and Spearman rank correlation analyses will be used to examine the relationship between parameters. In all analyses, significance will be declared at $P < 0.05$.

6.2 Primary Outcome

6.2.1 Primary study endpoint: This study is designed to be a two-stage crossover experiment in which individuals are randomized to AZ9668 or placebo for six months, and then crossed-over to another 6 months of protocol therapy on the other treatment arm. The cross-over design was chosen in order to reduce the overall target sample size, as well as to enable the study to investigate, in an exploratory fashion, whether or not delayed AZ9668 administration (at 6 months) is inferior to the upfront AZ9668 treatment (at trial start) on IR improvement, metabolic measures, and beta cell function. A cross-over design is generally used when an intervention may have an effect, but not a cure. That is, typically after the treatment is removed, the subject returns to a baseline level of disease activity (insulin resistance, IR), assessed by the 80 mU/m^2 min^{-1} hyperinsulinemic-euglycemic clamp method, and then the response to the second treatment is measured from this baseline. In this study, there will be a new baseline selected at the beginning of the second treatment period. Even though starting from a new baseline, it is assumed that the relative response in the second period is the same as it was in the first period. As others have found, that all participants will receive the intervention might make enrollment easier. As well, generally, a comparison of treatment effects on the same participant is more precise which translate into a smaller sample size. Hence, the sample size for this crossover design is about 2/3 of the sample size of a design not employing a cross-over (e.g. 2-arm parallel design).

The linear model for the standard 2x2 cross-over design is

$$Y_{ijk} = m + S_{ik} + P_j + F_{(j,k)} + C_{(j-1,k)} + e_{ijk}$$

where i = 1-n, the number of subjects, j represents the treatment period (1 or 2), and k the sequence (first or second). S is the subject effect, P the period effect, F the treatment effect and C the carry-over effect. While the assumption is that there is no carry-over effect, this assumption can be tested using a t-test. The treatment effect (our main interest) is also tested using a t-test and, in a similar fashion we can test for period effects in the same way (i.e., does the result depend upon whether the intervention was received upfront or delayed) which is a secondary endpoint.

6.3 Other Outcomes

6.3.1 Secondary study endpoints: Secondary end points include

i) Improvement in glycated HbA1c levels by a minimum of 0.2 or better (range=0.2 – 1.5) at 6 months compared to baseline;

ii) Improvement in OGTT-derived measurements, namely: glucose tolerance, Matsuda Index of insulin resistance [6-8], insulinogenic index using C-peptide [1-5];
iii) Improvement in fasting plasma glucose by 6% or better at 6 months compared to baseline; and
iv) decreased concentrations of serum/plasma IL-6, high sensitivity-C-reactive protein, fibrinogen, TNF-alpha, soluble TNF-alpha receptors at 6 months compared to baseline.

Based on the assumption that the individual within-treatment arm HbA1c level standard deviation would be no more than 1% after treatment, that the difference in HbA1c levels between treatment arms would be at least 0.6% in paired analyses; at a power of .89 and at a two-sided 0.05 significance level, a total of 30 study subjects are needed.

The proposed enrollment of 42 study subjects to account for potential study dropout to achieve the primary study aim fully powers this secondary objective. If all 42 of the study subjects complete the trial, the power increases to > .96. All the other secondary measures are summarized by their means and standard deviations.

The ratio of the difference in means to the standard deviation is a commonly accepted measure of the standard deviation to the mean defines the effect size in standard deviation units. While exact data from prior studies are not available for each of the secondary outcome measures, the analysis plan is the same and we would expect that if the study met the accrual projects for the primary stratum that it will produce sufficient numbers to detect a moderate effect size on the secondary outcome measures as well. As these are considered secondary, no special provision in the sample size target is made for multiple testing although caution will be exercised in interpreting significance levels close to 0.05.

6.3.2 Exploratory Endpoints: To investigate whether or not delayed AZ9668 administration (at 6 months) is inferior to the upfront AZ9668 treatment (at trial start) on IR improvement, metabolic measures, and beta cell function, we will compare the groups using a 12 month endpoint. Hence, the planned analysis would not consider the 6 month endpoint that is observed for each stage of the cross- over design. In this context the study is one of a parallel design with approximately 20 participants on each arm. With this number of participants, the study would have 80% power to detect a 59% change in IR (effect size 0.662) at a significance level of 0.5, two-sided test.

6.4 Interim Analyses

There are no interim analyses planned.

6.5 SAMPLE SIZE AND POWER

Primary Outcome

Table 3

Total Sample Size (N)	Mean Ratio Under H0 (R0)	Mean Ratio Under H1 (R1)	Effect Size (ES)	Coefficient of Variation (COV)	Significance Level (Alpha)	Beta
200	1.000	1.150	0.199	0.528	0.0500	0.1991
59	1.000	1.300	0.374	0.528	0.0500	0.1935
37	1.000	1.400	0.480	0.528	0.0500	0.1901
26	1.000	1.500	0.578	0.528	0.0500	0.1926

Based upon previous effect ranges of metformin+DPPIV inhibitor on insulin sensitivity/resistance (from 10%-20% improvement over baseline at 6 months; [155,157,158,160-162,185,186], a two-sided T- test achieves 80% power to reject the null hypothesis (e.g., the ratio of the 6-month placebo vs. AZ9668 IR=1) in favor of the alternative hypothesis (the IR ratio is 1.15) when the total sample size of a 2x2 cross-over design is 200 and the coefficient of variation (SD/M) is .528 (=2.3/5. 8). The sample size

requirement drops sharply when considering a larger effect size as Table 3 above demonstrates (all at 80% power).

From this, should the study be powered at 80% to detect a 48% effect size (which represents at least a a 20% improvement OVER the expected 10-20% improvement due to the MET+SAXA combination alone), the total sample size required would be 37.

This compares quite favorably to a straight parallel design in which case the required sample size would be 64 to have 80% power to detect the same effect size.

The above estimates do not include any provision for dropouts toward the end of the trial arms in Treatment Period B. Under ideal conditions, the target would be increased by 10% to provide for such a contingency. Should the dropout rate exceed this number, additional participants would be enrolled. Nevertheless, if end-of-trial dropout occurs, the primary analysis will be based on all participants enrolled (intent-to-treat).

Considering all this, we propose a total study population of 42 individuals in two arms (n=21 placebo and n=21 AZD9668).

6.6 Secondary Outcomes

Based on the assumption that the individual within-treatment arm HbA1c level standard deviation would be no more than 1% after treatment, that the difference in HbA1c levels between treatment arms would be at least 0.6% in paired analyses; at a power of .89 and at a two-sided 0.05 significance level, a total of 30 study subjects are needed.

The proposed enrollment of 42 study subjects to account for potential study dropout to achieve the primary study aim fully powers this secondary objective. If all 42 of the study subjects complete the trial, the power increases to > .96.

All the other secondary measures are summarized by their means and standard deviations The ratio of the difference in means to the standard deviation is a commonly accepted measure of the standard deviation to the mean defines the effect size in standard deviation units. While exact data from prior studies are not available for each of the secondary outcome measures, the analysis plan is the same and we would expect that if the study met the accrual projects for the primary stratum that it will produce sufficient numbers to detect a moderate effect size on the secondary outcome measures as well. As these are considered secondary, no special provision in the sample size target is made for multiple testing although caution will be exercised in interpreting significance levels close to 0.05.

6.7 Interim Analyses

6.7.1 Interim Analysis of Efficacy Data

No formal interim analyses are planned.

6.7.2 Interim Analysis of Safety Data

Safety results will be listed, tabulated using descriptive statistics, and plotted when appropriate. Parameters to be studied may include incidence of AEs, vital signs, laboratory parameters, physical examination findings, cytokine levels, and other safety analyses as deemed clinically appropriate. For some safety parameters, results may be summarized and plotted separately for obese vs. non-obese individuals (subjects who were obese at the time of randomization). Analyses of AEs may include special categories of AEs, such as bacterial/viral infections. Adverse events may also be summarized by time of onset.

6.7.3 Futility Analysis

Since no formal efficacy analyses are planned, a futility analysis without a priori knowledge of effects on efficacy is unwarranted.

6.8 Missing Data

The participating trial investigators are well aware of the problems caused by missing data, and subscribe to the credo that the best solution to the problems caused by missing data is the elimination or reduction of missing data. To prevent or minimize the amount of missing data, careful data collection, data management and quality control procedures must be implemented. These measures may include, among others, training and certification of all study personnel, careful design of data collection forms, clear and thorough documentation of all study procedures, the implementation of a data management system that minimizes the probability of data entry errors and reporting procedures that help track subjects in longitudinal studies.

However, it is prudent to acknowledge up front that some loss to follow-up and some missing data may occur in any study. In this case, it is critical to understand what events or factors are responsible for data that are missing. That is, are the missing data: (1) missing completely at random (MCAR), (2) missing at random (MAR) or (3) non-ignorable. By knowing how the data are missing (e.g., MAR), the most appropriate analytic approach can be selected. To understand how those individuals with missing data differ from those without missing data, comparisons of the baseline characteristics and the last observed measurement of subjects with missing data will be made to those without missing data and tests for MAR and MCAR will be applied when applicable.

6.9 Laboratory Values Below Limits of Quantification and Repeat Testing

Laboratory values below the level of quantification (BLQ) will be set to one-half the limit of quantification (unless noted otherwise) in computations for the aggregate analyses, but will be listed as BLQ in the listings.

If a laboratory test is repeated because of an apparent error, the repeat test result will be used in place of the original test result. Both the original test result and the repeat test result will be shown in the listings.

7.0 Ethical considerations and compliance with good clinical practice

7.1 Statement of compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the IRB. Any amendments to the protocol or to the consent materials will also be approved by the IRB before they are implemented.

7.2 Privacy and confidentiality

A participant's privacy and confidentiality will be respected throughout the study. All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA recommendations for subject privacy. Each participant will be assigned a study identification code and this code rather than names will be used to collect, store, and report study subject information. Any identifiable participant information will be kept in locked offices of study investigators and staff or on a password-protected, encrypted computer. This data will not be used for any other purpose than conducting the study. The only times when unblinding of the data will be requested is if an SAE has occurred that warrants the identity of the study subject to be known to the medical monitor, the study physician and/or the IRB for treatment of the SAE. Otherwise, data will be de-identified before analysis and before sharing with blinded study investigators and staff.

8.0 Identification and access to source data

8.1 Identifying source data

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The results of all clinical and clinical laboratory evaluations will be maintained in the participant's medical records and the data will be transferred to clinical CRFs.
Safety data will be recorded on CRFs specifically designed for this purpose. All the SAEs will be reported on an SAE report form as well as on individual CRFs. The DSMB and/or the IRB have the authority to withdraw any participants and/or terminate the study because of safety findings.

8.2 Permitting access to source data

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the participants in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals. The investigational site will normally be notified before auditing visits occur.

8.3 Study Subject Confidentiality

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.
Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored within the research offices of the study team. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number that cannot be used to identify any individual subject. The study data entry and study management systems used by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at AHN.

8.4 Sample and Data Storage

Data collected for this study will be analyzed and stored at AHN. After the study is completed, the de-identified, remaining blood samples could be available to other scientists for research purposes.

The relevance of left-over samples being made available to the research community is that such samples could be used for research into the causes of diabetes and obesity, its complications and other conditions for which individuals with diabetes are at increased risk and to improve treatment.

Per expert determination, the data collected for this study is deemed de-identified. Dates of laboratory values may be distinguishing, but they are rarely independently replicable, therefore, the risk of identification is low. In addition, at no point will the study sponsors have access to any data source that reveals the identity of the corresponding individuals.

8.5 Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual site will not report the data collected from its site alone. All presentations and publications using trial study data must protect the main objectives of the trial. Specifically, all presentations and publications will be generated and coordinated so that the main objectives of the trial are not compromised, such as might occur by early or ill-timed publication of an ancillary study. Data that could be perceived as threatening the equipoise of the trial will not be disclosed prior to release of the primary study outcomes. Timing of presentations or publications of data and the venue where they will be presented or published will be determined or approved by the Steering Committee. Study results should be discussed with the news media only upon authorization of the Steering Committee, and never before the results are presented. Any written statements about this study that are shared with national media should be approved by the Steering Committee before release.

8.6 Quality control and quality assurance

The site clinical investigators are required to keep accurate records to ensure that the conduct of the study is fully documented.

The sponsor is responsible for regularly reviewing the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data. Astra-Zeneca follows SOP QC and QA concerning the production, testing and validation of the study agents (placebo, AZD9668, and saxagliptin).

8.7 Data handling

The site clinical investigators are required to keep accurate records to ensure that the conduct of the study is fully documented. The investigators are required to ensure that all CRFs are completed for every participant entered in the trial. The PI and study team will regularly review the conduct of the trial, verifying adherence to the protocol, and confirming the completeness, consistency, and accuracy of all documented data. Study staff at the site will collect source information on paper CRFs and enter information into the electronic database. Data quality will be ensured through research team and database processes for detection and correction of errors. The database will be maintained on a secure, restricted access computer system. All paper CRFs will be maintained to provide an appropriate paper trail.

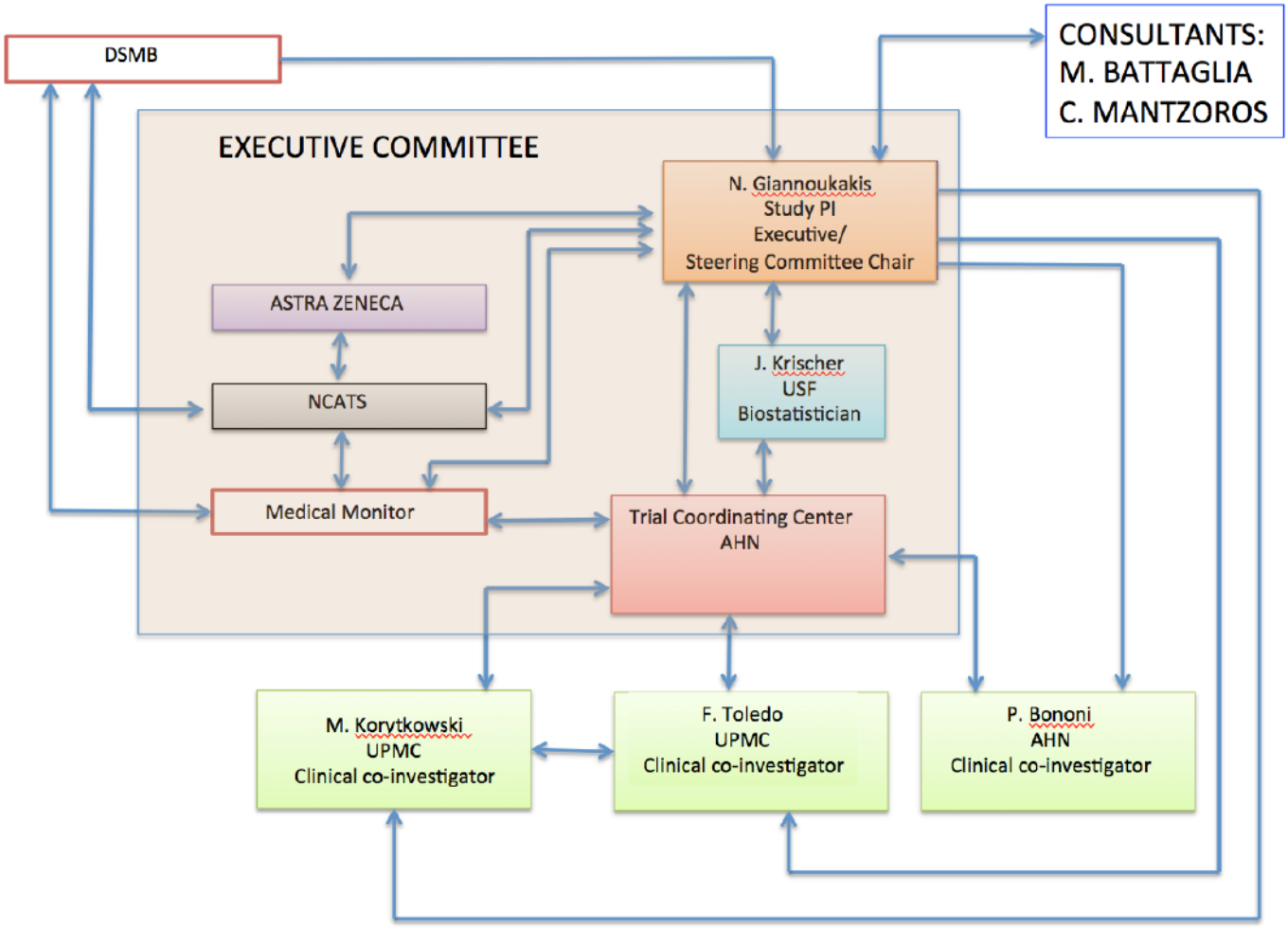
9.0 STUDY ADMINISTRATION

9.1 STUDY LEADERSHIP AND GOVERNANCE

9.2 The **Steering Committee** will govern the conduct of the study. The Steering Committee will be composed of the Study Principal Investigator who will also be the Study Chairman, a representative of the Trial Coordinating Center, the clinical principal investigators with expertise in T2D, the Biostatistician, an independent Medical Monitor who may also be a representative of the DSMB, and a representative from NCATS.

The Steering Committee will meet in person at least twice annually.

9.3 The **Executive Committee** will consist of the Study Chairman, the representative of Trial Coordinating Center, the Biostatistician, the independent Medical Monitor, and a representative from NCATS. The Executive Committee will manage the day-to-day conduct of the study. It will meet by phone regularly, with administrative and other support staff in attendance to discuss the progress of the study and provide frequent guidance and supervision. Members of the Steering Committee as well as study staff who are managing specific areas of the protocol will also join the Executive Committee call, as needed.



9.4 RESEARCH CENTER AND CHAIRMAN'S OFFICE

The trial research team will be located at Allegheny Health Network and specifically, the Institute of Cellular Therapeutics and the Allergy and Autoimmunity Institute of the Allegheny Health Network.

It will be responsible for study subject management, clinical visit and study agent procurement coordination, visit scheduling, test scheduling, study support and management, and for data storage, and management.

The Biostatistics Core will be under the leadership of Dr. Jeffrey Krischer at the University of South Florida and will interface with the AHN research staff on a frequent basis. It will be responsible for the randomization and the statistical analyses of the data.

The Study Chairman's Office is in the Institute of Cellular Therapeutics of the Allegheny Health Network under the direction of Dr. Nick Giannoukakis, the Study PI. He will provide overall administrative, clinical and scientific leadership for the conduct of this trial. He is also the designated PI for the NIH NCATS grant that supports this trial.

9.5 DATA AND SAFETY MONITORING BOARD AND PLAN

An independent physician familiar with clinical trials will be designated to serve as the Medical Monitor who will maintain regular contact with the TCC, the Study Site Clinical PI, the DSMB and IRB. (S)he will review all SAE reports that meet reporting requirements as described in Section 5.1.3..

Research staff will receive all the test results and medical reports in a timely manner, and enter into the database and will ensure that paper copies are stored in clearly labeled binders (with the study subject's alphanumeric identifier code) which shall be placed in a locked room.

The study site coordinators, the Study Site Clinical PIs and participating clinical staff will have regular communication with each study subject during the run-in and then the first 3 months of the trial as detailed in the study schematic above. The study subject will be encouraged to communicate with the study site coordinator should they experience any health anomaly. The Study Site Clinical PI will be responsible for determining whether the anomaly constitutes an AE, SAE, UP, or non-compliance and will proceed with safety reporting according to the process detailed in section 5.1.3. For events that meet FDA Expedited Safety Reporting requirements, the PI, clinical PI, Medical Monitor and DSMB Study Chair may determine whether the event requires a full meeting of the DSMB. The Medical Monitor can also make suggestions to the Clinical PI regarding initiation of any therapeutic course of treatment for relief of SAEs as appropriate and in consultation with the study subject's regular physician.

The DSMB will routinely meet approximately every 3 months following the point where the first subject enters the trial run-in and as needed to review indicators of safety. The DSMB will independently evaluate whether there are grounds to modify or discontinue the study. In all instances, the AHN research staff will ensure that the trial remains blinded, that no breach of identity has occurred, that all the data are protected in the database and that all primary records and test results are stored under controlled access. Unblinding could be requested by the Medical Monitor if needed to assess an event if subject management or safety requires it. Furthermore, the DSMB can request unblinded data at any time. Minutes of, as well as any data deliberated by the DSMB meetings will be available to the IRB at

the time of continuing review.

10.0 Conflict of interest/duality of interest

If any of the study team, including but not limited to the principal investigator, co-investigators, individual site-specific investigators and staff directly related to the conduct of this trial and data interpretation and publication has a real or potential conflict of industry/duality of interest relating directly to the study agents it will be disclosed on the consent and assent forms in a clear manner.

The independence of this study from any actual or perceived influence by the pharmaceutical industry is critical and, therefore, the pharmaceutical industry will play a supportive and advisory role in the trial through its representative on the Steering Committee. The study leadership and their institutions in conjunction with the NIH/NCATS have established policies and procedures for all study group members to disclose all conflicts of interest and should any be disclosed, mechanisms for their management, in line with NIH policies, will be instituted.

11.0 Publication policy

The principal investigator's institutional guidelines and policies on publication and public dissemination of study results will apply for this trial.

12.0 Literature cited/Bibliography

1. Pacini G, Mari A (2003) Methods for clinical assessment of insulin sensitivity and beta-cell function. Best Pract Res Clin Endocrinol Metab 17: 305-322.
2. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Jarvinen H, et al. (2000) Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 23: 295-301.
3. Hanson RL, Pratley RE, Bogardus C, Narayan KM, Roumain JM, et al. (2000) Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. Am J Epidemiol 151: 190-198.
4. Phillips DI, Clark PM, Hales CN, Osmond C (1994) Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. Diabet Med 11: 286-292.
5. Seltzer HS, Allen EW, Herron AL, Jr., Brennan MT (1967) Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest 46: 323-335.
6. Lorenzo C, Haffner SM, Stancakova A, Kuusisto J, Laakso M (2014) Fasting and OGTT-Derived Measures of Insulin Resistance as Compared with the Euglycemic-Hyperinsulinemic Clamp in Non-diabetic Finnish Offspring of Type 2 Diabetic Individuals. J Clin Endocrinol Metab: jc20142299.
7. Stancakova A, Javorsky M, Kuulasmaa T, Haffner SM, Kuusisto J, et al. (2009) Changes in insulin sensitivity and insulin release in relation to glycemia and glucose tolerance in 6,414 Finnish men. Diabetes 58: 1212-1221.
8. Matsuda M, DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 22: 1462-1470.
9. Martelletti P, Katsarava Z, Lampl C, Magis D, Bendtsen L, et al. (2014) Refractory chronic migraine: a consensus statement on clinical definition from the European Headache Federation. J Headache Pain 15: 47.
10. Martelletti P, Lampl C, Russell MB, Mitsikostas DD (2014) Gleaning after the European headache federation consensus statement on refractory chronic migraine. J Headache Pain 15: 75.
11. Halban PA, Polonsky KS, Bowden DW, Hawkins MA, Ling C, et al. (2014) beta-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. J Clin Endocrinol Metab 99: 1983-1992.
12. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, et al. (2014) The many faces of diabetes: a disease with increasing heterogeneity. Lancet 383: 1084-1094.
13. Chmelar J, Chung KJ, Chavakis T (2013) The role of innate immune cells in obese adipose tissue inflammation and development of insulin resistance. Thromb Haemost 109: 399-406.
14. Ferrante AW, Jr. (2013) The immune cells in adipose tissue. Diabetes Obes Metab 15 Suppl 3: 34-38.
15. Lee CT, Harris SB, Retnakaran R, Gerstein HC, Perkins BA, et al. (2014) White blood cell subtypes, insulin resistance and beta-cell dysfunction in high-risk individuals--the PROMISE cohort. Clin Endocrinol (Oxf) 81: 536-541.
16. Rao X, Zhong J, Sun Q (2014) The heterogenic properties of monocytes/macrophages and neutrophils in inflammatory response in diabetes. Life Sci.
17. Ryder E, Diez-Ewald M, Mosquera J, Fernandez E, Pedreanez A, et al. (2014) Association of obesity with leukocyte count in obese individuals without metabolic syndrome. Diabetes Metab Syndr.
18. Talukdar S, Oh da Y, Bandyopadhyay G, Li D, Xu J, et al. (2012) Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med 18: 1407-1412.
19. Tateya S, Kim F, Tamori Y (2013) Recent advances in obesity-induced inflammation and insulin resistance. Front Endocrinol (Lausanne) 4: 93.

20. Viardot A, Heilbronn LK, Samocha-Bonet D, Mackay F, Campbell LV, et al. (2012) Obesity is associated with activated and insulin resistant immune cells. Diabetes Metab Res Rev 28: 447-454.
21. Mansuy-Aubert V, Zhou QL, Xie X, Gong Z, Huang JY, et al. (2013) Imbalance between neutrophil elastase and its inhibitor alpha1-antitrypsin in obesity alters insulin sensitivity, inflammation, and energy expenditure. Cell Metab 17: 534-548.
22. Talukdar S, Oh da Y, Bandyopadhyay G, Li D, Xu J, et al. (2012) Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med 18: 1407-1412.
23. DeFronzo RA (2004) Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 88: 787-835, ix.
24. Adebayo O, Willis GC (2014) The changing face of diabetes in America. Emerg Med Clin North Am 32: 319-327.
25. Lam DW, LeRoith D (2012) The worldwide diabetes epidemic. Curr Opin Endocrinol Diabetes Obes 19: 93-96.
26. Federation ID (2014) IDF Diabetes Atlas (2014 Update). 6th Edition http://www.idf.org/diabetesatlas.
27. Boyle JP, Thompson TJ, Gregg EW, Barker LE, Williamson DF (2010) Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. Popul Health Metr 8: 29.
28. Association AD (2012) Economic costs of diabetes in the US in 2012. Diabetes Care 36: 1033-1046.
29. Shamseddeen H, Getty JZ, Hamdallah IN, Ali MR (2011) Epidemiology and economic impact of obesity and type 2 diabetes. Surg Clin North Am 91: 1163-1172, vii.
30. Campbell RK (2009) Type 2 diabetes: where we are today: an overview of disease burden, current treatments, and treatment strategies. J Am Pharm Assoc (2003) 49 Suppl 1: S3-9.
31. Cizza G, Brown RJ, Rother KI (2012) Rising incidence and challenges of childhood diabetes. A mini review. J Endocrinol Invest 35: 541-546.
32. Badaru A, Pihoker C (2012) Type 2 diabetes in childhood: clinical characteristics and role of beta-cell autoimmunity. Curr Diab Rep 12: 75-81.
33. Kim G, Caprio S (2011) Diabetes and insulin resistance in pediatric obesity. Pediatr Clin North Am 58: 1355-1361, ix.
34. Petitti DB, Klingensmith GJ, Bell RA, Andrews JS, Dabelea D, et al. (2009) Glycemic control in youth with diabetes: the SEARCH for diabetes in Youth Study. J Pediatr 155: 668-672 e661-663.
35. Mayer-Davis EJ, Bell RA, Dabelea D, D'Agostino R, Jr., Imperatore G, et al. (2009) The many faces of diabetes in American youth: type 1 and type 2 diabetes in five race and ethnic populations: the SEARCH for Diabetes in Youth Study. Diabetes Care 32 Suppl 2: S99-101.
36. Control CfD (2014) National Diabetes Statistics Report (2014). http://wwwcdcgov/diabetes/data/statistics/2014StatisticsReporthtml.
37. Liu LL, Lawrence JM, Davis C, Liese AD, Pettitt DJ, et al. (2010) Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth study. Pediatr Diabetes 11: 4-11.
38. Pettitt DJ, Talton J, Dabelea D, Divers J, Imperatore G, et al. (2014) Prevalence of diabetes in U.S. youth in 2009: the SEARCH for diabetes in youth study. Diabetes Care 37: 402-408.
39. Li Y, Schouten EG, Hu X, Cui Z, Luan D, et al. (2008) Obesity prevalence and time trend among youngsters in China, 1982-2002. Asia Pac J Clin Nutr 17: 131-137.
40. Dula SB, Jecmenica M, Wu R, Jahanshahi P, Verrilli GM, et al. (2010) Evidence that low-grade systemic inflammation can induce islet dysfunction as measured by impaired calcium handling. Cell Calcium 48: 133-142.
41. Nicol LE, Grant WF, Comstock SM, Nguyen ML, Smith MS, et al. (2013) Pancreatic inflammation and increased islet macrophages in insulin-resistant juvenile primates. J Endocrinol 217: 207-213.

42. Barbarroja N, Lopez-Pedrera C, Garrido-Sanchez L, Mayas MD, Oliva-Olivera W, et al. (2012) Progression from high insulin resistance to type 2 diabetes does not entail additional visceral adipose tissue inflammation. PLoS One 7: e48155.
43. Haffner S, Temprosa M, Crandall J, Fowler S, Goldberg R, et al. (2005) Intensive lifestyle intervention or metformin on inflammation and coagulation in participants with impaired glucose tolerance. Diabetes 54: 1566-1572.
44. Boni-Schnetzler M, Boller S, Debray S, Bouzakri K, Meier DT, et al. (2009) Free fatty acids induce a proinflammatory response in islets via the abundantly expressed interleukin-1 receptor I. Endocrinology 150: 5218-5229.
45. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, et al. (2002) Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. J Clin Invest 110: 851-860.
46. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259: 87-91.
47. Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117: 175-184.
48. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, et al. (2005) IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 11: 191-198.
49. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, et al. (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 11: 183-190.
50. Huang W, Metlakunta A, Dedousis N, Zhang P, Sipula I, et al. (2010) Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. Diabetes 59: 347-357.
51. Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel CR, et al. (2008) Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell Metab 7: 496-507.
52. Shoelson SE, Lee J, Goldfine AB (2006) Inflammation and insulin resistance. J Clin Invest 116: 1793-1801.
53. Tateya S, Rizzo NO, Handa P, Cheng AM, Morgan-Stevenson V, et al. (2011) Endothelial NO/cGMP/VASP signaling attenuates Kupffer cell activation and hepatic insulin resistance induced by high-fat feeding. Diabetes 60: 2792-2801.
54. Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, et al. (2012) Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest 122: 153-162.
55. Lynch L, Nowak M, Varghese B, Clark J, Hogan AE, et al. (2012) Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. Immunity 37: 574-587.
56. Hatanaka E, Monteagudo PT, Marrocos MS, Campa A (2006) Neutrophils and monocytes as potentially important sources of proinflammatory cytokines in diabetes. Clin Exp Immunol 146: 443-447.
57. Borregaard N (2010) Neutrophils, from marrow to microbes. Immunity 33: 657-670.
58. Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 11: 519-531.
59. Battaglia M (2014) Neutrophils and type 1 autoimmune diabetes. Curr Opin Hematol 21: 8-15.
60. Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, et al. (2010) Evidence for a cross-talk between human neutrophils and Th17 cells. Blood 115: 335-343.
61. Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, et al. (2008) Neutrophil secretion products pave the way for inflammatory monocytes. Blood 112: 1461-1471.
62. Lefrancais E, Roga S, Gautier V, Gonzalez-de-Peredo A, Monsarrat B, et al. (2012) IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. Proc Natl Acad Sci U S A 109: 1673-1678.
63. Yipp BG, Kubes P (2013) NETosis: how vital is it? Blood 122: 2784-2794.

64. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A (2010) Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol 191: 677-691.
65. Andrade VL, Petruceli E, Belo VA, Andrade-Fernandes CM, Caetano Russi CV, et al. (2012) Evaluation of plasmatic MMP-8, MMP-9, TIMP-1 and MPO levels in obese and lean women. Clin Biochem 45: 412-415.
66. Elgazar-Carmon V, Rudich A, Hadad N, Levy R (2008) Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. J Lipid Res 49: 1894-1903.
67. Talukdar S, Oh da Y, Bandyopadhyay G, Li D, Xu J, et al. (2012) Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med 18: 1407-1412.
68. Vieira VJ, Ronan AM, Windt MR, Tagliaferro AR (2005) Elevated atopy in healthy obese women. Am J Clin Nutr 82: 504-509.
69. Trellakis S, Rydleuskaya A, Fischer C, Canbay A, Tagay S, et al. (2012) Low adiponectin, high levels of apoptosis and increased peripheral blood neutrophil activity in healthy obese subjects. Obes Facts 5: 305-318.
70. Wang Q, Xie Z, Zhang W, Zhou J, Wu Y, et al. (2014) Myeloperoxidase deletion prevents high-fat diet-induced obesity and insulin resistance. Diabetes 63: 4172-4185.
71. Cai TQ, Wright SD (1996) Human leukocyte elastase is an endogenous ligand for the integrin CR3 (CD11b/CD18, Mac-1, alpha M beta 2) and modulates polymorphonuclear leukocyte adhesion. J Exp Med 184: 1213-1223.
72. Soehnlein O, Weber C, Lindbom L (2009) Neutrophil granule proteins tune monocytic cell function. Trends Immunol 30: 538-546.
73. Soehnlein O, Zernecke A, Weber C (2009) Neutrophils launch monocyte extravasation by release of granule proteins. Thromb Haemost 102: 198-205.
74. Korkmaz B, Horwitz MS, Jenne DE, Gauthier F (2010) Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacol Rev 62: 726-759.
75. Kahn SE, Cooper ME, Del Prato S (2014) Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet 383: 1068-1083.
76. Cornell S, D'Souza J (2014) Pharmacotherapy considerations in diabetes and obesity: setting patients up for success. Postgrad Med 126: 100-109.
77. Springer SC, Silverstein J, Copeland K, Moore KR, Prazar GE, et al. (2013) Management of type 2 diabetes mellitus in children and adolescents. Pediatrics 131: e648-664.
78. Namba M, Katsuno T, Kusunoki Y, Matsuo T, Miuchi M, et al. (2013) New strategy for the treatment of type 2 diabetes mellitus with incretin-based therapy. Clin Exp Nephrol 17: 10-15.
79. Mudaliar S (2013) Choice of early treatment regimen and impact on beta-cell preservation in type 2 diabetes. Int J Clin Pract 67: 876-887.
80. Morsink LM, Smits MM, Diamant M (2013) Advances in pharmacologic therapies for type 2 diabetes. Curr Atheroscler Rep 15: 302.
81. Reid J, Macdougall AI, Andrews MM (1957) Aspirin and diabetes mellitus. Br Med J 2: 1071-1074.
82. Reid J, Lightbody TD (1959) The insulin equivalence of salicylate. Br Med J 1: 897-900.
83. Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, et al. (2002) Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 109: 1321-1326.
84. Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, et al. (2010) The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 152: 346-357.
85. Larsen AH, Puggaard L, Hamalainen U, Aagaard P (2008) Comparison of ground reaction forces and antagonist muscle coactivation during stair walking with ageing. J Electromyogr Kinesiol 18: 568-580.

86. Osborn O, Brownell SE, Sanchez-Alavez M, Salomon D, Gram H, et al. (2008) Treatment with an Interleukin 1 beta antibody improves glycemic control in diet-induced obesity. Cytokine 44: 141-148.
87. Donath MY, Mandrup-Poulsen T (2008) The use of interleukin-1-receptor antagonists in the treatment of diabetes mellitus. Nat Clin Pract Endocrinol Metab 4: 240-241.
88. Sloan-Lancaster J, Abu-Raddad E, Polzer J, Miller JW, Scherer JC, et al. (2013) Double-blind, randomized study evaluating the glycemic and anti-inflammatory effects of subcutaneous LY2189102, a neutralizing IL-1beta antibody, in patients with type 2 diabetes. Diabetes Care 36: 2239-2246.
89. Rissanen A, Howard CP, Botha J, Thuren T, Global I (2012) Effect of anti-IL-1beta antibody (canakinumab) on insulin secretion rates in impaired glucose tolerance or type 2 diabetes: results of a randomized, placebo-controlled trial. Diabetes Obes Metab 14: 1088-1096.
90. Ridker PM, Howard CP, Walter V, Everett B, Libby P, et al. (2012) Effects of interleukin-1beta inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. Circulation 126: 2739-2748.
91. Geiler J, McDermott MF (2010) Gevokizumab, an anti-IL-1beta mAb for the potential treatment of type 1 and 2 diabetes, rheumatoid arthritis and cardiovascular disease. Curr Opin Mol Ther 12: 755-769.
92. Hensen J, Howard CP, Walter V, Thuren T (2013) Impact of interleukin-1beta antibody (canakinumab) on glycaemic indicators in patients with type 2 diabetes mellitus: results of secondary endpoints from a randomized, placebo-controlled trial. Diabetes Metab 39: 524-531.
93. Ridker PM, Thuren T, Zalewski A, Libby P (2011) Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J 162: 597-605.
94. Noe A, Howard C, Thuren T, Taylor A, Skerjanec A (2014) Pharmacokinetic and Pharmacodynamic Characteristics of Single-Dose Canakinumab in Patients With Type 2 Diabetes Mellitus. Clin Ther.
95. Howard C, Noe A, Skerjanec A, Holzhauer B, Wernsing M, et al. (2014) Safety and tolerability of canakinumab, an IL-1beta inhibitor, in type 2 diabetes mellitus patients: a pooled analysis of three randomised double-blind studies. Cardiovasc Diabetol 13: 94.
96. Bernstein LE, Berry J, Kim S, Canavan B, Grinspoon SK (2006) Effects of etanercept in patients with the metabolic syndrome. Arch Intern Med 166: 902-908.
97. Dominguez H, Storgaard H, Rask-Madsen C, Steffen Hermann T, Ihlemann N, et al. (2005) Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. J Vasc Res 42: 517-525.
98. Lo J, Bernstein LE, Canavan B, Torriani M, Jackson MB, et al. (2007) Effects of TNF-alpha neutralization on adipocytokines and skeletal muscle adiposity in the metabolic syndrome. Am J Physiol Endocrinol Metab 293: E102-109.
99. Anderson R, Theron AJ, Gravett CM, Steel HC, Tintinger GR, et al. (2009) Montelukast inhibits neutrophil pro-inflammatory activity by a cyclic AMP-dependent mechanism. Br J Pharmacol 156: 105-115.
100. Barletta KE, Ley K, Mehrad B (2012) Regulation of neutrophil function by adenosine. Arterioscler Thromb Vasc Biol 32: 856-864.
101. Barnes PJ (2007) New molecular targets for the treatment of neutrophilic diseases. J Allergy Clin Immunol 119: 1055-1062; quiz 1063-1054.
102. Barnes PJ (2011) Glucocorticosteroids: current and future directions. Br J Pharmacol 163: 29-43.
103. Gaudreau R, Le Gouill C, Venne MH, Stankova J, Rola-Pleszczynski M (2002) Threonine 308 within a putative casein kinase 2 site of the cytoplasmic tail of leukotriene B(4) receptor (BLT1) is crucial for ligand-induced, G-protein-coupled receptor-specific kinase 6-mediated desensitization. J Biol Chem 277: 31567-31576.

104. Gravett CM, Theron AJ, Steel HC, Tintinger GR, Cockeran R, et al. (2010) Interactive inhibitory effects of formoterol and montelukast on activated human neutrophils. Eur Respir J 36: 1417-1424.
105. Gravett CM, Theron AJ, Steel HC, Tintinger GR, Cockeran R, et al. (2010) Interactive inhibitory effects of formoterol and montelukast on activated human neutrophils. Eur Respir J 36: 1417-1424.
106. Gravett CM, Theron AJ, Steel HC, Tintinger GR, Cockeran R, et al. (2010) Interactive inhibitory effects of formoterol and montelukast on activated human neutrophils. Eur Respir J 36: 1417-1424.
107. Hatzelmann A, Schudt C (2001) Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhibitor roflumilast in vitro. J Pharmacol Exp Ther 297: 267-279.
108. Lazaar AL, Sweeney LE, MacDonald AJ, Alexis NE, Chen C, et al. (2011) SB-656933, a novel CXCR2 selective antagonist, inhibits ex vivo neutrophil activation and ozone-induced airway inflammation in humans. Br J Clin Pharmacol 72: 282-293.
109. Leonardi C, Matheson R, Zachariae C, Cameron G, Li L, et al. (2012) Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. N Engl J Med 366: 1190-1199.
110. Nair P, Gaga M, Zervas E, Alagha K, Hargreave FE, et al. (2012) Safety and efficacy of a CXCR2 antagonist in patients with severe asthma and sputum neutrophils: a randomized, placebo-controlled clinical trial. Clin Exp Allergy 42: 1097-1103.
111. Ohnishi H, Miyahara N, Gelfand EW (2008) The role of leukotriene B(4) in allergic diseases. Allergol Int 57: 291-298.
112. Sivertson KL, Seeds MC, Long DL, Peachman KK, Bass DA (2007) The differential effect of dexamethasone on granulocyte apoptosis involves stabilization of Mcl-1L in neutrophils but not in eosinophils. Cell Immunol 246: 34-45.
113. Stankova J, Turcotte S, Harris J, Rola-Pleszczynski M (2002) Modulation of leukotriene B4 receptor-1 expression by dexamethasone: potential mechanism for enhanced neutrophil survival. J Immunol 168: 3570-3576.
114. Steel HC, Theron AJ, Cockeran R, Anderson R, Feldman C (2012) Pathogen- and host-directed anti-inflammatory activities of macrolide antibiotics. Mediators Inflamm 2012: 584262.
115. Steel HC, Theron AJ, Tintinger GR, Anderson R (2009) Posaconazole attenuates leukotriene B4 release and uptake of calcium by chemoattractant-activated human neutrophils: a potential strategy to control neutrophil-mediated inflammation. J Antimicrob Chemother 64: 1008-1012.
116. Tannheimer SL, Sorensen EA, Haran AC, Mansfield CN, Wright CD, et al. (2012) Additive anti-inflammatory effects of beta 2 adrenoceptor agonists or glucocorticosteroid with roflumilast in human peripheral blood mononuclear cells. Pulm Pharmacol Ther 25: 178-184.
117. Tintinger GR, Feldman C, Theron AJ, Anderson R (2010) Montelukast: more than a cysteinyl leukotriene receptor antagonist? ScientificWorldJournal 10: 2403-2413.
118. Tintinger GR, Steel HC, Theron AJ, Anderson R (2009) Pharmacological control of neutrophil-mediated inflammation: strategies targeting calcium handling by activated polymorphonuclear leukocytes. Drug Des Devel Ther 2: 95-104.
119. Tsuchihashi Y, Oishi K, Yoshimine H, Suzuki S, Kumatori A, et al. (2002) Fourteen-member macrolides suppress interleukin-8 production but do not promote apoptosis of activated neutrophils. Antimicrob Agents Chemother 46: 1101-1104.
120. den Uyl D, van Raalte DH, Nurmohamed MT, Lems WF, Bijlsma JW, et al. (2012) Metabolic effects of high-dose prednisolone treatment in early rheumatoid arthritis: balance between diabetogenic effects and inflammation reduction. Arthritis Rheum 64: 639-646.
121. Rafacho A, Ortsater H, Nadal A, Quesada I (2014) Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes. J Endocrinol 223: R49-R62.
122. Huang HY, Caballero B, Chang S, Alberg AJ, Semba RD, et al. (2006) The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a

systematic review for a National Institutes of Health state-of-the-science conference. Ann Intern Med 145: 372-385.
123. Iwata K, Doi A, Ohji G, Oka H, Oba Y, et al. (2010) Effect of neutrophil elastase inhibitor (sivelestat sodium) in the treatment of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): a systematic review and meta-analysis. Intern Med 49: 2423-2432.
124. Makino H, Kunisaki C, Kosaka T, Akiyama H, Morita S, et al. (2011) Perioperative use of a neutrophil elastase inhibitor in video-assisted thoracoscopic oesophagectomy for cancer. Br J Surg 98: 975-982.
125. Miyoshi S, Hamada H, Ito R, Katayama H, Irifune K, et al. (2013) Usefulness of a selective neutrophil elastase inhibitor, sivelestat, in acute lung injury patients with sepsis. Drug Des Devel Ther 7: 305-316.
126. Tsujii S, Okabayashi T, Shiga M, Takezaki Y, Sugimoto T, et al. (2012) The effect of the neutrophil elastase inhibitor sivelestat on early injury after liver resection. World J Surg 36: 1122-1127.
127. Elborn JS, Perrett J, Forsman-Semb K, Marks-Konczalik J, Gunawardena K, et al. (2012) Efficacy, safety and effect on biomarkers of AZD9668 in cystic fibrosis. Eur Respir J 40: 969-976.
128. Gunawardena KA, Gullstrand H, Perrett J (2013) Pharmacokinetics and safety of AZD9668, an oral neutrophil elastase inhibitor, in healthy volunteers and patients with COPD. Int J Clin Pharmacol Ther 51: 288-304.
129. Kuna P, Jenkins M, O'Brien CD, Fahy WA (2012) AZD9668, a neutrophil elastase inhibitor, plus ongoing budesonide/formoterol in patients with COPD. Respir Med 106: 531-539.
130. Stockley R, De Soyza A, Gunawardena K, Perrett J, Forsman-Semb K, et al. (2013) Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. Respir Med 107: 524-533.
131. Vogelmeier C, Aquino TO, O'Brien CD, Perrett J, Gunawardena KA (2012) A randomised, placebo-controlled, dose-finding study of AZD9668, an oral inhibitor of neutrophil elastase, in patients with chronic obstructive pulmonary disease treated with tiotropium. COPD 9: 111-120.
132. Delbosc S, Rouer M, Alsac JM, Louedec L, Philippe M, et al. (2014) Elastase inhibitor AZD9668 treatment prevented progression of experimental abdominal aortic aneurysms. J Vasc Surg.
133. Stockley R, De Soyza A, Gunawardena K, Perrett J, Forsman-Semb K, et al. (2013) Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. Respir Med 107: 524-533.
134. Chaicha-Brom T, Yasmeen T (2013) DPP-IV inhibitor-associated arthralgias. Endocr Pract 19: 377.
135. Crickx E, Marroun I, Veyrie C, Le Beller C, Schoindre Y, et al. (2014) DPP4 inhibitor-induced polyarthritis: a report of three cases. Rheumatol Int 34: 291-292.
136. Yokota K, Igaki N (2012) Sitagliptin (DPP-4 inhibitor)-induced rheumatoid arthritis in type 2 diabetes mellitus: a case report. Intern Med 51: 2041-2044.
137. Sasaki T, Hiki Y, Nagumo S, Ikeda R, Kimura H, et al. (2010) Acute onset of rheumatoid arthritis associated with administration of a dipeptidyl peptidase-4 (DPP-4) inhibitor to patients with dibaetes mellitus. Diabetol Int 1: 90-92.
138. Ahren B (2009) Clinical results of treating type 2 diabetic patients with sitagliptin, vildagliptin or saxagliptin--diabetes control and potential adverse events. Best Pract Res Clin Endocrinol Metab 23: 487-498.
139. Cobble ME, Frederich R (2012) Saxagliptin for the treatment of type 2 diabetes mellitus: assessing cardiovascular data. Cardiovasc Diabetol 11: 6.
140. Gautier JF, Sauvanet JP (2011) Efficacy of saxagliptin as an add-on to oral monotherapy in the phase 3 clinical development program: predictive factors of the treatment response in type 2 diabetes. Ann Endocrinol (Paris) 72: 287-295.
141. Gerrald KR, Van Scoyoc E, Wines RC, Runge T, Jonas DE (2012) Saxagliptin and sitagliptin in adult patients with type 2 diabetes: a systematic review and meta-analysis. Diabetes Obes Metab 14: 481-492.

142. Kania DS, Gonzalvo JD, Weber ZA (2011) Saxagliptin: a clinical review in the treatment of type 2 diabetes mellitus. Clin Ther 33: 1005-1022.
143. Neumiller JJ, Campbell RK (2010) Saxagliptin: a dipeptidyl peptidase-4 inhibitor for the treatment of type 2 diabetes mellitus. Am J Health Syst Pharm 67: 1515-1525.
144. Scheen AJ (2012) Metformin + saxagliptin for type 2 diabetes. Expert Opin Pharmacother 13: 139-146.
145. Schwartz SL (2012) Saxagliptin for the treatment of type 2 diabetes mellitus: focus on recent studies. Ann Med 44: 157-169.
146. Yang LP (2012) Saxagliptin: a review of its use as combination therapy in the management of type 2 diabetes mellitus in the EU. Drugs 72: 229-248.
147. Stockley R, De Soyza A, Gunawardena K, Perrett J, Forsman-Semb K, et al. (2013) Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. Respir Med 107: 524-533.
148. Cersosimo E, Solis-Herrera C, Trautmann ME, Malloy J, Triplitt CL (2014) Assessment of pancreatic beta-cell function: review of methods and clinical applications. Curr Diabetes Rev 10: 2-42.
149. DeFronzo RA, Tobin JD, Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 237: E214-223.
150. Tam CS, Xie W, Johnson WD, Cefalu WT, Redman LM, et al. (2012) Defining insulin resistance from hyperinsulinemic-euglycemic clamps. Diabetes Care 35: 1605-1610.
151. Albarbarawi O, Barton A, Miller D, McSharry C, Chaudhuri R, et al. (2013) Characterization and validation of an isotope-dilution LC-MS/MS method for quantification of total desmosine and isodesmosine in plasma and serum. Bioanalysis 5: 1991-2001.
152. Fill JA, Brandt JT, Wiedemann HP, Rinehart BL, Lindemann CF, et al. (2006) Urinary desmosine as a biomarker in acute lung injury. Biomarkers 11: 85-96.
153. Ma S, Lin YY, He J, Rouhani FN, Brantly M, et al. (2013) Alpha-1 antitrypsin augmentation therapy and biomarkers of elastin degradation. COPD 10: 473-481.
154. Derosa G, Romano D, Bianchi L, D'Angelo A, Maffioli P (2014) Metformin powder formulation compared to metformin tablets on glycemic control and on treatment satisfaction in subjects with type 2 diabetes mellitus. J Clin Pharmacol.
155. Derosa G, Ragonesi PD, Fogari E, Cicero AF, Bianchi L, et al. (2014) Sitagliptin added to previously taken antidiabetic agents on insulin resistance and lipid profile: a 2-year study evaluation. Fundam Clin Pharmacol 28: 221-229.
156. Derosa G, Ragonesi PD, Carbone A, Fogari E, D'Angelo A, et al. (2013) Evaluation of the positive effects on insulin-resistance and beta-cell measurements of vildagliptin in addition to metformin in type 2 diabetic patients. Pharmacol Res 73: 20-26.
157. Derosa G, Ragonesi PD, Carbone A, Fogari E, D'Angelo A, et al. (2013) Evaluation of the positive effects on insulin-resistance and beta-cell measurements of vildagliptin in addition to metformin in type 2 diabetic patients. Pharmacol Res 73: 20-26.
158. Derosa G, Ragonesi PD, Carbone A, Fogari E, Bianchi L, et al. (2012) Vildagliptin added to metformin on beta-cell function after a euglycemic hyperinsulinemic and hyperglycemic clamp in type 2 diabetes patients. Diabetes Technol Ther 14: 475-484.
159. Derosa G, Carbone A, Franzetti I, Querci F, Fogari E, et al. (2012) Effects of a combination of sitagliptin plus metformin vs metformin monotherapy on glycemic control, beta-cell function and insulin resistance in type 2 diabetic patients. Diabetes Res Clin Pract 98: 51-60.
160. Derosa G, Carbone A, Franzetti I, Querci F, Fogari E, et al. (2012) Effects of a combination of sitagliptin plus metformin vs metformin monotherapy on glycemic control, beta-cell function and insulin resistance in type 2 diabetic patients. Diabetes Res Clin Pract 98: 51-60.
161. Derosa G, Carbone A, D'Angelo A, Querci F, Fogari E, et al. (2012) A randomized, double-blind, placebo-controlled trial evaluating sitagliptin action on insulin resistance parameters and beta-cell function. Expert Opin Pharmacother 13: 2433-2442.

162. Derosa G, Maffioli P (2011) Patient considerations and clinical utility of a fixed dose combination of saxagliptin/metformin in the treatment of type 2 diabetes. Diabetes Metab Syndr Obes 4: 263-271.
163. Lazaridis EN, Gonin R (1997) Continuously monitored stopping boundary methodologies: the issues of sensitivity, association and trial suspension. Stat Med 16: 1925-1941.
164. Lazaridis E, Gonin R (2000) A new program to compute and evaluate continuously monitored stopping boundaries for clinical trials. Comput Methods Programs Biomed 61: 187-194.
165. Simon R (1982) Patient subsets and variation in therapeutic efficacy. Br J Clin Pharmacol 14: 473-482.
166. Simon R (1989) Optimal two-stage designs for phase II clinical trials. Control Clin Trials 10: 1-10.
167. Simon R, Thall PF, Ellenberg SS (1994) New designs for the selection of treatments to be tested in randomized clinical trials. Stat Med 13: 417-429.
168. Cerutti A, Puga I, Magri G (2013) The B cell helper side of neutrophils. J Leukoc Biol 94: 677-682.
169. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, et al. (2009) Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell 16: 183-194.
170. Kamp VM, Pillay J, Lammers JW, Pickkers P, Ulfman LH, et al. (2012) Human suppressive neutrophils CD16bright/CD62Ldim exhibit decreased adhesion. J Leukoc Biol 92: 1011-1020.
171. Kamp VM, Pillay J, Lammers JW, Pickkers P, Ulfman LH, et al. (2012) Human suppressive neutrophils CD16bright/CD62Ldim exhibit decreased adhesion. J Leukoc Biol 92: 1011-1020.
172. Pillay J, Ramakers BP, Kamp VM, Loi AL, Lam SW, et al. (2010) Functional heterogeneity and differential priming of circulating neutrophils in human experimental endotoxemia. J Leukoc Biol 88: 211-220.
173. Puga I, Cols M, Barra CM, He B, Cassis L, et al. (2012) B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. Nat Immunol 13: 170-180.
174. Tsuda Y, Takahashi H, Kobayashi M, Hanafusa T, Herndon DN, et al. (2004) Three different neutrophil subsets exhibited in mice with different susceptibilities to infection by methicillin-resistant Staphylococcus aureus. Immunity 21: 215-226.
175. Christoffersson G, Vagesjo E, Vandooren J, Liden M, Massena S, et al. (2012) VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. Blood 120: 4653-4662.
176. Sica A, Mantovani A (2012) Macrophage plasticity and polarization: in vivo veritas. J Clin Invest 122: 787-795.
177. Xavier DJ, Takahashi P, Evangelista AF, Foss-Freitas MC, Foss MC, et al. (2015) Assessment of DNA damage and mRNA/miRNA transcriptional expression profiles in hyperglycemic versus non-hyperglycemic patients with type 2 diabetes. Mutation Research IN PRESS.
178. Takamura T, Honda M, Sakai Y, Ando H, Shimizu A, et al. (2007) Gene expression profiles in peripheral blood mononuclear cells reflect the pathophysiology of type 2 diabetes. Biochem Biophys Res Commun 361: 379-384.
179. Goertsches R, Zettl UK (2007) MS therapy research applying genome-wide RNA profiling of peripheral blood. Int MS J 14: 98-107.
180. Goertsches RH, Hecker M, Koczan D, Serrano-Fernandez P, Moeller S, et al. (2010) Long-term genome-wide blood RNA expression profiles yield novel molecular response candidates for IFN-beta-1b treatment in relapsing remitting MS. Pharmacogenomics 11: 147-161.
181. Goertsches RH, Zettl UK, Hecker M (2011) Sieving treatment biomarkers from blood gene-expression profiles: a pharmacogenomic update on two types of multiple sclerosis therapy. Pharmacogenomics 12: 423-432.
182. Meugnier E, Coury F, Tebib J, Ferraro-Peyret C, Rome S, et al. (2011) Gene expression profiling in peripheral blood cells of patients with rheumatoid arthritis in response to anti-TNF-alpha treatments. Physiol Genomics 43: 365-371.

183. Rosenberg A, Fan H, Chiu YG, Bolce R, Tabechian D, et al. (2014) Divergent gene activation in peripheral blood and tissues of patients with rheumatoid arthritis, psoriatic arthritis and psoriasis following infliximab therapy. PLoS One 9: e110657.
184. Rudkowska I, Paradis AM, Thifault E, Julien P, Barbier O, et al. (2013) Differences in metabolomic and transcriptomic profiles between responders and non-responders to an n-3 polyunsaturated fatty acids (PUFAs) supplementation. Genes Nutr 8: 411-423.
185. DeFronzo RA, Hissa MN, Garber AJ, Luiz Gross J, Yuyan Duan R, et al. (2009) The efficacy and safety of saxagliptin when added to metformin therapy in patients with inadequately controlled type 2 diabetes with metformin alone. Diabetes Care 32: 1649-1655.
186. Jadzinsky M, Pfutzner A, Paz-Pacheco E, Xu Z, Allen E, et al. (2009) Saxagliptin given in combination with metformin as initial therapy improves glycaemic control in patients with type 2 diabetes compared with either monotherapy: a randomized controlled trial. Diabetes Obes Metab 11: 611-622.

13.0 APPENDICES

Appendix A: Core labs
Appendix B: Study labs

Appendix A: Clinical Laboratory Measures - Core Lab

Performed as described in the Visit Schematic (Figure 4) in protocol Section 4.5

Hematology/ Coagulation Panel	Virology/Pregnancy Panel	Chemistry Panel	Urine Panel	OGTT Panel	Other labs (run individually)
White Blood Cells (total leukocyte count)	Cytomegalovirus IgM AB	Blood Urea Nitrogen	Bacteria	C-peptide	HgBA1C
Neutrophils (absolute and %)	Cytomegalovirus AB	Serum Creatinine	Bilirubin	Glucose	C-peptide
Lymphocytes (absolute and %)	Hepatitis B Surface AB	Fasting Plasma Glucose	Blood		
Monocytes (absolute and %)	Hepatitis B Surface AG EIA	Sodium	Calcium Oxalate Crystals		
Eosinophils (absolute and %)	Hepatitis C AB	Potassium	Casts		
Basophils (absolute and %)	HIV EIA	Chloride	Glucose		
Red Blood Cells	IM Antibody	Bicarbonate	Ketones		
MCV	Immunology Antibody	Calcium	Leukocyte, esterase		
Hematocrit	Acute Hepatitis Panel	Albumin	Nitrites		
Hemoglobin	HSV AB type 1	Total Serum Protein	pH		
Platelet (cell count)	HSV AB type 2	Alanine aminotransferase	Protein		
Fibrinogen	Serum Pregnancy (Visit 1 only)	Aspartate aminotransferase	Red Blood Cells		
		Lactic Acid Dehydrogenase	Specific Gravity		
		Total Bilirubin	Urobilinogen		
		Alkaline phosphatase	White Blood Cells		
		Total Cholesterol	eGFR		
		Triglycerides			
		Low Density Lipoprotein			
		High Density Lipoprotein			

Appendix B: Exploratory Laboratory Measures – Investigator Study Lab

Performed as described in the Visit Schematic (Figure 4) in protocol Section 4.5

Immunology Flow Cytometry	Immunology Neutrophils	Immunology Luminex	Genomics	Immunology Urine
T-Cells	NET formation	Response to viral CEF pool	Total genome expression analysis	Desmosine
CD4+	Neutrophil elastase activity in vitro	Response to allostimulation in vitro – IFNgamma or TNFalpha or Granzyme B ELISPOT	Total genome epigenotype – epG methylation and histone acetylation	
CD69+		Multi-cytokine panel		
CD8+ CD69+		hs-CRP		
CD25High		Serum leptin		
Foxp3+		Serum adiponectin		
B-Cells		IL-6		
CD19+		Insulin		
CD20+				
CD 19+ IL-10+				
CD19+ CD24+				
CD38+				
Dendritic Cells				
CD11c+/HLA-DR+				
CD11c+ BDCA2+ BDCA-4+				
ALDEFLUOR+				
CD11c+				
CD45+				
CD11c+ CD103+ALDEFLUOR+				
Neutrophils				
CD15+ CD16+ CD62L+				
CD15+ CD16+ CD68+				
Macrophages				
M1				
M2				