TITLE PAGE

Effect of Saxagliptin (DPP-4 Inhibitor) on Endothelial Progenitor Cells (EPCs) as a cellular biomarker for evaluating Endothelial Dysfunction in Early Type 2 Diabetes patients

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PROTOCOL SYNOPSIS

Protocol Title:	Effect of Saxagliptin (DPP-4 Inhibitor) on Endothelial Progenitor Cells (EPCs) as a cellular biomarker for evaluating Endothelial Dysfunction in Early Type 2 Diabetes patients
Site Numbers & Names:	GWU Medical Faculty Associates
Research Hypothesis:	We believe poor viability and function of EPCs in early diabetes ultimately affects the repair and regeneration of the endothelium and that prompts intervention using Saxagliptin with another oral hypoglycemic agent, Metformin, may reduce or reverse cardiovascular risk by improving EPC survival and function above and beyond adequate glucose metabolism control.
Study Schema: **Drugs / Doses / Length of Treatment)**	We propose a 2-arm randomized, parallel group, longitudinal study of 12-week intervention duration. The 12-week time interval has been previously shown adequate to observe changes in biochemistry, EPCs and, importantly, pulse wave velocity (PWV) changes. Patients will be randomized to 2 groups: **Control, (n=20), Metformin + Lifestyle + Placebo. Treatment, (n=20) Metformin+ Lifestyle+ Saxagliptin 5mg.**

Study Objectives: • **Primary:** • **Secondary:**	The primary objective is to ascertain if addition of Saxagliptin improves endothelial dysfunction in early type 2 diabetes patients. The secondary objective is to establish CD34+ cell number, function and gene expression as important bio-markers to monitor endothelial function. **Study Outcome Measures** **Primary:** To investigate the effect of Saxagliptin and lifestyle modification (diet and aerobic exercise intervention) separately on Endothelial function in patients inadequately controlled (HbA1C ≥6% to ≤9%) with Metformin ≥1.0 to ≤2.0 grams daily. • **Cellular markers.** We will study pre and post Saxagliptin treatment changes in number, function and gene expression of patients' peripheral blood-derived CD34+ cells. **Secondary:** • To investigate the effect of Saxagliptin treatment on serum endothelial inflammatory markers including high sensitivity C-reactive protein (hs-CRP), IL-6, TNF-alpha, and fasting lipid profile. Glycemic control will be evaluated by measuring fasting blood glucose, insulin, and HbA1c levels and assessing insulin resistance using HOMA-IR • Adiposity will be measured using the Tanita Body Composition Analyzer scale, measured as percentage body fat • Vessel health will be assessed by systolic and diastolic blood pressure and Arterial stiffness assessed using Vascular Flow and wave measurement equipment, SphygmoCor CP system from ATCOR. The sphygmocor system also allows us to estimate central aortic blood pressure.Resting Metabolic Rate (RMR, similar to Resting Energy expenditure measurement) at baseline, midway point (week 6) and post therapy.
Study Design:	Prospective, double-masked, randomized placebo-controlled trial

Accrual Goal: **(Total number of subjects)**	N=42
Accrual Rate: **(Number of subjects expected per month)**	**Recruit 42 patients in approximately 36 months.**
Estimated: **FPFV:** **LPFV:** **Follow Up:** **(dd-mm-yy)**	**FPFV: 01-10-12.** **LPFV: 01-06-15**
Correlative Studies: **(PK/PD, etc.)**	N/A
Inclusion Criteria:	1. Adults aged 40-70 years. 2. Diagnosis of type 2 diabetes within the previous 8 years using criteria of the American Diabetes Association 3. Currently treated with no hypoglycemic agents other than a stable dose (>3 months) of metformin (≥1.0 to ≤2 grams daily). 4. HbA1C between 6 to 9% (both inclusive) 5. BMI 25 to 39.9 kg/m^2 (both inclusive)

Exclusion Criteria:	Patients with: 1. Contraindications for moderate exercise 2. Implanted devices (e.g., pacemakers) that may interact with Tanita scale 3. Previous coronary or cerebrovascular event within 6 months of screening or active or clinically significant coronary and/or peripheral vascular disease 4. Low hematocrit (< 28 UNITS). 5. Pre-existing liver disease and/or ALT and AST >2.5X's UNL 6. Kidney disease at levels defined by: serum creatinine levels ≥1.5 mg/dL for men, ≥1.4 mg/dL for women, estimated CrCl ≤50 mL/min 7. History of pancreatitis, or cancer (except basal cell carcinoma) 8. Statin use started (or dose change) in the last 3 months, 9. Use of oral or injectable anti-diabetic medication other than Metformin 10. Daily use of a consistent long-term steroid medication (oral, inhaled, injected) within the last 3 months 11. Systolic BP> 140 mmHg and Diastolic BP> 90 mmHg at screening visit. 12. Active wounds or recent surgery within 3 months. 13. Inflammatory disease, or chronic current use of anti-inflammatory drugs within the last 3 months. 14. triglycerides >400 mg/dL 15. untreated hyper/hypothyroidism Additionally, patients who are active smokers, patients who are pregnant, nursing women, and post-menopausal women who are on hormone replacement therapy will be excluded. Patients on low dose oral contraceptives will be allowed to participate as these formulations contain lesser amount of estrogens.

Criteria for Evaluation: **(Efficacy, safety, stopping rules, etc.)**	Subjects will be screened at week -4, then evaluated for end points at weeks 0, 6 and 12. Study participation will be stopped for adverse effects at the discretion of the study investigator. Subjects may stop study participation at any time and for any reason.
Statistics:	The total sample size requested, accounting for attrition over the 12-week period, is 20 subjects per group or 40 subjects total. Sample size estimates were based on the effects of exercise on CD34+/KDR+ cells as described in the literature. The effect of a single session, as well as extended training, on healthy subjects or those with existing cardiovascular conditions appears to increase the CD34+/KDR+ cells. This will provide 73% power (see statistics section). A p-value of less than 0.05 will be considered statistically significant.

1 INTRODUCTION

Type 2 diabetes is a national epidemic [1,2] with significant macro and microvascular complications[3]. Insulin resistance in prediabetes and early and late diabetes are associated with endothelial dysfunction[4].

A few studies indicate that EPCs can act as a suitable bio-marker [5-7] for monitoring cardiovascular morbidity. In this proposal we suggest that EPCs or CD34 positive cells can act as a suitable cellular biomarker for estimating and following endothelial dysfunction in early type 2 diabetes patients.

EPCs have been used as a regenerative tool in ischemic myocardium and diabetic wound healing [8-13]. Endothelial dysfunction with associated inflammation may be a consequence of excess super-oxide presence in a setting of diabetes which is a pro-oxidative stress condition causing EPC dysfunction and senescence[14]. Therefore monitoring EPC number, function and gene expression may serve as a very useful cellular bio-marker for cardiovascular complications in early type 2 diabetes.

Though lifestyle modification has been proposed as a main stay for prevention and treatment of early type 2 diabetes, [2, 15-19] several new therapies for diabetes have been developed in recent years[2]. Incretins and incretin mimetics appear to hold promise. Oral DPP-4 inhibitors have been shown to increase EPCs in patients with type 2 diabetes [20] reportedly via SDF-1 alpha up-regulation. Interestingly, up-regulation of SDF-1 alpha and vascular endothelial growth factor (VEGF), both chemotactic factors increase mobilization and recruitment of EPCs in the face of acute ischemic injury for repair and regeneration.[21-24].

Several studies have shown positive effect of incretins (Glucagon like peptide, GLP-1) and incretin receptor agonists (GLP-1 receptor agonists) on cardiovascular risk factors in type 2 diabetes patients[20,25] and even in patients with chronic heart failure and left ventricular dysfunction who do not have diabetes[26,27].

DPP-4 Inhibitors may have cardio-protective effects of their own, as they increase bio-availability of endogenous GLP-1. They improve blood flow and nitric oxide production in endothelium[28, 29]. These are unique properties not demonstrated by other oral diabetes medications [28]. The mechanism underlying these effects may be mediated by increased nitric oxide bioavailability but is not completely known. It is possible that Saxagliptin, a member of DPP-4 inhibitor group of drugs may be able to improve number and function

of CD34+ endothelial progenitor cells by up-regulating chemotactic agent SDF1 alpha (DPP-4 degrades SDF-1) and its receptor CXCR4[7, 20, 21, 30, 31].

Poor viability and function of EPCs in early diabetes may ultimately affect the repair and regeneration of the endothelium and prompt intervention may reduce or reverse cardiovascular risk by improving EPC survival and function above and beyond adequate glucose metabolism control.

Therefore we would like to explore the effect of Saxagliptin in addition to lifestyle intervention, on number and function and gene expression of EPC and impact on endothelial dysfunction in type 2 diabetes.

This proposed study will recruit patients with type 2 diabetes of less than 8 years duration, who are already on Metformin (≥1.0 to ≤2 gm/d, stable dose for at least 3 months) but still remain poorly controlled with HbA1C between 6% and 9% (both values inclusive), but not overtly out of control (> 9% of HbA1C). In these patients we seek to compare the effect of saxagliptin and lifestyle modification versus lifestyle modification and placebo on endothelial function, particularly the cellular end-point. Initially life-style intervention with diet and regular aerobic activity (as per Standards of Diabetes Care: Diabetes Care Supplement: Jan 2014) will be undertaken for 4 weeks. Subjects will begin taking 5.0 mg of Saxagliptin or placebo after initial 4 weeks of stabilization of life style modification and will be withdrawn from the study if the medication or placebo is not tolerated. We will use the maximum approved dose of saxagliptin in order to discern a definitive effect.

1.1 Summary of Results of Investigational Program

1.1.1 Pharmacology of Saxagliptin

Saxagliptin has been shown to be a highly potent, reversible, competitive inhibitor of human DPP-4,[65] with a Ki value of 1.3 ± 0.3 nM (0.41ng/mL) at 37°C. The 5-hydroxyadamantyl metabolite of saxagliptin (5-hydroxysaxagliptin, BMS-510849), the major metabolite of saxagliptin both in vitro and in vivo in animals and humans, was also a competitive inhibitor of DPP-4 at 37°C, with a Ki value of 2.6 ± 1.0 nM (0.86 ng/mL. 2-fold less potent than saxagliptin).

The clinical pharmacology program consisted of 39 studies in 1090 subjects, 1037 of whom were dosed with saxagliptin. The Phase 2b and Phase 3 worldwide clinical development program in T2DM included 8 core studies in which 4607 subjects were randomized and treated. Of these, 3356 subjects received double-blind saxagliptin; an additional 66 subjects

received open-label saxagliptin 10 mg in CV181011. Over 1000 subjects have received saxagliptin for more than 76 weeks, including 260 who received saxagliptin 10 mg, and over 450 subjects have received saxagliptin for more than 102 weeks. In studies CV181011 and CV181014, 244 subjects have received saxagliptin for > 204 weeks. [66,67]

1.1.2 Preclinical Toxicology of Saxagliptin

The rat and dog were chosen as the standard species for in vivo safety studies based on comparative metabolism patterns and oral bioavailability. The cynomolgus monkey was added initially to model lymphocyte changes observed in humans (which could not be reproduced in the monkey) and subsequently at the request of the United States Food and Drug Administration (US FDA) for all sponsors developing DPP-4 inhibitors. Toxico-kinetic data collected in the repeat-dose studies confirmed that adequate exposure was achieved.

1.1.2.1 Repeat-Dose Studies

Saxagliptin was well tolerated in dogs at 1 mg/kg/day (AUC ≥ 286 ng•h/mL) for 12 months with effects at higher doses consisting primarily of GI (gastrointestinal) toxicity. A dose of 25 mg/kg/day (AUC 50803 ng•h/mL) was overtly toxic and resulted in euthanasia of 1 male after 9 doses due to saxagliptin-induced enteropathy with secondary minimal to mild decreases in serum protein and electrolytes related to intestinal dysfunction (diarrhea, emesis). Drug-related morphologic changes at 5 and 10 mg/kg/day were limited to minimal mixed hepatic infiltrates/inflammation (neutrophils, eosinophils, lymphocytes, and macrophages) around central veins and after 12 months, minimal to slight superficial erosions of the foot pad epidermis (corresponding to footpad cracking). All effects at 5 mg/kg/day in the 3-month study were reversible; recovery was not assessed in the 12-month study. The NOAEL was 1 mg/kg/day after 12 months of dosing, which resulted in respective AUC exposures for saxagliptin and metabolite of 4× and 2× the maximum recommended human dose (MRHD) of saxagliptin.

The repeat-dose oral toxicity of saxagliptin in cynomolgus monkeys was characterized at doses of 0.03, 0.3, and 3 mg/kg/day for 3 months. No drug-related changes were observed at 0.03 or 0.3 mg/kg/day. At 3 mg/kg/day, multifocal skin lesions/scabbing was observed on the feet and/or tails of 4 of 14 monkeys. Drug-related effects at 3 mg/kg/day included skin lesions, minimal multi-tissue mononuclear-cell infiltrates (considered an exacerbation of a common background change), and minimal splenic, thymic, and/or bone marrow lymphoid hyperplasia. Healing of the skin lesions occurred during the dosing period, and

all findings were reversible after a 3-month recovery period. Respective systemic exposures (AUC) for saxagliptin and the metabolite at the NOAEL of 0.3 mg/kg/day were 1 to 3× the MRHD.

1.1.2.2 Mutagenicity and Carcinogenicity

The mutagenic and clastogenic potential of saxagliptin was tested at high concentrations and exposures in a battery of genetic toxicity studies including an in vitro Ames bacterial assay, an in vitro cytogenetics assay in primary human lymphocytes, an in vivo oral micronucleus assay in rats, an in vivo oral DNA repair study in rats, and an oral in vivo/in vitro cytogenetics study in rat peripheral blood lymphocytes.[68] Saxagliptin was not mutagenic or clastogenic based on the combined outcomes of these studies. The major metabolite (BMS-510849) was not mutagenic in an in vitro Ames bacterial assay. Two-year carcinogenicity studies were conducted in mice and rats at oral doses of 50, 250, and 600 mg/kg/day and 25, 75, 150, and 300 mg/kg/day, respectively[4]. Saxagliptin did not induce tumors in either mice or rats at the highest doses evaluated. The highest doses evaluated in mice were equivalent to approximately 900× (males) and 1210× (females) the human exposure at the MRHD. In rats, exposures were approximately 370× (males) and 2300× (females) the exposures at the MRHD.

1.1.2.3 Reproductive and Developmental Toxicity

In a rat fertility study, males were treated with oral gavage doses of 100, 200, and 400 mg/kg/day for two weeks prior to mating, during mating, and up to scheduled termination (approximately 7 weeks total), and females were treated with oral gavage doses of 125, 300, and 750 mg/kg/day for 2 weeks prior to mating through gestation Day 7.[68] No adverse effects on fertility were observed at 200 mg/kg/day (males) or 125 mg/kg/day (females) resulting in respective exposures (AUC) of approximately 630× (males) and 805× (females) times human exposure at the MRHD. At higher, maternally toxic doses (300 and 750 mg/kg/day), increased fetal resorptions were observed (approximately 2150× and 6375× the MRHD). Additional effects on estrous cycling, fertility, ovulation, and implantation were observed at 750 mg/kg (approximately 6375× the MRHD). Saxagliptin was not teratogenic at any dose evaluated in rats or rabbits.[68] At high doses in rats, saxagliptin caused a minor and reversible developmental delay in ossification of the fetal pelvis at ≥240 mg/kg/day (≥1560× the MRHD). Maternal toxicity and reduced fetal body weights were observed at 900 mg/kg/day (8290× the MRHD). In rabbits, the effects of

saxagliptin were limited to minor skeletal variations observed only at maternally toxic doses (200 mg/kg/day, exposures 1420× the MRHD).

Saxagliptin administered to female rats from gestation Day 6 to lactation Day 20 resulted in decreased body weights in male and female offspring only at maternally toxic doses (≥250 mg/kg/day, exposures ≥1690× the MRHD).[68] No functional or behavioral toxicity was observed in offspring of rats administered saxagliptin at any dose.

1.1.3 Human Pharmacokinetics of Saxagliptin

Following a 5 mg single oral dose of saxagliptin tablet, the mean plasma AUC(INF) values for saxagliptin and BMS-510849 were 78 and 214 ng•h/mL, respectively. The corresponding maximum plasma concentration (Cmax) values were 24 and 47 ng/mL, respectively.

1.1.3.1 Absorption

Saxagliptin was rapidly absorbed after oral administration, with Cmax usually attained within 2 hours after administration in the fasted state. In general, the Cmax and AUC values increased approximately equal to the increment in the saxagliptin dose (CV181001). Following a single oral dose of 5 mg saxagliptin in the fasted state, the mean terminal half-life (T1/2) value for saxagliptin was 2.5 h, and no appreciable accumulation was observed upon repeated once-daily dosing at any dose.

1.1.3.2 Distribution

The in vitro protein binding of saxagliptin and its major metabolite in human serum is below measurable levels.[69] Thus, changes in blood protein levels in various disease states (e.g., renal or hepatic impairment) are not expected to alter the disposition of saxagliptin.

1.1.3.3 Elimination

Renal excretion is a major elimination pathway for systemic saxagliptin and BMS-510849. Following a single 50 mg dose of 14C-saxagliptin, 24%, 36%, and 75% (on average) of the dose was excreted in the urine as saxagliptin, BMS-510849, and total radioactivity, respectively (CV181004). These data suggest that saxagliptin was extensively absorbed from the gastrointestinal tract and that BMS-510849 is a major metabolite of saxagliptin in humans. The average renal clearance of saxagliptin (~200 L/min) was greater than the average estimated glomerular filtration rate (~120 mL/min), suggesting some active renal excretion. For BMS-510849, renal clearance values were comparable to estimated

glomerular filtration rate. A total of 22% (on average) of the administered radioactivity was recovered in feces representing the fraction of the saxagliptin dose excreted in bile and/or unabsorbed drug from the gastrointestinal tract.

1.1.3.4 Pharmacokinetics of Active Metabolites

Cmax and AUC values for BMS-510849 increased approximately equal to the increment in the saxagliptin dose. Following single oral doses of 5 to 100 mg saxagliptin in the fed or fasted states, the mean BMS-510849 AUC values were between 2- and 7-times higher than the parent saxagliptin exposures on a molar basis.[70] Following a single oral dose of 5 mg saxagliptin in the fasted state (CV181037), the mean terminal half-life (T1/2) value for BMS-510849 was 3.1 h, and no appreciable accumulation was observed upon repeated once-daily dosing at any dose (CV181002 and CV181010).

1.1.3.5 Dose and Time-dependencies

The single-dose pharmacokinetics of saxagliptin and BMS-510849 were predictive of their multiple-dose pharmacokinetics. No dose- or time-dependency was observed in any relevant pharmacokinetic parameter over 14 days of once-daily dosing with saxagliptin with doses ranging from 2.5 to 400 mg once daily (QD; CV181002 and CV181010). A linear pharmacokinetic model adequately described the plasma concentration time profile of saxagliptin and BMS-510849, suggesting the pharmacokinetics of saxagliptin and BMS-510849 were linear to dose within the tested dose range of 1-100 mg. The estimated pharmacokinetic parameters were time-invariant. These data suggest that saxagliptin does not inhibit or induce its own metabolism or active transport.

1.1.3.6 Special Populations

Saxagliptin may be administered without regard to meals. No dosage adjustment is considered necessary in elderly subjects on the basis of age. Body mass index (BMI), T2DM, gender, age, race, hepatic impairment, and mild renal insufficiency had no clinically meaningful effects on the pharmacokinetics of saxagliptin or BMS-510849. In subjects with moderate to severe renal impairment and end stage renal disease (i.e., creatinine clearance <50 mL/min), area under curve (AUC) values for saxagliptin and/or BMS-510849 were generally greater than 2-fold higher than the AUC values in healthy subjects.

1.1.3.7 Drug-drug interactions

Co-administration of saxagliptin with the following drugs had no meaningful effect on the pharmacokinetics of saxagliptin, BMS-510849, or the other drug tested: metformin, glyburide, pioglitazone, digoxin, simvastatin, rifampin, and combined oral contraceptives. Co-administration of saxagliptin and ketoconazole dosed to steady-state resulted in up to 1.6- and 2.5-times increase in saxagliptin maximum observed concentration (Cmax) and area under curve extrapolated to infinity [AUC(INF)] values, respectively, and 20- to 8-times decrease in the exposure to BMS-510849. However, the systemic exposures to the total active moieties of saxagliptin (saxagliptin + BMS-510849 corrected for relative differences in potency of DPP-4 inhibition) were not meaningfully altered by ketoconazole. Saxagliptin Cmax and AUC(INF) were also increased by 63% and 2.1-fold, respectively, when saxagliptin was co-administered with steady-state diltiazem. Higher geometric mean Cmax and AUV(TAU) for diltiazem were also found when co-administered with saxagliptin. The differences met statistical significance but were not considered clinically meaningful. Co-administration of saxagliptin with gastric acid controllers aluminum and magnesium hydroxides + simethicone, famotidine or omeprazole did not meaningfully alter the pharmacokinetics of saxagliptin or BMS-510849.

1.1.4 Clinical Safety with Saxagliptin

Once-daily, orally administered saxagliptin was safe and well-tolerated at doses of up to 400 mg QD for 2 weeks, 100 mg QD for 6 weeks, 40 mg QD for 12 weeks, and at doses of 2.5, 5, and 10 mg QD for up to 102 weeks.

1.1.4.1 Drug-Related Adverse Events

In an extensive Phase 2b/3 program, the majority of reported adverse events were of mild intensity and did not require treatment discontinuation. The safety profile was generally consistent with placebo or comparator when saxagliptin was given as monotherapy, as add-on combination treatment to metformin, SU, or TZD, and as initial therapy in combination with metformin. Although the rate of certain AEs was higher in subjects who received saxagliptin 10 mg compared with those who received 2.5 and 5 mg, saxagliptin 10 mg was also safe and well tolerated, providing a safety margin for the saxagliptin 5 mg dose.

A pre-specified pooled analysis (the 5-study pooled analysis) of the 2 monotherapy studies, (CV181011 and CV181038) the add-on to metformin study (CV181014), the add-on to TZD study (CV181013) and the add-on to glyburide study (CV181040)

demonstrated that the overall incidence of AEs in patients treated with saxagliptin 5 mg was similar to placebo (72.2% vs. 70.6%). The majority of reported AEs were of mild intensity and did not require treatment discontinuation. There was no discernible difference in the clinical AE profile between the saxagliptin 2.5 mg and 5 mg doses. The most frequently reported AEs (incidence ≥ 5%) in active vs. placebo group were upper respiratory tract infection (7.7% vs. 7.6%), headache (6.5% vs. 5.9%), and urinary tract infection (6.8% vs. 6.1%). In the combination study with metformin (CV181-039), headache (7.5% in saxagliptin, 5.2% in metformin) and Nasopharyngitis (6.9% in saxagliptin, 4.0% in metformin) were the most frequently reported AEs (incidence ≥ 5%). In September 2015, the FDA reported a new AE of severe and disabling joint pain (less than 5% incidence.)

The frequency of AEs leading to discontinuation from study therapy was generally low for all treatment groups. In the placebo-controlled pooled safety analysis, the overall frequency of AEs leading to discontinuation of study medication was comparable between the saxagliptin 2.5 mg and placebo groups (2.2% and 1.8%, respectively) but was greater for patients in the saxagliptin 5 group (3.3%). The most frequently reported AEs (> 1 subject) leading to study discontinuation, which were numerically higher in patients who received saxagliptin compared with placebo, were: lymphopenia, increased blood creatine kinase (CK), increased blood creatinine, nausea, and eye pain. Rates of discontinuation for rash were similar in patients who received saxagliptin and placebo, whereas the rate of discontinuation for AEs of increased weight, depression, and angina pectoris were higher in patients who received placebo compared with saxagliptin.

1.1.4.2 Drug-Related Serious Adverse Events

In the placebo-controlled pooled safety analysis, the frequency of SAEs was identical (3.4%) for the saxagliptin 5 mg and placebo groups. The frequencies of SAEs were comparable for the saxagliptin 5 mg plus metformin and metformin monotherapy groups (2.5% and 2.4%, respectively) in the combination with metformin study (CV181039).

1.1.4.3 Other Significant Drug-Related Adverse Events

Hypoglycemia: Treatment with saxagliptin led to rates of hypoglycemia that were generally similar compared with placebo. This is consistent with the mechanism of action of DPP-4 inhibitors, which exert their insulinotropic effects on the β-cell in a glucose-

dependent manner. In the add-on to sulphonylurea (SU) study, the rate of hypoglycemia was numerically higher in patients who received 2.5 or 5 mg of saxagliptin added on to an intermediate dose of glyburide compared with up-titration of glyburide monotherapy plus placebo.

Skin related AEs: The frequency of skin-related AEs was generally comparable between subjects who received saxagliptin 5 mg and placebo. Overall, evaluation of the Phase 3 clinical data has not revealed any signals that correlate to the skin findings during non-clinical toxicology studies in the monkey, including reversible erosive and/or ulcerative skin lesions with scab formation. Overall, evaluation of the Phase 3 clinical data with exposures up to 206 weeks did not reveal any signals that correlate to the skin findings in the cynomolgus monkey.

Local edema: The proportion of patients with AEs of localized edema, an event of special interest given reports of symptomatic edema of the hands and feet in patients who received another member of the DPP-4-inhibitor class, was generally similar in patients who received saxagliptin and placebo with the exception of the add-on to TZD study, where there was a higher rate of events constituting localized edema compared to placebo in patients treated with saxagliptin 5 mg. The majority of these events in the saxagliptin 5 mg plus TZD group were for pedal edema with no imbalance seen for events of hand edema. Across the clinical program, the majority of events of localized edema were of mild to moderate intensity and did not lead to study discontinuation.

Hypersensitivity Reactions

A 24-week analysis of a grouping of hypersensitivity-related events in a pool of 5 placebo-controlled Phase 3 studies showed an incidence of 1.5% and 0.4% in patients who received saxagliptin 5 mg and placebo, respectively. None of these events in patients who received saxagliptin required hospitalization or were reported to be life-threatening by the investigators.

Lymphopenia: The frequency of investigator reported AEs of lymphopenia was similar for subjects who received saxagliptin and placebo. Mean lymphocyte counts remained stable and within normal limits with daily dosing up to 102 weeks. Overall, a small decrease in mean absolute lymphocyte count was observed at a dose of saxagliptin 5 mg and above. At the 5 mg dose, the mean decrease was approximately 100 cells/μL relative to placebo (from baseline absolute lymphocyte count of approximately 2200 cell/μL) based on a pooled analysis of the 5 placebo-controlled clinical studies including saxagliptin as monotherapy,

and as add-on therapy to metformin, TZD and SU. While the clinical significance of the decreases in lymphocyte count relative to placebo is not known, the decreases were not associated with clinically relevant AEs.

Platelet counts: Results from the Phase 3 clinical studies demonstrated no clinically meaningful or consistent effect on platelet counts.

In the five pooled, monotherapy and placebo-controlled combination studies, the frequencies of AEs in the SOC Infection and Infestations were comparable in the saxagliptin 2.5 mg, saxagliptin 5 mg, and placebo groups (36.4%, 35.9%, and 34.8% respectively); a higher frequency of AEs was observed in the saxagliptin 10 mg group (40.1%).

There was no evidence for an association of saxagliptin treatment with an increased risk of elevated liver function tests or serum creatinine.

Effect on QTc interval: In the clinical pharmacology program, results from a thorough QTc study at daily doses of saxagliptin up to 40 mg QD and analyses from other studies at daily doses up to 400 mg QD demonstrated an absence of an effect of saxagliptin and BMS-510849 on QTc interval in humans.

1.1.4.4 Drug-Related Deaths

Overall, death was an infrequent event and occurred with comparable frequencies in the saxagliptin and comparator groups. In the core Phase 3 studies, a total of 24 patients died during the short-term and long-term periods: 4 subjects in the saxagliptin 2.5 mg group, 3 subjects in the 5 mg group, 5 subjects in the saxagliptin 10 mg group, 7 subjects in the placebo group, and 5 subjects in the metformin monotherapy group.

1.1.5 Clinical Efficacy of Saxagliptin

Saxagliptin Phase 2b and Phase 3 worldwide clinical development program in T2DM included eight studies in which over 4600 subjects combined with the results from clinical pharmacology studies support the oral dose of saxagliptin 5 mg once daily as the usual clinical dose in a wide range of subjects with T2DM, as either monotherapy, add-on combination therapy with metformin, a thiazolidinedione (TZD), or a sulfonylurea (SU), or initial combination therapy with metformin.

In the Phase 2b dose-ranging study, administration of saxagliptin 5 mg was associated with significant inhibition of plasma DPP-4 activity at the trough of the dosing interval as well

as clinically meaningful decreases in A1C, fasting plasma glucose (FPG), and postprandial serum glucose. The results from the short-term periods of the Phase 3 studies confirmed clinically meaningful benefits of saxagliptin 5 mg on A1C, as well as FPG, postprandial glucose, insulin, C-peptide, and glucagon levels. In the monotherapy studies (CV181011 and CV181038), add-on combination therapy studies (CV181014, CV181013, CV181040 with metformin, TZD, glyburide, respectively), and initial combination study with metformin (CV181039), treatment with saxagliptin produced statistically significant reductions in A1C relative to control. A greater percentage of subjects treated with saxagliptin achieved target glycemic goals including A1C levels < 7% compared with subjects treated with placebo or active comparator. The saxagliptin 5 mg groups achieved greater reductions from baseline in A1C than the saxagliptin 2.5 mg groups in five of the six core Phase 3 studies (one of the core studies, Study CV181039, did not have a 2.5 mg treatment group). There was no consistent evidence for an incremental efficacy benefit for 10 mg beyond that seen for the 5 mg dose.

Saxagliptin treatment consistently demonstrated a beneficial antihyperglycemic effect across subgroups of demographic and baseline diabetes characteristics.

1.2 Overall Risk/Benefit Assessment

The efficacy of saxagliptin for the treatment of patients with T2DM was demonstrated in a single Phase 2b study and confirmed in 6 double-blind, placebo- or active-controlled clinical studies. In these studies, 4673 subjects (4607 subjects randomized and treated and 66 patients treated in the open-label cohort of CV181011) received at least one dose of study medication, including 3422 receiving saxagliptin and 1251 receiving either placebo or metformin plus rescue medication if necessary. Treatment with saxagliptin 2.5, 5, and 10 mg was demonstrated to be generally safe and well tolerated. The overall frequencies of AEs for saxagliptin 2.5 and 5 mg were comparable to placebo. Also, the initial combination study with metformin showed that the safety profile of saxagliptin 5 mg plus metformin was generally comparable to either agent used as monotherapy.

Evaluation of the clinical safety and clinical efficacy data indicate an acceptable risk/benefit profile at the dose of saxagliptin planned for this study, 5 mg daily, as a monotherapy, and also in combination with metformin XR 1500 mg. Type 2 diabetes mellitus is a progressive disease, and a combination of 2 agents with complimentary mechanisms of action, such as metformin and saxagliptin, may offer considerable therapeutic advantage.

1.3 Research Hypothesis

We believe poor viability and function of EPCs in early diabetes ultimately affects the repair and regeneration of the endothelium and that prompt intervention using saxagliptin with another oral hypoglycemic agent, Metformin, may reduce or reverse cardiovascular risk by improving EPC survival and function above and beyond adequate glucose metabolism control.

1.4 Study Rationale

Though certain DPP-4 inhibitors have been shown to augment vascular function and endothelial progenitor cells, no such data is available for Saxagliptin.

2 STUDY OBJECTIVES

2.1 Primary Objective

To investigate the effect of saxagliptin and lifestyle modification (diet and aerobic exercise intervention) separately on Endothelial function in patients whose HbA1C is between 6 to 9% (both inclusive) despite treatment with metformin (≥1.0 to ≤2 gm/d), stable dose for at least 3 months)
Cellular markers[5-7,30].We will use patient's peripheral blood derived CD34+ cells looking at number, function and gene expression changes pre and post saxagliptin.

2.2 Secondary Objectives

To investigate the effect of saxagliptin treatment on serum endothelium inflammatory markers including: C-reactive protein (hs-CRP), IL-6, TNF-alpha, Leptin, Adiponectin, and fasting lipid profile [6, 30]

Glycemic control will be evaluated by measuring fasting blood glucose, insulin levels and HbA1c. Fasting blood glucose and insulin will be used to assess insulin resistance with HOMA-IR [32]

Adiposity will be measured using the Tanita Body Composition Analyzer scale, measured as percentage body fat [33]

Vessel health will be assessed by systolic and diastolic blood pressure and Arterial stiffness will be assessed using Vascular Flow and wave measurement equipment, SphygmoCor CP system from ATCOR. The sphygmocor system also allows us to estimate central aortic blood pressure.

The biochemical or bio-inflammatory markers are an important marker of endothelial inflammation and dysfunction along with glycemic markers and markers of arterial stiffness.

3 ETHICAL CONSIDERATIONS

3.1 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50).

The study will be conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent will receive Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion before initiation of the study.

All potential serious breaches must be reported to Astra-Zeneca (AZ) immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks.

This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure; debarment).

3.2 Institutional Review Board/Independent Ethics Committee

Before study initiation, the investigator must have written and dated approval/favorable opinion from the IRB/IEC for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The investigator or sponsor should also provide the IRB/IEC with a copy of the Investigator Brochure or product labeling information to be provided to subjects, and any updates.

The investigator should provide the IRB/IEC with reports, updates, and other information (e.g., expedited safety reports, amendments, and administrative letters) according to regulatory requirements or institution procedures.

3.3 Informed Consent

Investigators must ensure that subjects or, in those situations where consent cannot be given by subjects, their legally acceptable representative are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate.

Investigators must:

1) Provide a copy of the consent form and written information about the study in the language in which the subject is most proficient prior to clinical study participation. The language must be non-technical and easily understood.

2) Allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study.

3) Obtain an informed consent signed and personally dated by the subject or the subject's legally acceptable representative and by the person who conducted the informed consent discussion.

4) Obtain the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects, prior to the beginning of the study, and after any revisions are completed for new information.

5) If informed consent is initially given by a subject's legally acceptable representative or legal guardian, and the subject subsequently becomes capable of making and communicating their informed consent during the study, then consent must additionally be obtained from the subject.

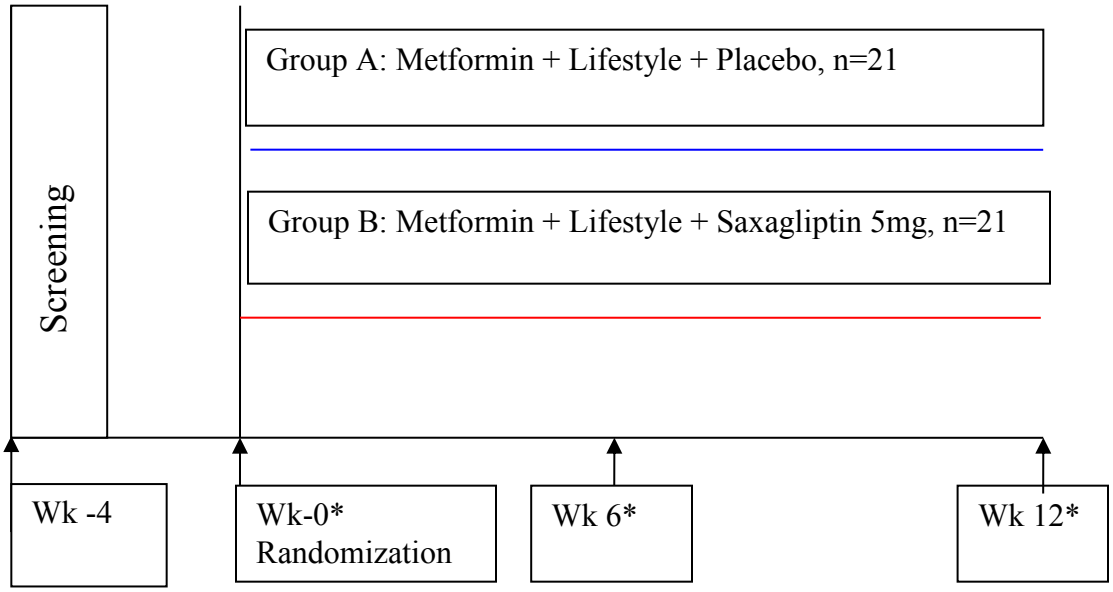
6) Revise the informed consent whenever important new information becomes available that is relevant to the subject's consent. The investigator, or a person designated by the investigator, should fully inform the subject or the subject's legally acceptable representative or legal guardian, of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented.

The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

4 INVESTIGATIONAL PLAN

4.1 Study Design and Duration

Study Schema, 42 Type 2 diabetic Subjects Aged 40-70 on stable dose of Metformin



+/- 3 days for visits Wk 0 Wk 6 and Wk 12, if the particular day is not feasible

*Assessed at week 0, 6 and 12: Biochemical and cellular markers of endothelial function and Pulse wave analysis and velocity (PWA and PWV).

Available related studies and Expected Results: Studies measuring changes to EPCs in type 2 diabetes patients are very limited. To the best of our knowledge there are no studies examining the effect of saxagliptin in early diabetes on patient's CD34/KDR+ve cells and comparing that to the effect of lifestyle + metformin on CD34+ve cell population. The regenerative potential of EPC is thought to be due to direct incorporation into vascular endothelium and/or the secretion of angio-supportive factors to enhance endothelial repair and function[17, 18, 19]. Our studies and others' [11,12,34], have shown that various disease states, including those seen in diabetes, can deplete and damage EPC, thereby diminishing their regenerative potential. The inability to maintain or repair damaged endothelial tissue leads to cumulative vascular dysfunction and cardiovascular disease.

We hypothesize that monitoring **number,** ***function and gene expression of endothelial progenitors will allow us to quantify cardiovascular disease risk at the onset and regenerative potential post intervention at a cellular level. It will also help us to identify and correlate the best endothelial function bio-chemical inflammatory marker as an early indicator of cardiovascular disease progression in early type 2 diabetes***.

Therefore, studying the effects of Saxagliptin which can increase SDF1 alpha levels and possibly CXCR4 receptor expression as an intervention on the number and health of EPCs may not only provide insight on the biological role of these cells, but they can be used as a tool to study vascular biology and lastly can be a potential target for prevention of diabetes vascular complications.

We expect to establish that saxagliptin improves endothelial function that is EPC number, function, gene expression and bio-inflammatory markers beyond its glycemic control effect in type 2 diabetes patients.

Saxagliptin 5 mg or placebo will be added to patients who have type 2 diabetes diagnosed less than 8 years with HbA1C of 6 to 9 % (both inclusive) while being on lifestyle modification and Metformin (≥1.0 to ≤2 gm/d orally).

Our study population will include equal numbers of adult male and female Type 2 diabetes (T2DM) patients aged 40-70 years, enrolled in both arms of the study.
Patients will be treated with 5 mg of Saxagliptin or placebo with lifestyle modification for 12 weeks. This time interval has been previously shown to be adequate to observe changes to Endothelial Progenitor Cells (EPCs)[5,6].

4.2 Study Population

For entry into the study, the following criteria MUST be met.

4.2.1 Inclusion Criteria

1) Signed Written Informed Consent

- Before any study procedures are performed, subjects will have the details of the study described to them, and they will be given a written informed consent document to read. Then, if subjects consent to participate in the study, they will indicate that consent by signing and dating the informed consent document in the presence of study personnel.

2) Target Population

- Subjects with a diagnosis of Type 2 diabetes mellitus within the previous 8 years using criteria of the American Diabetes Association.
- Currently treated with no hypoglycemic agents other than a stable dose (>3 months) of metformin (≥1.0 to ≤2 grams daily).

- HbA1C between 6% and 9% (both inclusive)
  - BMI 25-39.9 kg/m^2 both inclusive.

3) **Age and Reproductive Status**

- Men and women, 40 to 70 years of age.
- **Reproductive Status: Definition of Women of Child-Bearing Potential (WOCBP).** WOCBP comprises women who have experienced menarche and who have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who are not post-menopausal (see definition below).

Post-menopause is defined as:

- Women who have had amenorrhea for ≥ 12 consecutive months (without another cause) and especially with a documented serum follicle-stimulating hormone (FSH) level > 35 mIU/mL.
- Women who have irregular menstrual periods and a documented serum FSH level > 35 mIU/mL.
- Women who are taking hormone replacement therapy (HRT).

The following women are WOCBP:

- Women using the following methods to prevent pregnancy: Oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as intrauterine devices or barrier methods (diaphragm, condoms, spermicides).
- Women who are practicing abstinence.

- Women who have a partner who is sterile (e.g., due to vasectomy).WOCBP must be using an acceptable method of contraception to avoid pregnancy throughout the study and for up to 4 weeks after the last dose of study drug in such a manner that the risk of pregnancy is minimized.
- WOCBP must have a negative serum or urine pregnancy test result (minimum sensitivity 25 IU/L or equivalent units of HCG) within 0 to 72 hours before the first dose of study drug.
- Women must not be breast-feeding.

4.2.2 Exclusion Criteria

- Type 1 diabetes mellitus
- History of diabetic ketoacidosis or hyperosmolar nonketotic coma

- Hemoglobinopathies with low hematocrit (hematocrit below normal limits that may impair exercise tolerance) or abnormal CBC
- History of pancreatitis, or cancer (except basal cell carcinoma)
- Previous coronary or cerebrovascular event within 6 months of screening or active or clinically significant coronary and/or peripheral vascular disease

- Statin use started (or dose change) in the last 3months,
- Daily use of oral or injectable anti-diabetic medication other than Metformin
- use of any form of steroid medication (oral, inhaled, injected or nasal) within the last 3 months
- Systolic BP> 140 mmHg and Diastolic BP> 90mmHg at screening visit.
- Active wounds or recent surgery within 3 months
- Inflammatory disease, or current use of anti-inflammatory drugs
- Untreated hyper/hypothyroidism
- Contraindications for moderate exercise
- Implanted devices (e.g., pacemakers) that may interact with Tanita scale

Physical and Laboratory Test Findings

- Pre-existing liver disease and/or ALT and AST >2.5X's UNL,
- serum creatinine levels ≥1.5 mg/dL for men, ≥1.4 mg/dL for women, estimated CrCl ≤50 mL/min)
- Triglycerides >400 mg/dL

Allergies and Adverse Drug Reactions

- Subjects with a history of any serious hypersensitivity reaction to saxagliptin or DPP-4 inhibitor.

Sex and Reproductive Status

- WOCBP who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period.
- Women who are pregnant or breastfeeding.

See Section on WOCBP above (Section 4.2.1, item # 3.)

Prohibited Treatments and/or Therapies

- Treatment with systemic cytochrome P450 3A4 (CYP 3A4) inhibitors

Other Exclusion Criteria

- Prisoners or subjects who are involuntarily incarcerated.
- Subjects who are compulsorily detained for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

Additionally, patients who are active smokers, patients who are pregnant, nursing women, and post menopausal women who are on hormone replacement therapy will be excluded. Patients on low dose oral contraceptives will be allowed to participate as these formulations contain lesser amount of estrogens.

Eligibility criteria for this study have been carefully considered to ensure the safety of the study subjects and to ensure that the results of the study can be used. It is imperative that subjects fully meet all eligibility criteria.

4.2.3 Discontinuation of Subjects from Treatment

Subjects MUST discontinue investigational product (and noninvestigational product at the discretion of the investigator) for any of the following reasons:

- Withdrawal of informed consent (subject's decision to withdraw for any reason).
- Any clinical adverse event, laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject.
- Changes in estimated CrCL to ≤50 mL/min necessitating reduction in saxagliptin dose
- Treatment with systemic cytochrome P450 3A4 (CYP 3A4) inhibitors necessitating reduction in saxagliptin dose
- Pregnancy
  - Instruct WOCBP to contact the investigator or study staff immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of on-study pregnancy tests for WOCBP enrolled in the study.
  - The investigator must immediately notify BMS if a study subject becomes pregnant.
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

All subjects who discontinue should comply with protocol-specified follow-up procedures outlined in Section 6. The only exception to this requirement is when a subject withdraws consent for all study procedures or loses the ability to consent freely (i.e., is imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness). If a subject withdraws before completing the study, the reason for withdrawal must be documented appropriately.

5 TREATMENTS

5.1 Study Treatment: Saxagliptin

Definition of Investigational Product: A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical study, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorized form, or used for an unauthorized indication, or when used to gain further information about the authorized form. In this protocol, the investigational product is saxagliptin.

Definition of Non-Investigational Product: Other medications used in the study as support or escape medication for preventative, diagnostic, or therapeutic reasons as components of a given standard of care. In this protocol, the non-investigational product is Metformin. Patients will continue on Metformin as per their study entry dose added to Saxagliptin or placebo. Metformin is not an investigational drug and will not be provided by AstraZeneca Pharmaceuticals LP Bristol Myers Squibb. Subject will obtain continue to obtain Metformin as they did prior to study entry.

5.1.1 Identification

Saxagliptin 5 mg tablets are pink, biconvex, round, film-coated tablets with "5" printed on one side and "4215" printed on the reverse side, in blue ink.

5.1.2 Packaging and Labeling

Saxagliptin film-coated tablets in strengths of 1 mg, 2.5 mg, 5 mg, and 10 mg (as the free base) have been developed. The tablets are manufactured from saxagliptin and inactive ingredients including lactose, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, and film-coating materials (Opadry® II white and Opadry® II yellow). The tablets are supplied in high-density polyethylene bottles containing two desiccant and charcoal canisters, with cotton coil and tightly closed with a child resistant cap.

5.1.3 Handling and Dispensing

The investigational product should be stored in a secure area according to local regulations. The investigator is responsible for ensuring that it is dispensed only to study subjects and only from official study sites by authorized personnel, as dictated by local regulations.

The investigator is responsible for ensuring that the investigational product is stored under the appropriate environmental conditions (temperature, light, and humidity), as described below:

Saxagliptin tablets should be stored at temperatures between 15°C and 20°C (59°F and 77°F) in tightly closed containers. If concerns regarding the quality or appearance of the investigational product arise, do not dispense the investigational product, and contact BMS immediately.

5.2 Drug Ordering and Accountability

5.2.1 Initial Orders

Contact the BMS protocol manager for information.

5.2.2 Re-Supply

Contact the BMS protocol manager for information.

5.3 Method of Assigning Subjects to a Treatment

Subjects, as per selection criteria below will be randomized to treatments using a permuted block design, developed by the Epidemiology & Biostatistics Research Core. This approach ensures groups will be approximately balanced at any time during the study and at study completion.

5.4 Selection and Timing of Dose for Each Subject

The recommended dose of saxagliptin is 5 mg PO will be given once daily. Saxagliptin can be taken with or without food.

5.4.1 Dose Modifications

A single dosage adjustment to 2.5 mg daily is recommended for patients with moderate or severe renal impairment, or with end-stage renal disease (CrCl ≤50 mL/min, approximately corresponding to serum creatinine levels of ≥1.7 mg/dL in men and ≥1.5 mg/dL in women). Assessment of renal function is recommended before initiation of saxagliptin, and periodically thereafter. Given that this study protocol includes only saxagliptin 5 mg dose, the participation of any study subject requiring dose adjustment while on study medication will be discontinued (see section 4.2.3.).

5.5 Blinding/Unblinding

Blinding is critical to the integrity of this clinical study. However, in the event of a medical emergency or pregnancy in a subject, **in which knowledge of the investigational product is critical to the subject's management**, the blind **for that subject** may be broken.

Before breaking the blind of an individual subject's treatment, the investigator should have determined that the information is necessary, i.e., that it will alter the subject's immediate management. In many cases, particularly when the emergency is not investigational product-related, the problem may be properly managed by assuming that the subject is receiving active product without the need for unblinding.

5.6 Concomitant Treatments

5.6.1 Prohibited and/or Restricted Treatments

Ketoconazole significantly increased saxagliptin exposure. Similar significant increases in plasma concentrations of saxagliptin are anticipated with other strong cytochrome P450 3A4/5 (CYP3A4/5) inhibitors. The dose of ONGLYZA should be limited to 2.5 mg when coadministered with a strong CYP3A4/5 inhibitors (e.g., ketoconazole, atazanavir, clarithromycin, indinavir, itraconazole, nefazodone, nelfinavir, ritonavir, saquinavir, and telithromycin).

Given that this study protocol includes only saxagliptin 5 mg dose the participation of any study subject requiring dose adjustment while on study medication will be discontinued (see section 4.2.3.).

5.6.2 Other Restrictions and Precautions

Saxagliptin should not be used in patients with type 1 diabetes mellitus or for the treatment of diabetic ketoacidosis.

5.7 Treatment Compliance

Subjects will be given study drug at each dispensing visit with enough study drug to last until the next visit. The subjects will return all unused medication and compliance will be assessed. Subjects will be in compliance if they have taken at least 75% of study drug.

6 STUDY ASSESSMENTS AND PROCEDURES

6.1 STUDY SCHEMATIC

6.2 TIME AND EVENTS SCHEDULE

Time and Events Schedule for Protocol

Procedure	Screening Visit (Week -4)	Baseline/ Randomization Visit 1 (Week 0)	During Treatment Visit 2 (Week 6)	End-of-Treatment Visit 3 (Week 12)
Eligibility Assessments				
Informed Consent	X			
Inclusion/Exclusion Criteria	X			
Medical History	X			
Safety Assessments				
Physical Examination	X			X
Targeted Physical Examination		X	X	X

Time and Events Schedule for Protocol

Procedure	Screening Visit (Week -4)	Baseline/ Randomization Visit 1 (Week 0)	During Treatment Visit 2 (Week 6)	End-of-Treatment Visit 3 (Week 12)
Vital Signs (BP, RR, Temp, HR)	X	X	X	X
Assessment of Signs and Symptoms		X	X	X
Adverse Events Assessment	X	X	X	X
Laboratory Tests (Biochemical)	screening	X	X	X
Pregnancy Test (if applicable)	X	X	X	X
Efficacy Assessments				
Peripheral blood draw for CD34+cell harvest		X	X	X
Pulse wave analysis Pulse wave velocity assessment		X	X	X
Hip / Waist Measurements		X	X	X
Tanita Scale		X	X	X
REE		X	X	X
Clinical Drug Supplies				
Randomize		X		
Dispense Study Treatment		X	X	

Screening visit= week-4; Visit 1= week 0, Visit 2= week 6 and Visit 3= week 12.

+/- 3 day window for visits 1-3

Research Study Design and Methods:

Patient Definition and Selection: Adults aged 40-70 years will be recruited mainly from the MFA research patient database provided by MFA Information Technology. The remaining patients will be recruited from the endocrinology clinic at GWU and referring primary care physicians. Suitable advertisements for the study patient enrollment will be posted. Patients will be included in the study if they have been diagnosed with type 2 diabetes within the previous 8 years using criteria of the American Diabetes Association[2], and are currently treated with no other hypoglycemic agents other than a stable dose (>3 months) of metformin (≥1.0 to ≤2 grams daily). We plan to enroll subjects with HbA1C between 6% and 9% (both inclusive) and with BMI 25-34.9 kg/m^2 (both inclusive). Post enrollment, patients will be randomized to saxagliptin 5 mg or placebo as detailed in Study Design for direct comparison between the two regimens.

Before any study procedures are performed, subjects will have the details of the study described to them, and they will be given a written informed consent document to read. Then, if subjects consent to participate in the study, they will indicate that consent by signing and dating the informed consent document in the presence of study personnel.

Recruitment: Subjects will be recruited from the local general physician patient population and General Endocrinology and Diabetes department at GWU, Medical Faculty Associates, where Dr. Sen is a clinician. All patients will receive counseling regarding the study aims and methods.

Study Design Overview: We propose a 2-arm randomized, double-masked, placebo-controlled, parallel group, longitudinal study of 12-weeks duration. (See study design below), recruited over 2.5 years. The 12 week time interval has been previously shown adequate to observe changes in biochemistry, EPCs and, importantly, PWV changes[17-19,36]. Patients will be randomized to 2 groups:

Control, (n=20), Metformin + Lifestyle + Placebo

Treatment, (n=20) Metformin+ Lifestyle+ Saxagliptin 5mg

This proposed study is based on patients with type 2 diabetes of less than 8 years duration, who are already on Metformin (≥1.0 to ≤2 gm/d, stable dose for at least 3 months) but still remain poorly controlled with HbA1C between 6% and 9% (both inclusive), but not overtly out of control (> 9% of HbA1C). Our study design is similar to that of Fadini et al

[20]where Sitagliptin was compared to placebo with endothelial progenitor cells as a outcome measure in patients with type 2 diabetes over 4 weeks. Though our study is over 12 weeks we do not anticipate significant drop in HbA1C in between the two groups in this relatively short time period to act as a confounding factor on CD34+ cell number, function and gene expression. Most clinical studies looking at HbA1C reduction were of a much longer duration of 6 months or above and most DPP-4 inhibitor studies are with animal EPCs where comparison with human CD34+ population is difficult to make. In one study[58] where saxagliptin was used for 12 weeks in drug-naïve type 2 diabetes patients there was placebo-subtracted HbA1c reduction noted of 0.45-0.63%. It showed mean placebo-subtracted reductions in fasting serum glucose of 20 mg/dL. From our laboratory studies of in vitro human EPC studies we do not think glucose level change of 20 mg/dL is significant enough to account for changes in EPC number, function and gene expression. However we will take this possibility into account during statistical analysis of the results between the saxagliptin and placebo groups.

Lifestyle Intervention for Type 2 diabetes patients[2]:

To keep uniformity within the cohort regarding lifestyle during the length of the study duration, all patients at entry will ideally receive dietary advice from the research coordinator, a diabetes educator, a registered dietitian (as per American Diabetes Association clinical guidelines[2]), specifically geared towards type 2 diabetes patients. They will be advised to adhere to 150 minutes of weekly aerobic exercise (50-70% of maximal heart rate) as best as possible. Patient's activity will be monitored using accelerometers and will be downloaded at regular intervals by the research coordinator.

Subjects will be taking 5.0 mg of Onglyza (saxagliptin) or placebo. We choose to use the maximum approved dose of saxagliptin in order to discern a definite effect.

Visits with outcome measurements will be scheduled for morning following overnight 10 hours fast to reduce diurnal variability, and clinical parameters will be obtained at scheduled office visits as depicted in the study schema and will be measured at each outcome visit as follows:

Week -4: Subjects will be screened and eligibility will be verified based on physical and laboratory tests. We will aim to recruit equal numbers of male and female patients will be recruited. All patients will be educated on the recommended lifestyle changes of maintaining a level of aerobic activity of 150 minutes/ week (non-supervised)and ideally receive dietary advice from the research coordinator, a diabetes educator, or a registered

dietitian (as per American Diabetes Association clinical guidelines[2]) specifically geared towards type 2 diabetes patients. We plan to monitor activity using accelerometers. We will allow 4 weeks for the patients to reach a steady state while implementing life style (diet and exercise) changes.

Week 0: At this stage patients will be randomized into the study. Subjects will have the first set of measurements. We will make sure that patients still meet the inclusion criteria. They will be started on medication or placebo and will be encouraged to continue 150 minutes/week of moderate physical exercise and dietary management for the remainder of the study.

Weeks 0, 6 and 12: Subjects will all have the same measurements and will continue on study medication or placebo. All primary and secondary measures as outlined before will be carried out.

- Primary Measures: CD34+ cell number, function and gene expression.
- Mononuclear cell population (day 0 of harvest) will be analyzed by FACS to obtain cell counts of different endothelial precursor lineage cells. Combinations of following cell markers will be used, such as CD34, KDR(VEGFR2), CD31, CD144 (VE-Cadherin), CD133 (a progenitor cell marker of indeterminate lineage) and CXCR4 (SDF1α receptor) [5,6,8,10,20,21,59]

For:

- CD34/KDR+
- CD34/ CXCR4+
- CXCR4 + CD31
- KDR + CD34
- Some of the MNC population will be put through a human CD34 magnetic bead column using Miltenyi Biotech Column to obtain CD34+ and CD34- cells. The CD34+ cells will be used for
- Colony Forming Unit Assay (CFU Assay) from mononuclear cell population (prior to magnetic bead sorting)
- Migration assay in response to SDF-1 (0, 10, 100 ng/mL) and if sufficient cells are available we will note migration in response to SDF1 (0, 10, 100 ng/mL)
- Gene expression assays: Genes analyzed will be eNOS, PECAM-1, IGF-1, VEGF-alpha, VEGFR2, SDF-1, CXCR4 (receptor for SDF1), anti-oxidants MnSOD, Catalase, Cu-Zn SOD, Glutathione Peroxidase, extracellular SOD, pro-inflammatory markers such as IL6, TNF-alpha and Endothelin-1 genes and apoptosis cascade genes such as p53, p21, Bcl-2, GPX, Caspase-3 will be analyzed.

Secondary Measures:

- Highly selective C-reactive protein (hs-CRP), IL-6, TNF-alpha, Leptin [,18, 28], and fasting lipid profile
- Serum SOD activity and SDF1 alpha (ELISA), VEGF-A (ELISA) and GLP-1 (ELISA) levels will also be assessed[14,20,24,39].
- Assessment of insulin sensitivity using the HOMA-IR[32], calculated from individual serum measures (fasting glucose (mg/dl)* insulin (μU/mL)/405) [37].
- Adiposity will be measured using the Tanita Body Composition Analyzer scale, measured as percentage body fat, BMI, waist-hip ratio[5,55]
- Fasting lipid profile will be checked as a marker of insulin resistance and lipo-toxicity at weeks 0, 6, and 12 [5].
- We also intend to acquire Pulse wave analysis and Vascular Flow using SphygmoCor CP system from ATCOR to measure central arterial pressure and arterial stiffness[38,42]. The secondary measures are indirect measures of endothelial inflammation in early type 2 diabetes patients. [30, 31, 32, 55, 56, 57].
- Urine pregnancy testing for women of child bearing potential

We will obtain a total of85mL of peripheral blood per visit. Of these 85 mL, 60 mL will be used to obtain CD34+ cells from MNC population and 25 mL for biochemistry and serum ELISA assays.

Methodology of Cellular Measure of Endothelial Function:

This is the strongest and most direct endothelial functional parameter, yet the least explored.

Here we plan to measure stem cell precursors of endothelium in subjects with type 2 diabetes as a bio-marker of endothelial health.

Day 0: FACS Analysis for CD34/CD31/KDR/CD133/CD144+, CXCR4 cells (note percentage)

Sort MNC for CD34+ cells for Migration, Tube Formation Assay (Functional Assay)

And gene expression assays.

Functional Assay (Cellular): At Day 0, post cell sorting for CD34 + cells we will note functional assays of these cells.

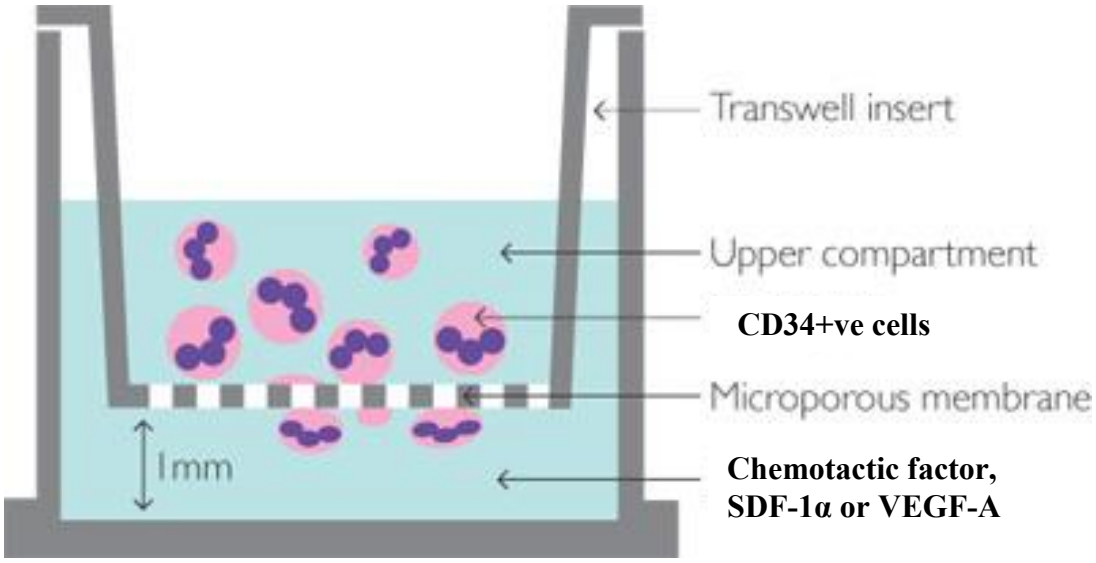
We plan to look at:

- Colony Forming Unit Assay
- Migration assay in response to SDF-1 and VEGF-A.

Colony Forming Unit Assay: MNC's (prior to CD34 magnetic bead sorting) will be plated in Human Fibronectin Coated 6 well (5 million) or 12 well plates (2 million) from BD Biosciences or Millipore. Number of CFU will be determined at Day 5, post plating.

Migration property of the patient's CD34+ cells in response to SDF-1 will be assessed immediately post cell sorting on day 0 using modified Boyden chamber. Briefly, CD34+ cells (1-2x10e5) will be placed in the upper compartment, as shown below. Concentrations tested will be SADF-1 at concentrations of 0, 10, 100 ng/ml and if sufficient cells remain, VEGF-A at concentrations of 0, 20, 50 ng/mL[12,34] dissolved in basal endothelial culture media. Other than this protocol one can also use Migration Assay kit using 3micron pore size from Cell BioLabs Inc. (catalog # CBA-103).

These tests will evaluate the functional (migration) property of CD34+ endothelial progenitor cells [60].



The CD34+ cells obtained at day 0 from peripheral blood derived mononuclear cell population will be analyzed for mRNA expression of various endothelial function related genes,

- The gene expression changes should indicate changes in paracrine properties of CD34+ cells pre and post saxagliptin therapy,
- Genes analyzed will be eNOS, and PECAM-1, VEGF, VEGFR2, SDF-1alpha, CXCR4, IGF-1 [36,42,43]. Intra-cellular antioxidant genes such as MnSOD, Catalase and GPX will also be noted[22,23,44,45]. eNOS is a critical gene regulating EPC's nitric oxide production capability[22]. PECAM-1 is platelet endothelial cell adhesion molecule and indicates inflammatory potential of endothelial lineage cells. VEGF and IGF-1 are growth factors produced by healthy EPCs. MnSOD and Catalase are anti-oxidants which may be important endothelial anti-inflammatory agents and may promote healing in hyperglycemic conditions [9,12,31,46]. Pro-inflammatory markers such as IL6, TNF-alpha and Endothelin-1 genes and apoptosis cascade genes such as p53, p21, Bcl-2, will be analyzed in adition to other genes previously mentioned.

5.8 Study Materials

Astra-Zeneca (AZ) will provide saxagliptin and matching placebo at no cost for this study.

5.9 Safety Assessments

Study drug toxicities will be assessed continuously. Adverse events will be evaluated on a continuous basis while the patient is on study and until 30 days after the last dose of study drug. Patients should be followed until all treatment-related adverse events have recovered to baseline or are deemed irreversible by the principal investigator.

6 ADVERSE EVENT REPORTING

6.1 Adverse Events

An ***Adverse Event (AE)*** is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or

disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

> Related: There is a reasonable causal relationship between study drug administration and the AE.
>
> Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

6.1.1 Serious Adverse Events

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence that at <u>any dose</u>:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed above. Examples of such events include but are not limited to intensive treatment in an emergency department or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization. Potential drug induced liver injury (DILI) us also considered an important medical event (see Section 7.6 for the definition of potential DILI)

Suspected transmission of an infectious agent (e.g., any organism, virus or infectious particle, pathogenic or non-pathogenic) via the study drug is an SAE.

Although pregnancy, overdose and cancer are not always serious by regulatory definition, these events must be handled as SAEs (See Section 7.5 for reporting pregnancies).

NOTE: The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event)
- elective surgery planned before signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

6.1.2 Non-serious Adverse Events

Non-serious adverse events are all adverse events that are not classified as SAEs.

6.1.3 Adverse Events of Special Interest

In this study, the following adverse events are to be reported to AstraZeneca, regardless of whether these reports are classified as serious or unexpected:

1. liver test abnormalities accompanied by jaundice or hyperbilirubinemia
2. opportunistic infections associated with the use of saxagliptin
3. pancreatitis
4. anaphylaxis
5. angioedema
6. Steven-Johnson's Syndrome

6.2 Collection and Reporting

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. To prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the event was administered, it should be recorded in the medical record. The investigator must supply AstraZeneca and the IRB/IEC with any additional information requested, notably for reported deaths of subjects.

6.2.1 Serious Adverse Event Collecting and Reporting

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, will be collected, including those thought to be associated with protocol-specified procedures. All SAEs will be collected that occur within 30 days of discontinuation of dosing or within 30 days of the last visit for screen failures. If applicable, SAEs will be collected that relate to any later protocol-specified procedure (e.g., a follow-up skin biopsy).

The investigator will report any SAE occurring after these time periods that is believed to be related to study drug or protocol-specified procedure at 90 days and 180 days period

An SAE report will be completed for any event where doubt exists regarding its status of seriousness.

If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship will be specified in the narrative section of the SAE Report Form.

All SAEs, whether related or unrelated to saxagliptin, and all pregnancies will be reported to BMS (by the investigator or designee) within 24 hours.

All SAEs should be reported via confirmed facsimile (fax) transmission, or scanned and reported via electronic mail to:

SAE Email Address: Worldwide.Safety@BMS.com

SAE Fax Number: 609-818-3804

SAEs will be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.

MedWatch SAE forms should be sent to the FDA at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

All SAEs should simultaneously be faxed or e-mailed to BMS at:

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to the BMS using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

6.2.2 Non-Serious Adverse Events (NSAEs) Collecting and Reporting

The collection of non-serious adverse event (NSAE) information will begin at initiation of study drug. NSAE information will be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects. NSAEs will be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up as required for NSAEs that cause interruption or discontinuation of study drug, or those that are present at the end of study treatment as appropriate will be undertaken. All identified NSAEs will be documented appropriately.

6.3 Laboratory Test Abnormalities

All laboratory test results captured as part of the study will be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the subject to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

6.4 Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose will be reported as an SAE.

6.5 Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half lifes after product administration, the investigational product will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety). Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated.

The investigator or designee will immediately notify the BMS (or designee) Medical Monitor of this event and complete and forward a Pregnancy Surveillance Form to BMS (or designee) within 24 hours and in accordance with SAE reporting procedures described in Section 7.2.1.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information will be reported on the Pregnancy Surveillance Form.

Any pregnancy that occurs in a female partner of a male study participant should be reported to the sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

6.6 Potential Drug-Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs (see Section 7.2.1 for reporting details).

Potential drug induced liver injury is defined as

1. AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)

AND

2. Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),

AND

3. No other immediately apparent possible causes of AT elevation and hyper-bilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

6.7 Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a nonserious or serious AE, as appropriate, and reported accordingly.

7 DATA MONITORING COMMITTEE

Not Applicable.

8 STATISTICAL CONSIDERATIONS

Statistical Analysis:

Sample Size Estimation:

The total sample size requested, accounting for attrition over the 12-week period, is 21 subjects per group or 42 subjects total.

Sample size estimates were based on the effects of exercise on CD34+/KDR+ cells and VEGF, as described in the literature [47-50]. The effect of a single session, as well as extended training, on healthy subjects or those with existing cardiovascular conditions appears to increase the CD34+/KDR+ cells and VEGF.

To compute sample size we used the approach suggested by Diggle, Liang, & Zeger (1994)[51] which compares the rates of change in the two study groups over time. This approach incorporates the number and interval of time points and the correlation among repeated measures. For this study, we will employ one baseline and two follow-up measures at 6 and 12 weeks. Further, we will assume a correlation 0.60 among repeated measures of the outcome. [We consider this a conservative estimate since Frison and Pocock (1992) [52] suggest a correlation of 0.65 as reasonable in the absence of an existing estimate. We also note that as this correlation increases, statistical power also increases.

The results in the table below show the expected mean difference in study groups at the end of follow-up, as well as the average rate of change in the two groups at 80% power and 90% power. To estimate the effect of saxagliptin on the CD34+/KDR+ cells, we expect that the effect would be at least 25% greater than the effect seen for exercise alone. Using the results from Sandri et al. (2005)[49] for the rate of change and the variability, the CD34+/KDR+ cells increased an average rate of about 4/wk with a standard deviation of about 15. Thus, for a 25% increase in the rate of change for the CD34+/KDR+ cells due to saxagliptin, a sample size of 18 subjects per group would provide about 84% power, assuming measures taken at baseline and 2 equally-spaced time points over 12 weeks. At the conclusion of follow-up, we would expect study groups to differ by an average of 12 cells. If the effect of saxagliptin is only 20%, a sample of 18 would provide about 70% power, whereas a sample of 20 would provide about 73% power.

For VEGF, the results from Sandri et al. (2005)[49] were mixed with one study group showing a .6 pg/mL per week increase and another showing a 0.5 pg/mL per week decrease after 4 weeks of training. If we assume that saxagliptin will increase VEGF consistently at 0.3 pg/mL per week difference in slopes compared with exercise alone, then assuming a standard deviation of 5 and correlation or 0.60 among repeated measures, a sample size of 18 per group would provide about 77% power to detect a difference in slopes of 0.3 pg/mL per week or a mean difference in the groups at the end

of the study of 3.6 pg/mL. A sample size of 27 would provide about 90% power to detect the same effects.

Thus, we feel that a sample size of at least 18 subjects per group with complete data would provide sufficient power for the study outcomes. In order to insure that we will have 18 per group who complete the study, we will enroll 20 subjects per group in order to account for attrition over the 12-week period.

Biochemical Measure	Mean difference at end of 12 weeks	Sample Size per group	Power
CD34+/KDR+ cells	12 cells (25% increase; 4/wk vs. 5/wk)	18	0.84
		22	0.90
VEGF, pg/mL	3.6 pg/mL (50% increase; (0.6/wk vs. 0.9/wk)	18	0.77
		27	0.90

Data Analysis.

The distributional assumptions of all measures will be examined. Means and standard deviations will be computed for continuous measures and proportions for categorical variables. Graphical representations of the mean group slopes and individual slopes will be generated and inspected. Study groups will be compared to determine whether any imbalance between the groups on patient characteristics remain after randomization. If imbalance is evident, by inspection, all models will be adjusted for the unbalanced covariates. Change in outcome over time will be examined using a multilevel approach with linear mixed models (LMM) [53,54]. The longitudinal multilevel modeling approach will enable us to examine characteristics of within-person change, as well as between-group differences that may influence change. LMMs handle missing data more efficiently than traditional ANOVA designs. In the linear mixed models, the patient is considered a random effect and the outcome measured at specific time points is nested within patient. In an initial model containing only the variable TIME, we will use a likelihood ratio test to determine whether a random effect for time should be modeled. The model below represents a random intercept for patient and a random coefficient for TIME.

$$Y_{ij} = \beta_0 + \beta_1 Time_{ij} + u_{i0} + u_{i1} Time_{ij} + \varepsilon_{ij}$$

We will examine whether TIME may be modeled linearly by testing the added contribution of TIME (represented with two indicator variables) to a model with TIME as a single continuous variable. Assuming time may be modeled as a continuous variable, subsequent analyses will add study group and other patient characteristics as covariates. We are particularly interested in whether there is a significant interaction between study group and time, adjusting for other patient level covariates. A significant interaction would indicate that the slopes in outcome over time differ by study group. If the global test of interaction is significant, subsequent significance testing of the pair wise comparisons of group slopes group will be adjusted for multiple comparisons. The model also includes coefficients for the group-by-time interactions and random effects for patient and time.

$$Y_{ij} = \beta_0 + \beta_1 Time_{ij} + \beta_2 Group_i + \beta_3 (Time_{ij} \times Group_i) + u_{i0} + u_{i1} Time_{ij} + \varepsilon_{ij}$$

In the event that TIME is modeled using indicator variables, two indicator variables will be used to represent the three time points, with baseline serving as the reference category. The interaction between GROUP and TIME will be modeled in a similar fashion using two additional indicator variables. The above multivariable model enables us to adjust for time-varying covariates, such as glucose. Since glucose will be measured contemporaneously with the outcomes at each visit, however, causal interpretations of glucose and any outcome are suspect. As such, we will consider glucose as a nuisance covariate, and consider any relationship between glucose and an outcome as merely associative.

9 STUDY MANAGEMENT

9.1 Compliance with the Protocol

9.1.1 Compliance with the Protocol and Protocol Revisions

The study shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with, and be prepared by, BMS. The investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB/IEC approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB/IEC for review and approval/favorable opinion
- AstraZeneca Pharmaceuticals LP
- Regulatory Authority(ies), if required by local regulations

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) will be sent to BMS.

If an amendment substantially alters the study design or increases the potential risk to the subject: (1) the consent form will be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion; (2) the revised form will be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and (3) the new form must be used to obtain consent from new subjects prior to enrollment.

If the revision is an administrative letter, investigators must inform their IRB(s)/IEC(s).

9.2 Records Retention

9.2.1 Records Retention

The investigator will retain all study records and source documents for the maximum period required by applicable regulations and guidelines, or institution procedures, or for the period specified by BMS, whichever is longer.

If the investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another investigator, IRB). Notice of such transfer will be given in writing to BMS.

9.2.2 Study Drug Records

Investigator will ensure that a current disposition record of investigational product (those supplied by the BMS) is maintained at each study site where study drug and non investigational product(s) is/are inventoried and dispensed. Records or logs will comply with applicable regulations and guidelines and will include:

- amount received and placed in storage area
- amount currently in storage area
- label ID number or batch number
- amount dispensed to and returned by each subject, including unique subject identifiers
- amount transferred to another area/site for dispensing or storage
- non-study disposition (e.g., lost, wasted)
- amount destroyed at study site, if applicable
- amount returned to the AstraZeneca Pharmaceuticals LP
- retain samples for bioavailability/bioequivalence, if applicable

- dates and initials of person responsible for Investigational Product (IP) dispensing/accountability, as per the Delegation of Authority Form.

10 GLOSSARY OF TERMS

Term	Definition
Adverse Reaction	An adverse event that is considered by either the investigator or the sponsor to be related to the investigational product
Expedited Safety Report	Rapid notification to investigators of all SAEs that are suspected (related to the investigational product) and unexpected (i.e., not previously described in the Investigator Brochure), or that could be associated with the study procedures.
SUSAR	Suspected, Unexpected, Serious Adverse Reaction as termed by the European Clinical Trial Directive (2001/20/EC).
Unexpected Adverse Reaction	An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator Brochure for an unapproved investigational product)

11 LIST OF ABBREVIATIONS

A1C	Glycosylated Hemoglobin
AE	Adverse Event
BMS	Bristol-Myers Squibb
CYP3A4	Cytochrome P450 3A4
DPP-4	Dipeptidyl peptidase 4
ESR	Expedited Safety Report
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
GIP	Glucose dependent insulinotropic peptide
HCG	Human Chorionic Gonadotropin
HRT	Hormone Replacement Therapy
IB	Investigator Brochure
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug (Application)
IRB	Institutional Review Board
IST	Investigator-Sponsored Trial
MWG	Mean Weighted Glucose
NSAE	Non-Serious Adverse Event
PPG	Post-Prandial Glucose
SAE	Serious Adverse Event
SU	Sulfonylurea
SUSAR	Suspected Unexpected Serious Adverse Reaction
TDZ	Thiazolidinedione
WOCBP	Women of Child-Bearing Potential

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