PROTOCOL

TITLE PAGE

Effect of Saxagliptin Treatment on Myocardial Fat Content, Left Ventricular Function, and Monocyte Inflammation in Patients with Impaired Glucose Tolerance

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PROTOCOL SYNOPSIS

Protocol Title:	Effect of Saxagliptin Treatment on Myocardial Fat Content, Left Ventricular Function, and Monocyte Inflammation in Patients with Impaired Glucose Tolerance
Site Numbers & Names:	Baylor College of Medicine, Houston, TX
Research Hypothesis:	**We hypothesize that** saxagliptin therapy (compared to placebo) would be associated with a reduction in myocardial and hepatic fat content, an improvement in left ventricular (LV) function, and a reduction in monocyte inflammation in patients with impaired glucose tolerance.
Study Schema: **Drugs / Doses / Length of Treatment)**	Saxagliptin 5 mg or placebo PO daily for 6 months
Study Objectives: • **Primary:** • **Secondary:**	The **primary objective** is to examine the effects of saxagliptin on myocardial and hepatic fat content in patients with impaired glucose tolerance (IGT). The **secondary objective** is to examine the effect of saxagliptin on left ventricular systolic and diastolic function and monocyte inflammation in patients with IGT.
Study Design:	Patients with IGT or prediabetes will be randomized to receive saxagliptin or placebo in a double blind placebo controlled single center trial.
Accrual Goal: **(Total number of subjects)**	40 patients
Accrual Rate: **(Number of subjects expected per month)**	3 patients per month
FPFV: **LPFV:** **Follow Up:** **(dd-mm-yy)**	**December 1, 2011** **December 1, 2012** **31-05-2013**
Correlative Studies: **(PK/PD, etc.)**	-
Inclusion Criteria:	Patients between the age of 30 and 70 years with IGT.

Exclusion Criteria:	Patients with type 1 or type 2 diabetes and patients on any anti-hyperglycemic medications
Criteria for Evaluation: **(Efficacy, safety, stopping rules, etc.)**	Changes in myocardial and hepatic fat, LV function and monocyte inflammation following saxagliptin versus placebo treatment for 6 months.
Statistics:	Between groups (i.e., saxagliptin therapy vs. placebo) comparisons will be made using two-way analysis of variance (ANOVA). Pre and post treatment within a group will be analyzed using paired t-tests. Simple correlation analysis will be performed with Pearson's correlation coefficients to examine the relationship between hepatic fat and myocardial fat and LV function. Multivariate analysis will be performed where appropriate to examine the impact of simultaneous changes (glucose levels) in experimental parameters on hepatic fat. All statistical analysis will be performed using SAS (Cary, NC). Sample size is calculated using SigmaPlot software (San Jose, CA). For the two major outcome variables i.e. myocardial fat and hepatic fat, the sample size of ~ 16 patients per treatment group (total of 32 patients) will have >90% power to detect a difference in the means of 20%, assuming a standard deviation of 15%, and with alpha= 0.025. Given that we expect about 10-15% of the patients enrolled may not complete the 6 month study, we would like to enroll 36 patients so that 32 patients may complete the study.

1 INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a progressive disease and is associated with long-term microvascular complications such as retinopathy, nephropathy, and neuropathy as well as cardiovascular morbidity and mortality. Intensive treatment to reduce plasma glucose to normal levels can minimize the risk of developing these complications (1). Currently available therapeutic agents are associated with side effects including hypoglycemia, weight gain, edema and changes in blood lipid profile. Agents with new mechanisms of action for the treatment of T2DM are being studied, and inhibition of dipeptidyl peptidase IV (DPP-IV) is emerging as a new therapeutic approach for treatment of type 2 diabetes. Bristol-Myers Squibb (BMS) and Astra-Zeneca (AZ) have jointly developed saxagliptin, a novel DPP-IV inhibitor, as a once daily oral therapy for the treatment of hyperglycemia in patients with T2DM.

Saxagliptin (BMS-477118) is a synthetic, potent, reversible, orally active DPP-IV inhibitor. DPP-IV is an enzyme that selectively cleaves dipeptides from the N-terminus of oligopeptides with proline or alanine in the penultimate position. DPP-IV converts the key insulinotropic hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) from active to inactive form, and is responsible for their short-half-lives in vivo. Inhibitors of DPP-IV increase levels of endogenous intact GLP-1 and GIP in a glucose-dependent manner, thereby potentiating their physiological actions, augmenting postprandial insulin secretion, and improving the glycemic profile in patients with T2DM. Because of the glucose-dependent mechanism of action, DPP-IV inhibitors are expected to present low risk of hypoglycemia.

Several lines of evidence indicate that preservation of active GLP-1 by treatment with a DPP-IV inhibitor will improve the insulin secretion pattern from pancreatic β-cells, enhance postprandial glucose control, and result in long-term improvements in both fasting and postprandial glycemia (2). Furthermore, experimental data suggest DPP-IV inhibitors may protect and/or promote pancreatic β-cell function and capacity as well as have pleiotropic effects on glucose homeostasis and/or pancreatic β-cell function (2). Possible mechanisms include inhibiting inactivation of GIP and effects on other relevant targets, such as decreasing the levels of the ‘counter-regulatory’ hormone glucagon.

Saxagliptin has been tested in a large developmental program including six Phase 3 studies and in over 4000 patients. Saxagliptin has shown statistically significant lowering

in all parameters of glucose control (glycated hemoglobin [A1C], fasting plasma glucose [FPG], and postprandial glucose [PPG]) either as monotherapy or in combination with metformin, glyburide or thiazolidinedione. Further studies are intended to evaluate the safety and efficacy of saxagliptin in comparison with commonly used strategies of treatment in clinical practice.

1.1 Summary of Results of Investigational Program

1.1.1 Clinical Toxicology

In clinical pharmacology studies, 620 subjects have received at least 1 single oral dose (1-400 mg) of saxagliptin.

Saxagliptin is rapidly and completely absorbed after oral administration, with maximum plasma concentration usually attained within 2 hours after dosing. It is primarily metabolized by hydroxylation to a pharmacologically active metabolite, BMS-510849, approximately 2-fold less potent than saxagliptin. BMS-510849 systemic exposure values are between 2 to 7 times higher than those of saxagliptin on a molar basis. The pharmacokinetics of saxagliptin and BMS-510849 appear linear and do not change with repeated daily dosing of saxagliptin doses up to 400 mg once daily for 2 weeks (i.e., 80 times the expected usual clinical dose of 5 mg). Evidence from in vitro experiments suggest that cytochrome P450 3A4 and 3A5 (CYP3A4 and 3A5) are the major enzymes responsible for the metabolism of saxagliptin and conversion to BMS-510849. Saxagliptin and BMS-510849 do not appear to inhibit or induce the major CYP enzymes or P-glycoprotein.

Approximately 24% and 36% of the dose was excreted into the urine as unchanged saxagliptin and BMS-510849, respectively. Renal excretion is a major elimination pathway for systemic saxagliptin and BMS-510849. In humans, following a single 50 mg dose of ^{14}C-saxagliptin, 24%, 36%, and 75% (on average) of the dose was excreted in the urine as saxagliptin, BMS-510849, and total radioactivity respectively (CV181004) (3). 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 (~230 mL/min) was greater than the average estimated glomerular filtration rate (~120 mL/min), suggesting saxagliptin undergoes active renal excretion by as yet unidentified transporter(s). 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. BMS-510849 was the predominant saxagliptin-related compound in human feces (8.4% of the dose), indicating that approximately one-fifth of the total amount of BMS-510849 formed is excreted via the bile in healthy subjects.

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.

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, or simvastatin. Co-administration of saxagliptin and ketoconazole or diltiazem 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 up to an 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-IV inhibition) were not meaningfully altered by diltiazem or ketoconazole. 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.

More detailed information on clinical pharmacokinetics of saxagliptin may be found in the Investigator Brochure (Sections 5.1 and 5.2) (3).

1.1.2 Summary of Efficacy Results in Subjects with Type 2 Diabetes Mellitus

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-IV 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.

1.1.3 Summary of Safety Results in Subjects with Type 2 Diabetes Mellitus

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. In an extensive Phase 2b/3 program, the majority of reported adverse events (AEs) was 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.

The placebo-controlled pooled safety analysis, which included the two monotherapy studies (CV181011 and CV181038), and the add on combination studies (CV181014, CV181013, CV181040 with metformin, TZD, glyburide, respectively), demonstrated that the overall incidence of AEs in subjects treated with saxagliptin 5 mg was similar to placebo (72.2% vs. 70.6%). The majority of reported AEs was 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 (7.0% vs. 5.9%), nasopharyngitis (6.0% vs. 6.8%), urinary tract infection (5.8% vs. 6.1%), diarrhea (5.2% vs. 6.1%), and back pain (3.9% vs. 5.1%). In the combination study with metformin (CV181-039) headache (7.9% in saxagliptin, 5.2% in metformin) and diarrhea (6.4% in saxagliptin, 7.3% in metformin) were the most frequently reported AEs (incidence ≥ 5%).

In the placebo-controlled pooled safety analysis the frequency of serious adverse events (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).

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 1.2%, respectively), but was greater for subjects in the saxagliptin 5 and 10 mg groups (3.3% and 3.9%, respectively). The most frequently reported AEs (> 1 subject) leading to study discontinuation, which were numerically higher in subjects 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 subjects who received saxagliptin and placebo, whereas the rate of discontinuation for AEs of increased weight, depression, and angina pectoris were higher in subjects who received placebo compared with saxagliptin.

Overall, death was an infrequent event and occurred with comparable frequencies in the saxagliptin and comparator groups. In the Phase 2/3 studies, a total of 16 subjects died during the short-term and long-term periods (as of January, 2008): 2 (0.2%) subjects each in the saxagliptin 2.5 and 5 mg groups, 3 (0.3%) subjects in the saxagliptin 10 mg group, 5 (0.5%) subjects in the placebo group, and 4 (1.2%) subjects in the metformin monotherapy group.

1.1.3.1 Adverse Events of Special Interest

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-IV 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 subjects 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.

Local edema: The proportion of subjects with AEs of localized edema, an event of special interest given reports of symptomatic edema of the hands and feet in subjects who received another member of the DPP-IV-inhibitor class, was generally similar in subjects 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 subjects 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 was of mild-to-moderate intensity and did not lead to study discontinuation.

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 system organ class (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.

1.2 Overall Risk/Benefit Assessment

The efficacy of saxagliptin for the treatment of subjects 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 subjects treated in the open-label (OL) 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 efficacy and clinical safety data, discussed in Sections 1.1.2 and 1.1.3, indicate an acceptable risk/benefit profile at the dose of saxagliptin 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 hypothesize that saxagliptin therapy (compared to placebo) would be associated with a reduction in myocardial and hepatic fat content, an improvement in left ventricular function, and a reduction in monocyte inflammation in patients with impaired glucose tolerance.

1.4 Study Rationale

Obese, insulin resistant individuals have an excess of fat in the liver which is not attributable to alcohol or other known causes of liver disease, a condition defined as nonalcoholic fatty liver disease (NAFLD) (4). The fatty liver is insulin resistant. Individuals with a fatty liver are more likely to have excess intra-abdominal fat as well as a reduction in circulating plasma adiponectin levels (4-9). We have previously shown that type 2 diabetes and its associated NAFLD is characterized by increased hepatic fat content, and hepatic and peripheral (muscle) insulin resistance (6,8). Treatment with PPAR-gamma agonists (thiazolidinediones) reduces hepatic fat content, improves hepatic insulin sensitivity, and is associated with a three-fold increase in plasma adiponectin in patients with type 2 diabetes (6,8). DPP4-deficient rats (10), characterized by high levels of active GLP-1 due to an inactive DPP4 enzyme, display reduced hepatic triglycerides, accompanied by down-regulation of lipogenesis enzymes and parallel up-regulation of carnitine palmitoyltransferase-1, a key enzyme in fatty acid beta-oxidation.

Mechanistically, GLP-1 increased AMP-Activated-Protein-Kinase (AMPK) activity in hepatocytes, resulting in reduced hepatic lipogenesis. Furthermore, DPP4- rats expressed lower levels of hepatic proinflammatory and profibrotic cytokines in response to nutrient stimuli (10).These results are similar to our own studies in DIO mice treated with exendin-4. We demonstrated that a single injection of an adenovirus exendin-4 expression vector to diet-induced obese mice reduced hepatic fat and enzymes involved in hepatic fatty acid synthesis independent of changes in insulin and glucagon (11).

Recent reports suggest that NAFLD is associated with an increased risk of cardiovascular disease independent of associated cardiovascular risk factors (12,13). Furthermore type 2 diabetics and subjects with impaired glucose tolerance are characterized by an increase in both hepatic and myocardial fat and left ventricular (LV) dysfunction, particularly diastolic dysfunction (14-17). Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus as well as impaired glucose tolerance (15, 17, 18). Pioglitazone therapy reduces myocardial fat and improves LV function (16); however, it is associated with fluid retention and an increased risk of pulmonary edema in those with pre-existing LV failure. However, the effect of saxagliptin therapy on liver and myocardial fat content, as well as LV systolic and diastolic function in patients with IGT or type 2 diabetes has not been previously studied. The pathophysiology of diabetic cardiomyopathy is complex, and the exact mechanisms of disease remain partly unknown. Increasing evidence is emerging that lipid oversupply to cardiomyocytes, which may lead to lipotoxic injury, plays a role in the development of

diabetic cardiomyopathy (14). Increased fatty acid (FA) fluxes arising from the disproportionate amount of insulin resistant (visceral) adipose tissue lead to excessive FA delivery and uptake in the heart. This FA uptake exceeds the oxidizing requirements of the organ, giving rise to fatty acyl-CoA esters, diacylglycerol, and ceramide as intermediates. Increasing evidence exists that accumulation of these FA intermediates causes mitochondrial dysfunction and reactive oxygen species, giving rise either directly to cell damage and apoptosis or indirectly through the induction of inflammatory cascades, which leads to myocardial dysfunction. In animal models, antisteatotic treatment with thiazolidinediones reduced myocardial triglyceride accumulation and ceramide content, and prevented myocardial dysfunction. Recently, it has been demonstrated that myocardial triglyceride content is increased in uncomplicated T2DM and is associated with impaired left ventricular diastolic function, independently of age, BMI, heart rate, visceral fat, and diastolic blood pressure (15). More recently, Iozzo et al (17) have shown that obese normal glucose tolerant subjects, obese subjects with IGT, and type 2 diabetic subjects have increased myocardial fat compared to lean subjects (Figure 1). In multiple regression analysis, myocardial fat was independently related with cardiac output and work in these subjects (17). Furthermore, Bajraktari et al (18) have shown that both type 2 diabetics and subjects with IGT have left ventricular diastolic dysfunction and that insulin resistance is an independent correlate of diastolic dysfunction. Thus, both IGT and type 2 diabetic subjects have increased myocardial steatosis and defects in LV function.

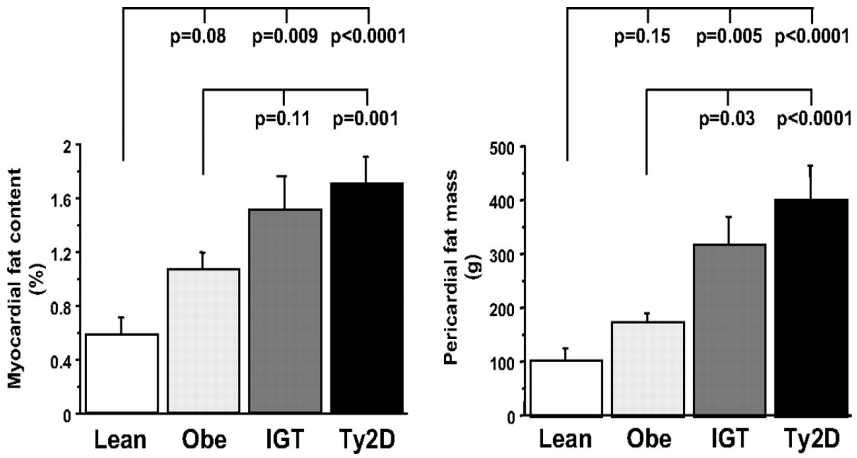

Figure 1.

GLP-1 has been shown to improve myocardial function and cardiac output in conscious chronically instrumented canine models of cardiac injury or heart failure. GLP-1 increased cardiac output and reduced left ventricular end diastolic pressure in association with reduced systemic vascular resistance, and it improved myocardial insulin sensitivity and myocardial glucose uptake in dogs with rapid pacing-induced dilated cardiomyopathy (19). A clinical study showed that GLP-1 improves left ventricular ejection fraction and functional status in patients with congestive heart failure, without affecting heart rate or blood pressure, suggesting a mechanism other than direct inotropic or chronotropic effects (20). Early studies demonstrated the presence of transcripts for GLP-1Rs in the heart (21), but only recently has the cellular distribution of the receptors been localized. Ban et al. (22) identified GLP-1Rs via immunohistochemistry in cardiomyocytes and coronary and vascular endothelial cells as well as smooth muscle in mice. Recent studies in rodents suggest that some of the cardiovascular effects of native

GLP-1 may be mediated by a mechanism independent of the known GLP-1R (22). However, no previous study has examined the effect of saxagliptin on myocardial fat or LV function in IGT or type 2 diabetic patients.

Finally, the effect of saxagliptin on vascular inflammation and monocyte NFkappaB activity remains to be studied. We have previously shown *in vitro* (23) that exenatide inhibits FFA-induced inflammation and NFkappaB activity in cultured skeletal muscle cells (L6 Myotubes). However, no previous study has examined the effect of GLP-1/saxagliptin on monocyte inflammation in patients with type 2 diabetes or impaired glucose tolerance.

Patients with Impaired Glucose Tolerance (IGT)/ Impaired Fasting Glucose (IFG) have insulin resistance as a well established defect. Hepatic insulin resistance is well established as the product of basal hepatic glucose production and fasting plasma insulin levels (basal hepatic insulin sensitivity index) is increased significantly compared with non-diabetic control subjects even though the fasting (and following a 75 gram OGTT) hyperglycemia is mild. Furthermore, as stated previously, both myocardial and hepatic steatosis as well as defects in LV function are well characterized in obese, insulin resistant patients with IGT. However, the effect of DPP-IV inhibitors on hepatic and myocardial steatosis and monocyte inflammation in insulin resistant patients with IGT have not been previously studied.

2 STUDY OBJECTIVES

2.1 Primary Objective

The **primary objective** is to examine the effects of saxagliptin on myocardial and hepatic fat content in patients with impaired glucose tolerance (IGT).

2.2 Secondary Objectives

The secondary objective is to examine the effect of saxagliptin on left ventricular systolic and diastolic function and monocyte inflammation in patients with IGT

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 Bristol-Myers Squibb (BMS) 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 (eg, 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 (eg, advertisements), and any other written information to be provided to subjects. The investigator 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 (eg, 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, the 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. Freely given written informed consent must be obtained from every subject (or, in those situations where consent cannot be given by subjects, the legally acceptable representative) before clinical study participation, including informed consent for any screening procedures conducted to establish subject eligibility for the study.

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 consent form must also include a statement that BMS and regulatory authorities have direct access to subject records.

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

Subjects:

40 obese subjects with impaired glucose tolerance (IGT) or prediabetes, (fasting plasma glucose between 100-125 mg/dl, or 2 hour post 75 gram OGTT plasma glucose between 140-199 mg/dl, or HbA1c 5.7-6.4%) who are free of other major organ system disease will be studied. Subjects will range in age from 30 to 70 years and in body mass index from 30-35 kg/m^2. Subjects who have previously received or are taking metformin, DPP-IV inhibitors, exenatide, liraglutide, or a thiazolidinedione for treatment of IGT will be excluded. Patients on statins and other lipid lowering therapy will be included provided they are on a stable dose of lipid lowering therapy for at least 3 months prior to enrollment. The dose of the lipid lowering therapy will not be changed for the entire duration of the study. The fasting plasma glucose must be ≤125 mg/dl, 2 hour post 75 gram OGTT plasma glucose must be less

than 200 mg/dl, and HbA1C must be < 6.5% to be included in the study. Subjects with clinically significant cardiac, hepatic, or renal disease will not be studied. Subjects will be recruited from the Endocrine/Diabetes/ Obesity Clinic at the Baylor Clinic. All subjects will be recruited by direct contact from Dr. Mandeep Bajaj or one of the co-investigators.

Study Design: All subjects will participate in an initial screening visit (medical history and physical exam, urinanalysis, EKG, CBC, liver function tests (LFTs), serum creatinine, lipid profile, HbA1c; and a fasting and 2 hour plasma glucose (during a 75 gram OGTT). During the 2 hour OGTT (75 grams), glucose, insulin, c-peptide, and FFA determinations will be made at baseline (fasting) and every 15-30 minutes and insulin sensitivity will be calculated by the Matsuda Index. In addition, plasma triglycerides will be measured at baseline and at 120 minutes during the OGTT.

Following determination of eligibility, 40 IGT or prediabetes subjects will be randomized (double blind) to participate in one of the following two treatment arms (i) saxagliptin 5 mg daily PO for 26 weeks or (ii) Placebo PO daily for 26 weeks (control arm). Two weeks prior to randomization, subjects will meet with a dietitian and will be instructed to consume a weight-maintaining diet containing 50% carbohydrate, 30% fat, and 20% protein. During the week prior to start of the study all subjects will receive: (i) baseline measurements of FPG, plasma adiponectin (HMW and total adiponectin), CRP, IL-1, IL6, TNF-alpha, MMP-9, ICAM, VCAM, Fibroblast Growth Factor 21 (FGF21), fasting

plasma lipids, postprandial plasma triglyceride level (at 2 hours following a mixed meal), and HbA1c; (ii) monocyte isolation from peripheral (venous) whole blood and quantification of monocyte TLR2, TLR4, SOCS-3, MYD88, NFkappaB (P65) and IkB-alpha, and IkB-beta protein as well as expression levels of CD36, MCP-1, MMP-9,VCAM1, ICAM1 and JNK. We will also measure of plasma lipopolysaccharide (LPS) and LPS- binding protein (LPS-BP) concentrations. Plasma samples used for LPS determination will be stored in LPS-free glass tubes to prevent loss of endotoxin to plastic tube walls. All materials for the assay will be rendered LPS-free; and (iii) liver and myocardial fat content with magnetic resonance (MR) spectroscopy and determination of left ventricular systolic and diastolic function by MR imaging as detailed below.

During the entire 6 month treatment period, subjects will return to the Clinical Research Center every 4 weeks at 0800 h following an overnight fast for measurement of FPG, body weight, and blood pressure. A urine pregnancy test will also be performed at each visit. On each visit, dietary adherence will be reinforced by the dietician. Fasting plasma lipids and postprandial triglyceride levels, HbA1c, and LFTs will be measured at 3 months and at the end of the 6 month study period. At the end of the treatment period of six months all subjects will also undergo a repeat 75 gram OGTT (for measurements of baseline FPG, and plasma glucose, FFA, c-peptide and insulin during OGTT as well as 2 hour plasma triglyceride measurements as described previously), plasma adiponectin,

CRP, IL-1, IL6, TNF-alpha, MMP-9, ICAM, VCAM, Fibroblast Growth Factor 21 (FGF21), FFA, insulin, LPS and LPS-BP. Liver and Myocardial fat and LV function as well as monocyte studies for inflammatory pathways as detailed previously will be repeated after 26 weeks of therapy.

Measurement of Liver Fat Content (MR Spectroscopy)

Localized 1H nuclear magnetic resonance spectra of the liver will be obtained on a 3.0 Tesla magnetic resonance imaging scanner (Department of Radiology, St Lukes Episcopal Hospital and Texas Heart Institute, Houston) using a standard body coil in transmitter and receiver mode. An initial T1-weighted spin-echo anatomical magnetic resonance scan for liver MRS localization will be performed with the following parameters: repetition time/echo time• = 130 msec/15 msec•160 degrees; slice thickness = 7 mm; field of view = 44 cm x 45 cm; number of excitations = 1; and an image matrix = 100 x 256. The slice with the largest gross dimensions of the liver will be chosen for the MRS study. MRS for water and fat quantification will be accomplished by using a point resolved spectroscopy sequence as described previously (3). The imaging parameters for point resolved spectroscopy sequence are as follows: repetition time/echo time• = 1500 msec/54 msec•90 degree; number of averages = 2; and data points = 512. A 3 cm x 3 cm x 3 cm volume (voxel) will be selected in the left, right anterior, and right posterior hepatic lobes for scanning to provide a more generalized distribution of fat within the liver. During the measurements, the

subject will be supine within the bore of the magnet. The total scan time is approximately 60 min. During the MRS examinations, identical areas of the liver are scanned in the pre- and post-treatment MRS studies of the same subject by the use of anatomical landmarks visualizing images.

After line broadening, phase and baseline correction, the peak area of the water at 4.77 ppm, and fat resonance (Sf) at 1.4 ppm will be measured. Quantification of the fat content will be done by comparing the area of the Sf with that of the unsuppressed water. Spectroscopic data will be processed using the operating system software. Hepatic fat percentage will be calculated by dividing (100 x Sf) by the sum of Sf and peak area of the water. This technique is highly reproducible, with a coefficient of variation less than 2% when the same subjects were studied on eight separate days.

Measurement of Cardiac Myocardial Triglyceride Content with Dual Gating

The MR studies will be performed on a commercial MR scanner (3.0 tesla) equipped with 1H MR Spectroscopic capabilities. The participating subjects will undergo MR scanning in the morning after fasting overnight. A 5-10 ml size voxel will be carefully positioned over the interventricular septum based on the four-chamber and the short-axis images acquired at end-systole. Based on a high-temporal resolution cine image acquired previously, the MR spectroscopy will be timed to occur during systole, and MR spectra will be acquired using the following acquisition parameters: sequence type: Point resolved

spectroscopy sequence (PRESS); TE: 25 ms; TR > 3000 msec; 1024 acquisition points acquired using 1 kHz spectral bandwidth, and 128 NSA. The long TR ensures a more complete recovery of the myocardial triglyceride signals. The cardiac MR spectroscopy sequence is both cardiac gated (vectorcardiography (VCG) gating), as well as respiratory gated to combat the adverse effects of both cardiac pulsation as well as bulk motion of the heart caused by respiratory motion. Gating (respiratory and cardiac gating) improves the reproducibility of cardiac MR spectra. During the MRI/MRS, we will also measure LV systolic and diastolic function.

MRI Measurement of Left Ventricular Function

Systolic LV Function: A series of scout images of the thoracic cavity along multiple axes will be obtained. Following these scout scans, a series of cardiac cine Steady State Free Precession (SSFP) images will be obtained along the conventional two-chamber and four-chamber orientations with the following acquisition parameters: TR/TE/flip: 3-3.2 ms/1.5-1.6 ms/; slice thickness: 8 mm; temporal resolution: 40-50 ms; acquired in-plane spatial resolution: 2 x 2 mm; breath-hold duration: 6-8 heart-beats per slice. Based on these two long-axis orientation slices, a series of contiguous short-axis slices were acquired to cover from the left-ventricular apex to the base at 8 mm intervals in a series of breath-holds. The endocardial contours will be manually traced by an experienced observer at end-diastole and end-systole to delineate the LV volume in diastole (EDV) and systole (ESV). From the knowledge of EDV, and

ESV, LV ejection fraction (LVEF) will be calculated as the normalized difference between EDV and ESV, or (EDV-ESV)/EDV.

Diastolic LV function: MRI flow measurements: Diastolic LV function will be obtained by measuring the blood-flow across the mitral-valve using a cardiac-gated phase-contrast MR sequence. A 5 mm slice oriented orthogonal to the mitral valve plane (positioned with the help of the four-chamber and the left-ventricular outflow tract (LVOT) views) will be acquired with the following parameters: TR/TE: 4.6 ms/15 ms; flip angle; in-plane acquired voxel size: 1.6 x 1.8 mm; reconstructed voxel size: 1.2 x 1.2 mm; velocity encoding value (Venc value): 100-125 cm/s; number of acquisitions: 2. The acquisition will be retrospectively cardiac gated to ensure that the entire diastolic period is captured.

MRI flow analysis: The flow data will be analyzed using a commercial post-processing workstation. The characteristic diastolic flow data will be quantified by calculating the peak filling rates during the early filling phase (E), and the active filling phase (A).

High temporal resolution cine MR imaging:

Using a combination of a state-of-the-art RF hardware (32 channel RF system), and fast imaging techniques (Sensitivity Encoding or SENSE), a high temporal resolution cine MR images (~ 6 ms/cardiac phase) of the LV will be acquired in a series of breath-holds. A total of three short-axis slices will be acquired with a temporal resolution of 5-6.5 ms using the following acquisition parameters: TR/TE/flip: 2.7-3.1 ms/1.5-1.6 ms; acquired voxel size: 2.5 x 2.5 x 8 mm; reconstructed voxel size: 1.76 x

1.76 x 8 mm; half-scan factor: 0.625; number of phase encoding steps acquired per heart-beat (turbo-field echo factor): 2; acquired temporal resolution: 5.5 – 6.2 ms/cardiac phase; SENSE factor: 3; total scan time: 18-20 hb/slice. From the high-temporal resolution cine images, the LV cavity will be segmented using a segmentation algorithm. Metrics quantifying diastolic function such as IVRT will be calculated using custom-built software. A series of blood-pressure measurements will be obtained throughout imaging at 5 minute intervals.

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**
   a) 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**
   a) Subjects with a diagnosis of Impaired Glucose Tolerance or Prediabetes i.e. fasting plasma glucose between 100-125 mg/dl, or 2 hour post 75 gram OGTT plasma glucose between 140-199 mg/dl, or HbA1c 5.7-6.4% as per revised ADA criteria (24)
   b) Age and Reproductive Status
   c) Men and women, ages 30 to 70 years.
      i) Women of childbearing potential (WOCBP) must be using an acceptable method of contraception to avoid pregnancy throughout the study in such a manner that the risk of pregnancy is minimized.
      ii) Women must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 72 hours prior to the start of investigational product.

iii) Women must not be breastfeeding.

Women of childbearing potential include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal. Post menopause is defined as:

- Amenorrhea ≥ 12 consecutive months without another cause and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL, or
- Women with irregular menstrual periods and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL, or

  NOTE: FSH level testing is not required for women ≥ 62 years old with amenorrhea of ≥ 1 year.
- Women on hormone replacement therapy (HRT).

Women who are using oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, or are practicing abstinence or where their partner is sterile (eg, vasectomy) should be considered to be of childbearing potential.

d) Patients must have been on a stable dose of allowed chronic medications such as statins and other lipid lowering therapy for 90 days prior to entering the study.
e) Patients must have a BMI between 30-35 kg/m^2. Only subjects whose body weight has been stable (±3-4 pounds) over the three months prior to study will be included.
f) Patients must have the following laboratory values:

Hematocrit	≥ 34 vol%
S. creatinine	< 1.5 mg/dl in men and 1.4 mg/dl in women
AST (SGOT)	< 2.5 times upper limit of normal
ALT (SGPT)	< 2.5 times upper limit of normal
Alkaline phosphatase	< 2.5 times upper limit of normal

4.2.2 Exclusion Criteria

1) Sex and Reproductive Status

a) WOCBP who are **unwilling or unable** to use an acceptable method to avoid pregnancy for the entire study period.

b) Women who are pregnant or breastfeeding.

2) Medical History and Concurrent Diseases

a) Type 1 or Type 2 diabetes mellitus (FPG > 125 mg/dl)

b) History of diabetic ketoacidosis or hyperosmolar nonketotic coma

3) Physical and Laboratory Test Findings

a) Patients with diabetic gastroparesis will be excluded.

b) Patients with a history of clinically significant heart disease (New York Heart Classification greater than class 2; more than non-specific ST-T wave changes on the EKG), peripheral vascular disease (history of claudication), or pulmonary disease (dyspnea on exertion of one flight or less; abnormal breath sounds on auscultation) will not be studied.

4) Allergies and Adverse Drug Reactions

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

5) Prohibited Treatments and/or Therapies

a) Treatment with strong systemic cytochrome P450 3A4/5 (CYP 3A4/5) inhibitors.

b) Patients must not be on or have received any antihyperglycemic treatment for treatment of IGT at any time. Patients must not be receiving any of the following medications: thiazide or furosemide diuretics, beta-blockers, or other chronic medications such as hormone replacement therapy with known adverse effects on glucose tolerance levels. Patients must not be on systemic glucocorticoids.

6) Other Exclusion Criteria

a) Prisoners, or subjects who are involuntarily incarcerated.

b) Subjects who are compulsorily detained for treatment of either a psychiatric or physical (eg, infectious disease) illness.

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 (AE), 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.
- Pregnancy
  - Instruct WOCBP to contact the investigator or study staff immediately if they suspect they might be pregnant (eg, 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. The mechanism for reporting pregnancy is described in Section 7.5.
- Termination of the study by BMS.
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (eg, infectious disease) illness
- Inability to comply with the protocol.

All subjects who discontinue should comply with protocol specified follow-up procedures as 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 (ie, is imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness). If a subject was withdrawn before completing the study, the reason for withdrawal must be entered on the appropriate case report form (CRF) page.

4.3 Data Safety Monitoring Plan

Protocol and consent form approval has been obtained from the Baylor College of Medicine IRB. Request for IND shall submitted to the FDA. Prior to screening evaluations, each subject eligible for the study will be given an orientation session in which the nature and purpose of the study and the risks and benefits will be described. If an eligible subject agrees to participate in the study, he/she will be required to sign an informed consent form.

The PI and his team will monitor the study on a daily basis. All treatment emergent AEs will be recorded on source documents (i.e. original documents, data, and records). AEs include those reported spontaneously by the subject and those noted incidentally or as observed by the investigator or study personnel. All clinically significant abnormalities noted upon physical examination, or other diagnostic test results should be reported as an AE, except for baseline measurements that may be considered part of the medical history. In addition, all clinically significant AEs that continue at Study Termination will be followed up by the investigator and evaluated with additional tests if necessary, until the underlying cause is diagnosed or resolution occurs. All AEs will be evaluated for intensity and causal relationship with use of the study medication and/or study procedures by the investigator and reported to the Baylor IRB. All SAEs will be reported to the Baylor IRB and the sponsor within 24 hours. In addition, a safety report will be submitted to Baylor IRB annually. Any new information regarding saxagliptin will be submitted to Baylor IRB.

5 TREATMENTS

5.1 Study Treatment: Saxagliptin

An investigational product, also known as investigational medicinal product in some regions, is defined as follows: 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.

Other medications used as support or escape medication for preventative, diagnostic, or therapeutic reasons, as components of the standard of care for a given diagnosis, may be considered as noninvestigational products. In this protocol, noninvestigational products are: ***no noninvestigational products will be used in this study***

5.1.1 Identification

Saxagliptin 5 mg tablets and placebo tablets are plain, yellow, biconvex, round, film coated tablets.

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 Storage, 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.

Saxagliptin tablets should be stored at temperatures between 20°C and 25°C (68°F and 77°F) in tightly closed containers. The investigator is responsible for ensuring that the investigational product is stored under the appropriate environmental conditions (temperature, light, and humidity), as determined by the sponsor.

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

Patients will be randomized to one of the two treatment groups i.e. saxagliptin or placebo. Randomisation numbers (patient numbers) will be assigned strictly sequentially

as patients become eligible for randomisation. Randomization will be computer-generated by the research pharmacy, and the investigators will be blinded to the treatment assignments. The research pharmacy will provide the randomisation number and the appropriate bottle numbers.

5.4 Selection and Timing of Dose for Each Subject

The recommended dose of saxagliptin is 5 mg once daily. Saxagliptin can be taken with or without food. We shall administer saxagliptin 5 mg once daily PO for 6 months.

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 by the treating physician.

Before breaking the blind of an individual subject's treatment, the investigator should have determined that the information is necessary, ie, that it will alter the subject's immediate management. In many cases, particularly when the emergency is clearly 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

The co-administration of saxagliptin and strong CYP3A4/5 inhibitors such as ketoconazole, atazanavir, clarithromycin, indinavir, itraconazole, nefazodone, nelfinavir, ritonavir, saquinavir, and telithromycin may result in increased plasma concentrations of saxagliptin and should be avoided.

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

Treatment compliance will be monitored by drug accountability as well as the patient's medical record.

6 STUDY ASSESSMENTS AND PROCEDURES

6.1 Time and Events Schedule

Table 1: Time and Event Schedule for Protocol [Insert Protocol Number]

Procedure	Visit 1 (Screening)	Visit 2 (OGTT)	Visit 3 (Pre randomization)	Visit 4 (Randomization)	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11
Study week	**-4**	**-2**	**-1**	**0**	**2**	**6**	**10**	**14**	**18**	**22**	**26**
Obtain Informed Consent	X										
Confirm Eligibility	X										
Medical History	X										
Review Concomitant	X		X		X	X	X	X	X	X	X
Physical Examination	X				X	X	X	X	X	X	X
Vital Signs	X		X		X	X	X	X	X	X	X
Run 12-Lead Electrocardiogram	X										
Diet Counseling		X									
Pregnancy Test (a)	X		X		X	X	X	X	X	X	X
Urinalysis	X										
CBC, Lipids, LFTs, Serum Creatinine	X							X			X
FPG	X		X		X	X	X	X	X	X	X
HbA1c	X		X					X			X
OGTT		X									X

Insulin/Cytokines/FFA			X								X
Monocyte isolation			X								X
MRI Studies			X								X
Randomize				X							
Study drug				Start	x	x	x	x	x	x	Stop
Medication Compliance Check					X	X	X	X	X	X	X
Assess for Adverse Events		X	X	X	X	X	X	X	X	X	X

6.2 Study Materials

Bristol-Myers Squibb (BMS) will provide saxagliptin at no cost for this study.

6.3 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. Patients will report every 4 weeks while on the study drug and a complete safety assessment including a complete history and physical and fasting blood glucose will be performed to evaluate for the following specific adverse events

- Headache, URI, nasopharyngitis

-Nausea, abdominal discomfort, gastroenteritis

-Hypersensitivity-related reactions including skin rash (rare)

-Hypoglycemia (mild to moderate). 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-IV inhibitors, which exert their insulinotropic effects on the β cell in a glucose dependent manner. Severe hypoglycemia has not been reported in patients on saxagliptin monotherapy.

6.4 Efficacy Assessments

6.4.1 Primary Efficacy Assessment

The primary efficacy objective is to determine whether treatment with saxagliptin compared with placebo will result in a reduction in hepatic and myocardial fat in patient with IGT.

6.4.2 Secondary Efficacy Assessments

The secondary efficacy objective is to determine whether treatment with saxagliptin compared with placebo will result in an improvement in left ventricular systolic and diastolic function and a reduction in monocyte inflammation in patients with IGT.

7 ADVERSE EVENT REPORTING

7.1 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.

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.)

7.1.1 Serious Adverse Events

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

- 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 upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (eg, 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) is also considered an important medical event (see Section 7.7 for the definition of potential DILI)

Suspected transmission of an infectious agent (eg, 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 for data transmission purposes (See Section 7.5.2 for reporting pregnancies).

NOTE: The following hospitalizations are not considered SAEs in BMS clinical studies:

- 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 (eg, 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 (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

7.1.2 Adverse Events of Special Interest

In this study, the following adverse events are to be reported to BMS, 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, and
3. pancreatitis
4. anaphylaxis
5. angioedema and
6. Stevens' Johnson syndrome.

7.1.3 Nonserious Adverse Events

Nonserious adverse events are all adverse events that are not classified as SAEs.

7.2 Assignment of Adverse Event Intensity and Relationship to Investigational Product

All adverse events, including those that are serious, will be graded by the investigator as follows:

7. Mild (Grade 1): awareness of event but easily tolerated
8. Moderate (Grade 2): discomfort enough to cause some interference with usual activity
9. Severe (Grade 3): inability to carry out usual activity
10. Very Severe (Grade 4): debilitating; significantly incapacitates subject despite symptomatic therapy.

The following categories and definitions of causal relationship to investigational product as determined by a physician should be used:

11. **Related:** There is a reasonable causal relationship to investigational product administration and the adverse event.
12. **Not Related:** There is not a reasonable causal relationship to investigational product administration and the adverse event.

The expression "reasonable causal relationship" is meant to convey in general that there are facts (eg, evidence such as de-challenge/re-challenge) or other arguments to suggest a positive causal relationship.

7.3 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 BMS and the IRB/IEC with any additional information requested, notably for reported deaths of subjects.

7.3.1 Serious Adverse Events and Adverse Events of Special Interest

Following the subject's written consent to participate in the study, all SAEs and all AEs of Special Interest (defined in Section 7.1.2), whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur during the screening period and within 30 days of discontinuation of dosing. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy). The investigator should report any SAE occurring after these time periods that is believed to be related to study drug or protocol-specified procedure.

An SAE report should 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 should be specified in the narrative section of the SAE Report Form.

All SAEs, whether related or unrelated to saxagliptin, and all pregnancies must be reported to BMS (by the investigator or designee) within 24 hours of study personnel becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

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

For studies conducted under an **Investigator IND**, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible **and no later than 7 days** (for a death or life-threatening event) **or 15 days** (for all other SAEs) **after the investigator's or institution's initial receipt of the information.** The investigator must provide BMS with a copy of all adverse events filed with the FDA. SAEs should 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 BMS using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization. All pregnancies must be followed to outcome.

7.3.2 Handling of Expedited Safety Reports

In accordance with local regulations, BMS will notify investigators of all SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the Investigator Brochure). In the European Union, an event meeting these criteria is termed a Suspected Unexpected Serious Adverse Reaction (SUSAR). BMS will send investigators an expedited safety report (ESR) to notify them of such an event.

Other important findings that BMS may report as ESRs include increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety findings from a nonclinical (eg, animal) study, important safety recommendations from a study data monitoring committee, or the decision by BMS to end or temporarily halt a clinical study for safety reasons.

Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the Investigator Brochure. Where required by local regulations or when there is a central IRB/IEC for the study, BMS will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. When BMS has a written agreement with a local IRB/IEC, BMS will directly submit ESR(s). The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

In addition, BMS will report suspected serious adverse reactions (whether expected or unexpected) to the relevant health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

7.3.3 Nonserious Adverse Events

The collection of nonserious AE (NSAE) information should begin at initiation of study drug. NSAE information should also 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 should be followed to resolution or stabilization, or reported as SAEs if they become serious (see Section 7.3.1). Follow-up is also required for NSAEs that cause interruption or discontinuation of study drug, or those that are present at the end of study treatment as appropriate. All identified NSAEs must be recorded and described on the NSAE page of the CRF.

Completion of supplemental CRFs may be requested for AEs and/or laboratory abnormalities that are reported/identified during the course of the study.

7.4 Laboratory Test Abnormalities

All laboratory test results captured as part of the study should be recorded following institutional procedures. When reporting a test result that constitutes an adverse event, the clinical term should be used; for example, the event should be reported as “anemia” not “low hemoglobin.” Test results that constitute SAEs should be documented and reported as such.

The following laboratory abnormalities should be captured on the NSAE CRF page or SAE Report Form as appropriate:

- 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.

7.5 Pregnancy

WOCBP must use an effective method of birth control during the course of the study, in such a manner that the risk of failure is minimized.

7.5.1 Requirements for Pregnancy Testing

All WOCBP MUST have a negative pregnancy test within 72 hours before receiving saxagliptin. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive saxagliptin and must not continue in the study.

In addition, all WOCBP must be instructed to contact the investigator and/or other study personnel immediately if they suspect they might be pregnant (eg, missed or late menstrual period) at any time during study participation.

7.5.2 Reporting of 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-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, 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 (eg, x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated.

The investigator must immediately notify the BMS Medical Monitor of this event and complete and forward a Pregnancy Surveillance Form to BMS within 24 hours and in accordance with SAE reporting procedures described in Section 7.3.1

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must 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.

7.6 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 must be reported as an SAE (see Section 7.3.1 for reporting details).

Once-daily, orally administered saxagliptin has been shown to be safe and well tolerated, with no clinically meaningful effect on QTc interval or heart rate at doses up to 400 mg daily for 2 weeks (80 times the recommended human dose). In the event of an overdose, appropriate supportive treatment should be initiated as dictated by the patient's clinical status. Saxagliptin and its major metabolite are removed by hemodialysis (23% of dose over 4 hours).

7.7 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 (7.3.1 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 hyperbilirubinemia, 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.

7.8 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.

8 STATISTICAL CONSIDERATIONS

8.1 Sample size determination

Sample size is calculated using SigmaPlot software (San Jose, CA). For the two major outcome variables i.e. myocardial fat and hepatic fat, the sample size of ~ 16 patients per treatment group (total of 32 patients) will have >90% power to detect a difference in the means of 20%, assuming a standard deviation of 15%, and with alpha= 0.025. Given that we expect about 20% of the patients enrolled may not complete the 6 month study, we would like to enroll 40 patients so that 32 patients may complete the study.

8.2 Population for analysis

All patients used in the analyses of the primary and secondary objectives will belong to the intention-to-treat (ITT) population. This population is defined as all randomised patients. The patients will be analysed according to the treatment group to which they were randomized.

8.3 Endpoint Definitions:

Primary Efficacy Endpoint:is defined as the effect of saxagliptin compared with placebo on reduction in hepatic and myocardial fat.

Secondary Efficacy endpoint: is defined as the effect of saxagliptin compared with placebo on improvement in left ventricular systolic and diastolic function and a reduction in monocyte inflammation.

Safety endpoints: The number and percent of patients with an AE will be summarised for each treatment group. In addition, the number and percent of patients with a predefined marked abnormality in clinical laboratory tests will be summarised by treatment group.

8.4 Analyses

8.4.1 Demographic and baseline characteristics : Demographic and baseline characteristics will be summarised using summary statistics based on the ITT data set, for each treatment group as well as for all patients combined. Key baseline characteristics will be summarised. No statistical test will be performed for comparison of any baseline measurement among treatment groups. Demographic and baseline characteristics will be summarised for the total study population.

8.4.2 Efficacy Analysis: Between groups comparisons will be made using two-way analysis of variance (ANOVA.) to analyze changes in hepatic/myocardial fat following saxagliptin

treatment in comparison to placebo (primary efficacy analysis). Secondary efficacy analysis will include the effect of saxagliptin (versus placebo) on LV function and monocyte inflammation. Pre and post treatment within a group will be analyzed using paired t-tests. Simple correlation analysis will be performed with Pearson's correlation coefficients to examine the relationship between hepatic fat and myocardial fat/ LV function . Spearman Rank Correlation will be used for data which is not normally distributed. All statistical analysis will be performed using SAS (Cary, NC).

8.4.3 Safety Analysis

Safety is evaluated by the following endpoints:

o Incidence of overall AEs and AEs of special interest

Other safety endpoints:

o Changes from baseline in laboratory tests, pulse, BP, and body weight.

o Incidence of hypoglycaemic events.

9. Compliance with the Protocol and Protocol Revisions

The study must be conducted as described in the final IRB/IEC-approved protocol. Documentation of approval, signed by the IRB/IEC chairperson or designee, must be sent to the BMS protocol manager.

All protocol amendments and revisions to the informed consent must be submitted to the BMS protocol manager and to the IRB/IEC. No protocol amendments will be implemented until written approval has been given by the IRB/IEC, except when necessary to eliminate an immediate hazard to study subjects. Administrative letters should also be sent to the BMS protocol manager and IRB/IEC; however, they do not require approval.

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 must be submitted to:

- IRB/IEC for review and approval/favorable opinion
- Bristol-Myers Squibb
- Regulatory Authority(ies), if required by local regulations.

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

If an amendment substantially alters the study design or increases the potential risk to the subject: (1) the consent form must be revised and submitted to the IRB(s)/IEC(s) for review and

approval/favorable opinion; (2) the revised form must 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.

9.1Records Retention

The investigator will retain, in a confidential manner, all data pertinent to the study for all treated subjects as well as those entered as control subjects. The investigator will retain source documents and accurate case histories that record all observations and other data pertinent to the investigation (eg, the medical record) for the maximum period required by applicable regulations and guidelines or following institutional procedures. If the investigator withdraws from the study (eg, relocation or retirement), the records must be transferred to a mutually agreed upon designee, such as another investigator or an IRB. Written documentation of such transfer must be provided to BMS.

The investigator will ensure that a current record of disposition of investigational product is maintained at each study site where the investigational product is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines and should include:

13. amount received and placed in storage area
14. amount currently in storage area
15. label identification number or batch number and use date or expiry date
16. dates and initials of person responsible for each inventory entry/movement
17. amount dispensed to and returned by each subject, including unique subject identifiers
18. amount transferred to another area/site for dispensing or storage
19. non-study disposition (eg, lost, wasted, broken)
20. amount destroyed at study site, if applicable
21. amount returned to the sponsor, if applicable.

9.2 Destruction of Investigational Product

If the study drugs are to be destroyed on site, it is the investigator's responsibility to ensure that arrangements have been made for disposal, and that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal must be maintained.

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 (ie, 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 (eg, Investigator Brochure for an unapproved investigational product)

LIST OF ABBREVIATIONS

A1C	Glycosylated Hemoglobin
AE	Adverse Event
BMS	Bristol-Myers Squibb
CYP3A4	Cytochrome P450 3A4
DPP-IV	Dipeptidyl peptidase IV
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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