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CLINICAL STUDY PROTOCOL

Impact of Timed Bromocriptine-QR Therapy upon Measures of Sympathetic Tone and Vascular Biology in Type 2 Diabetes Subjects.

Date of Original Protocol

Single center study
US sites only

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TABLE OF CONTENTS

PROTOCOL OUTLINE

Study number

Title

Impact of Timed Bromocriptine-QR Therapy upon Measures of Sympathetic Tone and Vascular Biology in Type 2 Diabetes Subjects

Investigator(s), Study Site(s)

Aaron Vinik, M.D. (vinikai@evms.edu), **The Strelitz Diabetes Center, Norfolk, VA**

Sponsor-Funded, Single Center, Investigator Initiated Trial

Study Duration and Dates	The duration of this study is expected to be approximately 38 months (31-month subject recruitment, up to 3 week screening phase, and 6-month treatment), with subject recruitment to start in June 2015 and the last subject to finish by August 2018.	**Phase** IV (Double Blind, Placebo Controlled Study of Bromocriptine-QR vs Placebo added to Usual Diabetes Treatment)

Objectives

Primary Objective:

To demonstrate the effects of dopaminergic activation on the autonomic nervous system in subjects with type 2 diabetes.

Secondary Objective:

To demonstrate the effects of dopaminergic activation with bromocriptine-QR on the regulation of hypothalamic-pituitary-axis (HPA) hormones and on the plasma levels of markers of inflammation and oxidative/nitrosative stress in type 2 diabetes subjects. The study will evaluate treatment effects on inflammatory markers, the leptin/adiponectin system, and hormonal levels of renin-angiotensin system (RAS), aldosterone and cortisol. A co-secondary objective of the study will be to assess the impact of bromocriptine-QR vs placebo on measures of insulin resistance and glycemic control (e.g., OGTT glucose and insulin, Matsuda index, HOMA-IR, HbA1c).

The treatment effects on all of the above primary and secondary outcomes will also be assessed as a function of the duration of diabetes and other baseline demographics such as HbA1c, concomitant medications and metabolic status.

Study Design

This is a single-site, prospective, interventional, twenty-four week, randomized, double blind, placebo-controlled trial with bromocriptine QR in subjects with early diabetes and established T2DM to evaluate its effects on the cardiovascular and peripheral autonomic nervous system, as well as inflammatory markers, the leptin/adiponectin system, hormonal levels of RAS and HPA axis, indices of insulin resistance and glycemic control, and measures of oxidative and nitrosative stress. At least twenty (20) but no more than forty (40) early diabetes subjects and at least forty (40) but no more than sixty (60) subjects with established diabetes will be enrolled in the study.

Entry criteria will be checked at the screening visit. Subjects will be required to be on a stable anti-diabetes regimen consisting of diet and/or oral anti-diabetic agents consisting of metformin alone or metformin plus an insulin secretion enhancer (sulfonylurea, DPP4 Inhibitors, or GLP-1 analogs) for at least 60 days prior to randomization. Following a 2 week lead-in period, subjects will be randomized in a 1:1 ratio to receive UDT (usual diabetes therapy) plus bromocriptine-QR or UDT plus placebo. The circadian timing of the interventional therapy will be within 2 hours of waking in the morning.

Following randomization, subjects will be titrated to the maximum tolerated dose of the study drug (bromocriptine-QR or placebo) over a 4 week period.

Subjects will return at 4 weeks after randomization, at 12 weeks after randomization, and then at study end (week 24 or early termination) for analyses of the primary and secondary endpoints. A primary analysis of the study data will be performed after 12 weeks with a secondary analysis conducted at 24 weeks. Subjects will be contacted 30 days after stopping the study drug to record any adverse events that occurred after cessation.

All visits will be conducted at The Strelitz Diabetes Center, Norfolk, VA.

Number Of Subjects

Planned enrollment; A total of 80 subjects are planned to be enrolled in the study. At least 20 but no more than 40 early (diabetes duration of < 4 years) diabetes subjects and at least 40 but no more than 60 subjects with established diabetes (diabetes duration of ≥ 4 years) will be enrolled in the study.

Primary Subject Characteristics at Enrollment

T2DM male or female subjects between the ages of 30 and 80 years with HbA1c ≤ 10% on a stable anti-diabetes regimen of diet and/or metformin alone therapy or metformin plus an insulin secretion enhancer (sulfonylureas, DPP4 Inhibitors, GLP-1 analogs) therapy for a 60 day period prior to randomization. Subjects must have a documented C-peptide level of >2 ng/ml from the screening visit.

Study Treatments

Bromocriptine-QR, 0.8 mg/day, with the dose increased by 0.8 mg/day every week to a maximum of 3.2 mg/day, or as tolerated to a minimum dose of 1.6 mg/day, or matching placebo, added on to usual diabetes therapy consisting of a stable anti-diabetes regimen of diet and/or metformin

alone therapy or metformin plus an insulin secretion enhancer (sulfonylureas, DPP4 Inhibitors, GLP-1 analogs) therapy for a 60 day period prior to randomization.

Study Endpoints

The primary endpoint is the effect of bromocriptine-QR vs placebo on changes in autonomic function measured by assessing sympathetic and parasympathetic function using conventional measures of autonomic function, including power spectral analysis of heart rate as well as peripheral autonomic function using sudorimetry and laser scanning of peripheral microvascular autonomic control.

Secondary endpoints will be bromocriptine-QR vs placebo effects on inflammatory markers, markers of oxidative/nitrosative stress, the leptin/adiponectin system, and hormonal levels of the renin-angiotensin system (RAS), aldosterone and cortisol. The following plasma markers of inflammation and oxidative/nitrosative stress will be evaluated: 1) CRP, 2) IL6,3) TNF α, 4) PAI1, SOD, TBARS and ADMA will be assessed as well as nitrotyrosine and other markers of oxidative/nitrosative stress .

Statistical Procedures

We propose that an effect size of 10% mean change from baseline in treated groups represents a clinically meaningful difference, and we propose that sample size reflects the ability to detect a 10% change due to intervention factor.

We intend to use the parametric statistical procedures ANOVA and MANOVA in these analyses, based on our previous experience that the data from these tests is normally distributed in raw form or in some cases after simple log-transformations. If data are not normally distributed, the Wilcoxon signed-rank (within group) or Mann Whitney (between group) tests may be employed or Fisher's exact test in case of small sample sizes. The level of significance will be set at $p<0.05$. Relationships and/or trends between the effect of Cycloset on neurovascular function and diabetic neuropathy will be determined with Spearman's rank correlation.

Based on these considerations, we calculate a total of 80 participants will result in a power greater than 0.80 for observing statistical significance at the $p < 0.05$ level. Specifically, we plan to recruit approximately 40 in the placebo group and approximately 40 in the treatment group. In total, approximately 120 people will be screened in order to enroll approximately 80 participants in this study. JMP statistical Software version 9.3 will be used to perform all statistical analyses.

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A statistical analysis plan (SAP), providing details of the analyses and presentation structure of the results, will be developed and finalized before the database is locked.

STUDY SCHEDULE

Data collected and/or action	Screen	Baseline	Treatment Period (24 weeks)									
	Visit 0	Baseline Visit 1 (Visit 0 + <30 days)	Phone call 1 (Visit 2 + 7 days)	Phone call 2 (Call 1 + 7 days)	Phone call 3 (Call 2 + 7 days)	Visit 2 Week 4	Phone call 4 Week 8	Visit 3 Week 12	Phone call 5 Week 16	Phone call 6 Week 20	Visit 4 Week 24 (or Early Term)	Phone call 7 Visit 4 +30 days
		Random-ization										
Informed Consent	X											
Entry Criteria	X											
Medical History	X					X		X			X	
Physical Exam	X										X	
Neurological Exam	X										X	
ECG	X										X	
Hematology and chemistry	X										X	
Lipids		X						X			X	
HbA1c	X	X						X			X	
Urine Microalbumin		X						X			X	
Inflammatory oxidative/ nitrosative stress and neuroendocrine Markers		X				X		X			X	
OGTT (with glucose and insulin analyses)		X						X			X	
C-peptide	X							X			X	
HOMA-IR (fasting glucose and insulin)						X						
Autonomic Function Tests		X				X		X			X	
Sudorimetry		X				X		X			X	
Skin Blood Flow		X				X		X			X	
Quantitative Sensory Tests		X				X		X			X	
Study Drug instruction (first dose next AM)		X										
Study Drug Titration reminder			X	X	X							
AE/SAE query/ Study Drug assessment			X	X	X	X	X	X	X	X	X	
Final follow up for AE/SAE resolution												X
Concomitant meds		To be assessed throughout the study										
Adverse Events		To be assessed throughout the study										
Serious Adverse Events		To be assessed throughout the study **Report serious adverse events to sponsor within 24 hours**										
Mandatory Contact		Weekly titration phone contacts as described in the protocol										

ABBREVIATIONS AND DEFINITIONS

AE	Adverse Event
BG	Blood Glucose
BMI	Body Mass Index
CNS	Central Nervous System
CRF	Case Report Form
CV	Cardiovascular
DM	Diabetes Mellitus
FPG	Fasting Plasma Glucose
GCP	Good Clinical Practice
HbA1c	Hemoglobin A1C
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
MACE	Major Adverse Cardiac Event
QR	Quick Release
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
T2DM	Type 2 diabetes mellitus
UDT	Usual Diabetes Therapy

1 INTRODUCTION AND STUDY RATIONALE

A. Summary

Type 2 diabetes is a growing health concern and diabetic subjects are at high risk for atherosclerotic cardiovascular complications (1). The CNS and the hypothalamus in particular have emerged as fundamental regulators of whole body homeostasis, including glucose and lipid metabolism (2). Several studies on animal models of type 2 diabetes (T2DM) and insulin resistance have shown multiple hypothalamic neurophysiologic derangements characterized mainly by low dopaminergic tone, and elevated adrenergic and serotoninergic tone (3, 3A). This cluster of derangements contributes to the pituitary hypothalamic release of ACTH and cortisol, but more importantly to the overactivity of sympathetic drive to the liver, adipose tissue and cardiovascular system. Increased sympathetic drive to adipose tissue stimulates lipolysis with release of free fatty acids (FFA) and hepatic synthesis of VLDL, raising triglycerides that cause lipotoxicity with secretion of a number of inflammatory cytokines including TNFα and IL6. This induces a state of chronic low grade inflammation with increased oxidative/nitrosative stress and resistance to the action of insulin. Increased sympathetic drive to the liver induces hepatic glucose production and decreases hepatic glucose disposal elevating fasting and postprandial glucose levels (3). Sympathetic overactivity affects the cardiovascular system causing vasoconstriction of resistance vessels and inducing hypertension. This is a major contributor to cardiovascular disease including tachycardia, arrhythmias, sudden death and myocardial cell apoptosis with impaired ventricular function and failure. Furthermore, reduction of parasympathetic function with loss of sympathetic/parasympathetic balance affects the anti-inflammatory state, levels of anti-inflammatory cytokines such as IL10 and the Leptin/Adiponectin ratio, thus increasing the inflammation and insulin resistance in T2DM (4).

We have shown that loss of sympathovagal balance occurs early in newly diagnosed T2DM and may precede the appearance of inflammation and oxidative/nitrosative stress, predicting macrovascular damage and cardiovascular events (5). Recently, three prominent studies, ACCORD, ADVANCE and VADT, have shown that diabetic cardiac autonomic neuropathy (CAN) may predict risk of sudden death with intensification of glycemic control (6-11). While numerous likely mechanisms for the elevated incidence of cardiovascular events in these studies have been proposed, the presence of autonomic dysfunction is emerging as one of the strongest predictors of cardiovascular risk. Furthermore, there are reports that directly associate autonomic dysregulation with inflammatory cytokines and adipocytokines, which subsequently increase and promote cardiovascular risk. Quick-release (QR) bromocriptine has shown to improve HbA1C, fasting plasma glucose and postprandial glucose tolerance in subjects with T2DM (12-15). We have recently demonstrated that this drug can reset dopaminergic tone and, in one year, reduce cardiovascular death by 39% and major adverse cardiovascular events (MACE) by 52% (see preliminary data). This raises the question about the mechanisms implicated for this effect. We hypothesize that bromocriptine-QR, a dopamine D2 receptor agonist, resets dopaminergic tone, thus improving the sympathovagal balance and mitigating inflammation, oxidative/nitrosative stress and the metabolic consequences of dyslipidemia, hyperglycemia and hypertension. In this study, we propose to investigate the effects of bromocriptine QR on the unique relationship between autonomic function and inflammation in newly diagnosed versus established T2DM subjects. These studies should shed new light on the mechanisms of cardioprotection by resetting autonomic balance. This may lead, in time, to a new therapeutic focus for managing cardiovascular risk in T2DM.

B. Detailed Background

Obesity, insulin resistance, and T2DM are growing health concerns, and the incidence and prevalence of these diseases are increasing worldwide (1). The CNS has been identified as a key regulator of whole body homeostasis. Evidence indicates that, within the brain, the hypothalamus in particular is responsible for the day-to-day regulation of a number of factors including body temperature, blood pressure, thirst, and hunger; and is a fundamental structure for the integration of the nervous and endocrine systems. Over the past decade, it has been shown that the CNS senses: 1) hormones, namely insulin, leptin, and glucagon-like peptide (GLP)-1, and 2) nutrients, namely fatty acids and glucose, to regulate both energy and glucose homeostasis (2).

Quick-Release (QR) Bromocriptine: Bromocriptine is an ergot derivative that exerts activities as a sympatholytic dopamine D2 receptor agonist, adrenergic alpha-1 antagonist, alpha-2 agonist, and also as a modulator of serotonin and prolactin levels. It activates D2 receptors, which results in reduced norepinephrine secretion and suppression of sympathetic nervous system activity (16). A QR formulation has recently been approved by the FDA for the treatment of T2DM. Based on animal and human studies, bromocriptine-QR administration within 2 h of awakening is believed to augment low hypothalamic dopamine activity and inhibit excessive sympathetic tone within the CNS. This results in reduced postprandial plasma glucose levels due to improvements in impaired hypothalamic fuel sensing mechanisms and subsequent impaired insulin- and non-insulin mediated glucose disposal and enhanced suppression of hepatic glucose production. Such treatment also reduces fasting and postprandial plasma free fatty acid (FFA) and triglyceride levels. Studies have shown that, in subjects with poorly controlled T2DM treated with diet alone, metformin, sulfonylureas, or thiazolidinediones, the addition of bromocriptine produces a 0.5–0.91 decrement in HbA1c and improves fasting plasma glucose and postprandial glucose tolerance (12-14) (see preliminary data). A prospective 1-year study in type 2 diabetic subjects demonstrated a significant 40% relative risk reduction among bromocriptine-QR treated subjects of a composite pre-specified cardiovascular end point that included ischemic (myocardial infarction and stroke) and non-ischemic (hospitalizations for unstable angina, congestive heart failure and revascularization) related endpoints (15). In a further analysis it reduced MACE by 39% and mortality by 50% within a year of treatment (see preliminary data). This is the first drug approved for the treatment of hyperglycemia that has shown a reduction on CV events. **The mechanism of the drug's beneficial effect on cardiovascular events has not been clearly delineated.**

Mechanism of action of bromocriptine: Bromocriptine's effects are mediated via resetting of dopaminergic and sympathetic tone within the CNS. Mammalian species living in the wild have the ability to alter their metabolism from the insulin-sensitive/glucose-tolerant state to the insulin-resistant/glucose-intolerant state seasonally for survival. Such metabolic changes are governed by changes in monoaminergic concentrations/activity in the suprachiasmatic nuclei (SCN) of the hypothalamus and in the ventromedial hypothalamus (VMH) (3). Numerous studies implicate shifts in the circadian phase relationship between endogenous dopaminergic and serotonergic activity rhythms in SCN in the transition from the insulin-sensitive to insulin-resistant state (reviewed in 3A). The VMH plays a pivotal role in modulating autonomic nervous system function, hormonal secretion, peripheral glucose/lipid metabolism, and feeding behavior (17,18). Importantly, Humans also do manifest circadian oscillations and seasonal changes in metabolism (3B). Available evidence suggests that the same circadian neuroendocrine events that control peripheral metabolism and cardiovascular biology in species of all the major vertebrate classes is also operative in humans (3B,

3C). Hypothalamic centers that regulate these circadian rhythms receive inputs via numerous centers throughout the CNS, neurogenic stimuli from peripheral tissues and gastrointestinal tract, hormonal signals, and signals from circulating metabolites. Interventions, such as timed daily bromocriptine administration to coincide with the daily peak in endogenous CNS dopaminergic activity which is diminished in insulin resistant states and, which alter monoamine neurotransmitter levels within these hypothalamic circadian centers, can exert significant improvements on glucose and lipid dysmetabolism (19). It is believed that type 2 diabetic subjects have an early morning diminution in dopaminergic tone, which leads to increased sympathetic activity (16). In lean, normal individuals, plasma prolactin concentrations peak at night during sleep and CNS dopaminergic activity peaks at the onset of waking. These neuroendocrine rhythms along with others function to entrain a circadian pattern of normal fuel metabolism. In contrast, obese insulin-resistant individuals have elevated day time plasma prolactin levels (20), and reduced morning dopaminergic tone (21). **Administration of bromocriptine-QR reduces the elevated day-time prolactin levels (12,20,22) and restores central dopaminergic activity, thereby reducing sympathetic activity, hepatic gluconeogenesis, postprandial plasma glucose, triglycerides, and FFA concentrations, all without increasing plasma insulin levels (Figure 2) (23).**

Cardiovascular risk in DM and bromocriptine: It is well known that type 2 diabetic subjects are at high risk for atherosclerotic cardiovascular complications. More than 50% of adults with T2DM have coronary artery disease (CAD), and death from CAD is two to five-times more likely in a diabetic than in a nondiabetic patient (24). Although hyperglycemia is a risk factor for cardiovascular events, it is relatively weak when compared with other risk factors such as dyslipidemia, hypertension, obesity, insulin resistance and metabolic syndrome (25). However, even after correction of these more established risk factors, type 2 diabetic subjects still remain at high risk for atherosclerotic cardiovascular complications (26). Moreover, a number of recent trials (27) including ADVANCE (6) and VADT (7), which assessed the impact on cardiovascular events of intensive glucose-lowering therapy, were not able to demonstrate a significant reduction of cardiovascular events in the intensive group as compared to the standard group. The primary endpoints in these studies were similar, mainly MACE including: non-fatal MI, stroke, PVD, CABG and sudden death. None of these studies showed a significant reduction in MACE. Furthermore, in ACCORD, the study with the most ambitious goal (HbA1c < 6%), the overall and cardiovascular mortality was greater in the intensive group, with a 22% increase in the likelihood of sudden death. The presence of autonomic dysfunction increased the risk of an event 2.8 times and the presence of peripheral neuropathy raised the risk to 4.4. Similarly, the presence of autonomic dysfunction combined with peripheral neuropathy, was the strongest predictor of cardiovascular events in the DIAD study. In the first three studies, the hypoglycemic risk was indeed increased in the intensive group. However, hypoglycemia was not the cause of sudden death in the ACCORD study but a marker for susceptibility to the event (8). It now seems that autonomic imbalance coupled with peripheral nerve dysfunction are lead candidates conferring susceptibility to CV risk (11). **Therefore, anti-diabetic agents that not only improve glycemia but also reduce cardiovascular risk by altering sympathetic/parasympathetic balance are highly desirable (3).**

Diabetic CAN is a serious complication found in one fourth of type 1 and one third of type 2 diabetic subjects. It has long been recognized that cardiac autonomic neuropathy is associated with increased cardiovascular events and mortality in diabetes and may have greater predictive power than traditional risk factors for cardiovascular events. Moreover, recent studies have shown that autonomic imbalance may be a predictor for risk of sudden death with intensification of glycemic control (4,7,11). This can be attributable to autonomic imbalance between the sympathetic and

parasympathetic nervous system regulation of cardiovascular function (4,28). Chang et al. demonstrated that cardiac autonomic dysfunction (as measured by spectral analysis and expiratory-inspiratory ratio) may occur prior to the development of insulin resistance in individuals with 1 or 2 components of the metabolic syndrome (29). The Finnish Diabetes Prevention Study showed that cardiovascular autonomic dysfunction was common in subjects with impaired glucose tolerance and metabolic syndrome, especially in the overweight, obese subgroup (30). We recently demonstrated the presence of cardiac autonomic dysfunction both in newly diagnosed and established diabetic subjects (see preliminary data) (5). Thus, parasympathetic tone may decline with an autonomic imbalance shifting toward augmented sympathetic tone during the development from normal glucose tolerance to impaired glucose tolerance and finally diabetes (31). It is then not surprising to find abnormalities in autonomic function in the early stages of the disease, but without established autonomic neuropathy, suggesting a potential reversibility at the stage of newly diagnosed diabetes. In the cardiovascular safety trial (15), bromocriptine-QR reduced HbA1c, blood pressure, heart rate, and plasma triglycerides. However, these changes were modest and do not seem to explain the 40% decrease in composite cardiovascular outcome. We propose that bromocriptine's beneficial effect on cardiac events is that it decreases overactivity of the sympathetic nervous system and attenuates overactivity of the HPA axis and the RAS. In animal studies, bromocriptine has been shown to attenuate the effect of CNS sympathetic overactivity on the vasculature (32,33). Furthermore, in humans, bromocriptine has been shown to decrease plasma norepinephrine levels in the resting state as well as with maneuvers that increase catecholamine levels, such as tilting (34-37). Although bromocriptine is no longer circulating or bound to D2 receptors throughout the 24-h period, changes in catecholamine levels persist because of the appropriately timed dopamine pulse. As increased sympathetic activity is associated with both sustained ventricular arrhythmias and heart failure, the reduction in sympathetic activity that occurs with bromocriptine could improve ventricular function and decrease cardiac arrhythmias in the diabetic patient who has a higher prevalence of these cardiac complications (38). **Thus, bromocriptine- QR's potential to restore a diurnal variation in sympathetic activity in addition to its ability to decrease total sympathetic nervous system activity may explain its cardioprotective effect. Yet, this has not been further investigated.**

Postprandial hyperglycemia, which is decreased by QR bromocriptine, has been shown to be associated with an increased risk of CV events in subjects with and without T2DM (39). Elevations of postprandial glucose especially when accompanied by potentially more important factors such as increased triglyceride and FFA levels, lead to inflammation, oxidative stress and endothelial dysfunction, which in turn may lead not only to increases in the volume of arterial atheroma but also to increased cardiac events. Pharmacological lowering of postprandial glucose has been shown to decrease cardiac events in some studies (STOP-NIDDM trial) (40-42) but not in others (NAVIGATOR trial) (43,44). Bromocriptine-QR significantly lowers postprandial glucose. This places it in a select group of drugs (α-glucosidase inhibitors, incretin mimetics, DPP4-inhibitors, pramlitide, thiazolidinediones and fast-acting insulins) that have been shown to effectively lower postprandial glucose. Therefore, the decrease in cardiac events seen with this drug could also be due to its effects on postprandial glucose and lipid levels (postprandial dysmetabolism) (38). However the mechanisms whereby these agents lower postprandial glucose may be very different. **Here we will examine the contribution of changes in autonomic balance on postprandial blood glucose responses.**

Inflammation and Autonomic Imbalance: IL6, IL12 and C reactive protein (CRP) have been associated with reduced heart rate variability (HRV) and sympathovagal balance (4). Other markers

of inflammation such as TNF alpha, ADMA, and adipose-derived hormones have also been linked to CAN (4). It has been hypothesized that inflammation alters HRV, but the opposite directional relationship suggesting that autonomic changes could be pro-inflammatory is also conceivable (45). Experimental data support both possibilities. IL6 and CRP were found to be associated with reduced heart rate variability (HRV) in a study of 264 middle-age male twins free of symptomatic coronary artery disease (45). The results of this cross-sectional study suggest that autonomic dysregulation may lead to inflammation providing a pathway through which traditional risk factors promote the development of cardiovascular disease. Both exposure to acetylcholine and direct vagal stimulation inhibits release of cytokines by macrophages (46). Conversely, sympathetic activation is pro-inflammatory. In isolated adipocytes, β-adrenergic stimulation increases IL6 (47), whereas β – blockers dampen the IL6 increase normally seen in response to stress in rats (48). Given that cause–effect relationships cannot be determined from cross-sectional studies; prospective studies are needed to determine whether autonomic dysfunction mediates the inflammatory process or if autonomic imbalance is a consequence of inflammation. In the study performed in our lab we demonstrated an association between cardiac autonomic dysfunction and markers of inflammation (IL6, PAI1) both in newly diagnosed and established diabetic subjects (see preliminary data) (5). An association between both CRP and IL6 and diabetic polyneuropathy has also recently been demonstrated (49).

Oxidative/Nitrosative Stress and Autonomic Dysfunction: Growing evidence suggests that enhanced oxidative/nitrosative stress and, in particular, increased production of the potent oxidant peroxynitrite is a characteristic feature of both experimental and clinical diabetes mellitus (50). Peroxynitrite causes damage to a variety of tissues, including peripheral nerve, spinal cord, dorsal root ganglion neurons, and vasa nervorum (51). Several markers of oxidative stress in plasma, including superoxide and peroxynitrite, are elevated in subjects with diabetes and cardiac autonomic neuropathy (52). These findings suggest the presence of peroxynitrite cytotoxicity at both early and advanced stages of diabetes and, furthermore, at the pre-diabetic stage. **Here we will examine the relationship between modulation of autonomic balance and the amelioration of oxidative and nitrosative stress.**

Leptin and Autonomic Dysfunction: It is well documented that leptin, secreted by the adipose tissue, plays a critical role in the regulation of energy, body weight and glucose homeostasis (2). Recent observations strongly suggest that leptin, just as insulin, can also regulate glucose homeostasis independent of its effects on weight loss (53). Leptin's glucose homeostatic regulation also has a central component and the hypothalamic arcuate nucleus (ARC) has been spotlighted as the key CNS site. Hyperleptinaemia may be an important player in the activation of the sympathetic nervous system in humans. Leptin levels were associated with a shift of sympathovagal balance toward increased sympathetic activation in a study of 120 non-obese adults (54). Leptin receptor-deficient db/db mice develop T2DM, hypertension and obesity, with a disrupted circadian blood pressure, higher resting heart rates as well as loss of heart rate variability (55). In our study (5) we showed a fall in the adiponectin/leptin ratio in newly diagnosed diabetes with further progression in established diabetes which accompanied the changes in autonomic balance (see preliminary data). **This evidence shows that alterations in leptin homeostasis may have important consequences in the balance of the autonomic nervous system and changes in leptin in relation to autonomic balance with bromocriptine QR treatment will be monitored.**

Adiponectin and Autonomic Balance: Another adipocyte-derived protein that may be regulated by the sympathetic nervous system is adiponectin. In humans with autonomic imbalance (i.e. predominant sympathetic activation), low levels of circulating adiponectin have been demonstrated

(56). Adiponectin acts in the hypothalamic paraventricular nucleus to coordinate neuroendocrine and autonomic functions (57). Parasympathetic input to adipose tissue has been demonstrated by Kreier et al (58), illustrating that adipose tissue does receive dual autonomic control. This is important in the regulation of cytokine release, as well as in the control of release of FFA and the development of oxidative/nitrosative stress. In our study we showed decreased adiponectin/leptin ratios in both newly diagnosed and established diabetes and a significant correlation between these ratios and abnormalities in the ANS function (see preliminary data) (5). There appears to be a closed-loop system, wherein the adipose tissue mass regulates the hypothalamic autonomic system, with both the sympathetic and parasympathetic nervous system impacting the metabolic and inflammatory potential of adipose tissue.

Significance of the proposal: Reduced HRV, the hallmark of autonomic dysfunction, has been shown to have consequences in terms of morbidity (e.g. progression of coronary atherosclerosis) and mortality (59), independent of traditional cardiovascular risk factors in various populations, including those with pre-diabetes and diabetes (60,61). With a growing understanding of bi-directional interactions between the sympathetic and parasympathetic efferent pathways at different levels of the neuro-axis and at target organs involved in inflammation (62), it is possible that this neuroinflammatory pathway may be a key component involved in both the etiology and the clinical course of cardiovascular disease (4). The question arises as to what the mechanism could be. A number of biochemical and molecular abnormalities involved in the development of atherosclerosis, as well as multiple cardiovascular risk factors (hyperglycemia, hypertriglyceridemia, elevated FFA and hypertension) have been shown to improve with bromocriptine-QR therapy. **Actually, it is not clear whether bromocriptine's reduction on cardiovascular events is due to the drug's beneficial effect on any of these pathologic processes since the response is so rapid and of such large magnitude. Recent preclinical studies suggest that anti-inflammatory mechanisms in liver, other metabolic tissues, and the vessel wall itself as well as an effect to reduce reactive oxygen/nitrogen species generation in the vessel will contribute to the effects of bromocriptine-QR to reduce CVD outcomes (62A, 62B) Further mechanistic studies are necessary to establish the mechanism of action providing cardiovascular protective benefit of this therapy.** Trials with bromocriptine have been performed in subjects with more established diabetes. We propose to conduct an interventional study with bromocriptine-QR in subjects with newly diagnosed versus established T2DM to evaluate its effects on cardiovascular autonomic function, HPA axis hormones and inflammatory markers of disease. This will aid into further understanding of the mechanisms by which this drug improves glucose tolerance and reduces cardiovascular risk in these subjects. The possibility of reversing autonomic imbalance by early intervention may have important impact in the development of new potential therapeutic strategies to abrogate the CV complications of diabetes.

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2 STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

To demonstrate the effects of dopaminergic activation on the autonomic nervous system in subjects with. The primary endpoint is the effect of bromocriptine-QR on changes in autonomic function measured by assessing sympathetic and parasympathetic function using conventional measures of autonomic function, including power spectral analysis of heart rate as well as peripheral autonomic function using sudorimetry and laser scanning of peripheral microvascular autonomic control.

2.2 SECONDARY OBJECTIVES

To demonstrate the effects of dopaminergic activation with bromocriptine-QR on the regulation of plasma neuroendocrine factors such as the hypothalamic-pituitary-axis (HPA) hormones, and on the plasma levels of markers of inflammation and oxidative/nitrosative stress in type 2 diabetes subjects. The study will evaluate treatment effects on inflammatory markers, the leptin/adiponectin system, and hormonal levels of renin-angiotensin system (RAS), aldosterone and cortisol. Specifically, we will evaluate the following markers of inflammation and oxidative/nitrosative stress: 1) CRP, 2) IL6, 3) TNF α, 4) PAI1, SOD, TBARS and ADMA and nitrotyrosine as well as other markers of oxidative/nitrosative stress. The study will evaluate the leptin/adiponectin system by measuring total adiponectin (TA), high molecular weight (HMW) adiponectin, leptin, and their ratios (TA/leptin and HMW adiponectin/leptin). A co-secondary objective of the study will be to assess the impact of bromocriptine-QR vs Placebo on measures of insulin resistance and glycemic control (e.g., OGTT glucose and insulin, Matsuda index, HOMA-IR, HbA1c).

The treatment effects on all of the above primary and secondary outcomes will also be assessed as a function of the duration of diabetes and other baseline demographics such as HbA1c, concomitant medications and metabolic status.

3 STUDY DESIGN, DURATION AND DATES

3.1 STUDY DESIGN

This is an interventional, twenty-four week, randomized, double blind, placebo-controlled trial with bromocriptine-QR in subjects with early diabetes and established T2DM to evaluate its effects on the cardiovascular and peripheral autonomic nervous system, as well as on inflammatory markers, the leptin/adiponectin system, hormonal levels of RAS and HPA, indices of insulin resistance and glycemic control, and measures of oxidative and nitrosative stress. A total of 80 subjects are planned to be enrolled in the study. At least 20 and no more than 40 early diabetes subjects and at least 40 but no more than 60 subjects with established diabetes will be enrolled in the study and each randomized to treatment with bromocriptine-QR or placebo. Subjects will be required to be on a stable anti-diabetes regimen consisting of either diet and/or oral anti-diabetic agents consisting of

metformin alone or metformin plus an insulin secretion enhancer (sulfonylurea, DPP4 Inhibitors, or GLP-1 analogs) for at least 60 days prior to randomization. Following a 2 week lead-in period, subjects will be randomized in a 1:1 ratio to receive UDT (usual diabetes therapy) plus bromocriptine-QR or UDT plus placebo. Subjects must have a documented C-peptide level of >2 ng/ml from the screening visit. The circadian timing of the interventional therapy will be within 2 hours of waking in the morning. Following randomization subjects will be titrated to the maximum tolerated dose of the study drug over a four-week period. During these first 4 weeks, the daily dose of the study drug will be titrated up by one tablet (0.8 mg bromocriptine-QR or 1 matching placebo tablet) per day on a weekly basis until a maximal tolerated dose of at least two tablets (1.6 mg/day bromocriptine-QR or 2 placebo tablets) and no more than four tablets (3.2 mg/per day b-QR or 4 placebo tablets) is achieved. During the first 4 weeks of the study, subjects will be called weekly to monitor for possible adverse events. Subjects will be maintained at their maximum tolerated dose of between two to four tablets per day (1.6 to 3.2 mg bromocriptine-QR per day or 2 to 4 placebo tablets) for the duration of the study. Subjects unable to tolerate two tablets per day of study drug will be terminated from the study and any further analyses. Subjects will be seen at 4 weeks after randomization, at 12 weeks after randomization, and then at study end (week 24 or early termination). A primary analysis of the primary and secondary endpoints will be performed after 12 weeks with a secondary analysis conducted on these study endpoints at 24 weeks. Subjects will be contacted 30 days after stopping the study drug to record any adverse events that occurred after cessation. During the study period subjects will be advised to perform home blood glucose monitoring at least on a daily basis. Subjects will be required to continue their usual anti-diabetes regimen during the study but will be allowed to alter the dosages of these medications and/or add or subtract medications as deemed necessary by the Principal Investigator to optimize blood glucose control to avoid hypoglycemia or persistent hyperglycemia. In any case, rescue therapy for hyperglycemia will be administered if a subject has two consecutive fasting glucose measurements >270 mg/dL spaced ≤7 days apart before week 12, or two consecutive fasting glucose measurements >250 mg/dL spaced ≤7 days apart or a HbA1c >11% at or after week 12. The choice of rescue therapy will be at the discretion of the Principal Investigator and may include adjustments in the dosages of the medications that the subject is already on and/or addition of other glucose lowering therapies. The subjects with concurrent antidiabetes medication changes may remain "on study" for the remainder of the study until week 24 undergoing all evaluations if in the opinion of the investigator this does not pose any serious risk to the subject or the study objectives unless the rescue therapy requires insulin, in which case the subject will be withdrawn from the study as per the exclusion criteria for the study. Subjects will receive a complete physical and neurological exam and clinical laboratory assessments at baseline and at the completion of the study. The primary and secondary endpoints will be evaluated at baseline, week 4, week 12, and week 24 or at early termination. Subjects will be evaluated using the procedures listed below.

STUDY PROCEDURES

Fasting blood chemistries- We will obtain blood samples using standard venipuncture techniques for determination of: Fasting serum glucose, HbA1c, and C-peptide (C-peptide may be non-fasting); lipid profile including total serum cholesterol, HDL, LDL, FFA and triglycerides; comprehensive metabolic panel including creatinine, AST and ALT; TSH, renin, angiotensin, aldosterone, ACTH, cortisol, prolactin, and serotonin. Oral glucose tolerance tests and HOMA measure of insulin resistance and beta cell function will also be determined. All of these assays are performed routinely by Sentara Healthcare System. We will also collect samples to assess markers

of inflammation and oxidative/nitrosative stress which include 1) CRP, 2) IL6, 3) TNF alpha, 4) PAI1, SOD, TBARS and ADMA and nitrotyrosine as well as other measures of oxidative/nitrosative stress. We will measure total adiponectin (TA), high molecular weight (HMW) adiponectin, leptin, and their ratios (TA/leptin and HMW adiponectin/leptin). The analyses of plasma markers of inflammation, oxidative/nitrosative stress, neuroendocrine factors and the adiponectin/leptin ratio will be conducted at Vero Science LLC (Tiverton, RI).

Autonomic function tests- ANS function will be assessed by the measurement of heart rate variability (HRV), which is reduced early in the development of autonomic imbalance in diabetic subjects, and has been associated with increased risk for death after myocardial infarction. Power spectral analysis of HRV will be assessed using ANSAR (ANX 3.0 software; ANSAR Group, Inc., Philadelphia, PA). The ANSAR Parasympathetic and Sympathetic (P&S) monitoring method was developed and validated by the Harvard Medical School and Massachusetts Institute of Technology (MIT) Biomedical Engineering department (Akselrod and Aysin). The P&S monitoring method received US FDA Market Clearance in 1995. Time- and frequency-domain analyses will be performed. Time-domain analysis provides a measure of the sympathetic and parasympathetic control of the heart beat (the R-R interval on an electrocardiogram) recorded with maneuvers including deep breathing, Valsalva, and standing from the supine position while frequency-domain analysis is performed under resting conditions. Given their complementary nature both will be utilized in this study. Specifically, total spectral power (TSP) is the average low frequency plus high frequency for each phase during baseline, deep breathing, Valsalva, and postural stimulations. The sample difference of the beat to beat (NN) intervals measures heart rate variability, and was significantly impaired in both diabetic groups when compared to healthy controls in our prior study. The TSP will be calculated, as well as the standard deviation of all normal R-R intervals (sdNN), a measure of both sympathetic and parasympathetic action on HRV, and the root-mean square of the difference of successive R-R intervals (rmSSD), a measure primarily of parasympathetic activity. It is also a measure of changing heart rate variability. ***These time and frequency domain analyses will allow quantification of ANS components, including sympathetic/parasympathetic balance before and after treatment with bromocriptine-QR. We anticipate that autonomic balance, lost early in T2DM subjects, will be re-established with bromocriptine-QR therapy.***

Sudorimetry- Sweat glands have a preganglionic sympathetic innervation that is regulated by acetylcholine and neuropeptidergic activity. The functional impairment of this system can cause abnormal sweating. The Sudoscan (Impeto Medical, Paris, France) will be used to measure the impact of autonomic imbalance on the sweat gland system using reverse iontophoresis. The Sudoscan will employ sudorimetry to assess sweat gland nerve fiber function. Test-retest reliability in 112 healthy controls before and after VO2max test was excellent for the feet (Correlation coefficient of 0.8, $p<0.0001$). ***Changes in peripheral autonomic function have now been shown to correlate with indices of insulin resistance and inflammation. We will determine if abnormal peripheral autonomic function occurs in newly diagnosed vs. established diabetes and if these are modified by bromocriptine-QR.***

Skin blood flow (SkBF): methods and procedures- Since activation of the sympathetic arm of the autonomic nervous system has profound effects on microvascular function we will quantify this using laser Doppler techniques. We anticipate that impaired microvascular perfusion seen in newly diagnosed and established diabetes will be correctible with bromocriptine-QR. Continuous laser Doppler (CLD) will be used to assess skin blood flow in response to several stimuli and allows us to evaluate sympathetic versus neuropeptidergic control of neurovascular perfusion. CLD measurements are a reliable index of SkBF and are uninfluenced by blood flow in the underlying muscle (63). Several pieces of equipment in our lab will be used: the Periflux Master Unit PF4001-2, the Peritemp heating module with sensor PF4005-3, and the (Pressure Unit) (all from Perimed, Inc., Smithtown, NY). After a 30-minute acclimation period 10 minutes of baseline blood flow will be recorded. Following this ten minute period heat will be applied to stimulate WT and NOCI blood flow. WT stimulation will be achieved at 40°C and NOCI stimulation will be achieved by heating the skin to 44°C for 10 and 40 minutes respectively. Skin perfusion will also be measured during cholinergic stimulation.

Laser Doppler Skin Blood Flow Imaging- *Laser Doppler imaging (Moor Instruments Inc., Wilmington, DE) is a standard non-invasive, non-contact technique used to monitor and measure blood flow in very small blood vessels of the microvasculature. Sympathetic overactivity affects the cardiovascular system causing vasoconstriction of small, resistance vessels and we will use this technique as a measure of peripheral sympathetic activity.* Skin blood flow images will be recorded using the Moor LDI2-VR imager. A visible red laser beam will be placed 14 inches from the exposed site and it will take a few minutes to scan over each site being measured. The information collected by the laser is digitally processed into a color coded image displaying the skin blood flow at that site.

Quantitative sensory tests (QST) - Sympathetic activation can be measured by quantifying the sensory perception of heat and cold stimuli. Enhanced responses to these stimuli (hyperesthesia and hyperalgesia) reflect increases in sympathetic tone and will be used as a measure of enhanced peripheral sympathetic activity mediated by the loss of central dopaminergic tone and correctible with bromocriptine. We will use our previously published methods and algorithms for measuring small fiber somatosensory function, including cold and warm thermal sensation, and cold- and heat-induced pain thresholds at the dominant great toe, forearm, and finger in all subjects (64). Generally for each of these non-noxious sensations we will use the method of limits, 4 ascending trials with an inter-stimulus interval randomly varying from 4 to 20 seconds using the Medoc TSA 2001 / VSA 3000 (Medoc Advanced Medical, Minneapolis, MN). Threshold is calculated as the mean stimulus intensity level over all 4 responses.

3.2 STUDY DURATION AND DATES

The duration of this study is expected to be approximately 38 months, with subject recruitment proposed to start in July 2015 and end by August 2018. The actual overall study duration or subject recruitment period may vary.

4 SELECTION OF SUBJECTS

4.1 NUMBER OF SUBJECTS

A total of up to 80 subjects are planned to be enrolled; At least twenty and no greater than forty early diabetes subjects (diabetes duration of < 4 years) and at least 40 but no greater than 60 subjects with established diabetes (diabetes duration of ≥4 years) will be enrolled in the study.

4.2 INCLUSION CRITERIA

1. Diagnosis of Type 2 Diabetes (as defined by the 2004 American Diabetes Association guidelines)
2. Age 30–80 years
3. HbA1c at screening ≤ 10
4. Male or Female (female of child bearing age must use definitive contraceptive therapy)
5. Type 2 Diabetes Mellitus subjects on a stable anti-diabetes regimen of diet and/or metformin alone therapy or on metformin plus an insulin secretion enhancer (sulfonylureas, DPP4 Inhibitors, GLP-1 analogs) therapy for a 60 day period prior to randomization. Subjects must have a documented C-peptide level (either fasting or random) of > 2 ng/ml from the screening visit.

4.3 EXCLUSION CRITERIA

1. Presence of type 1 diabetes mellitus.
2. Type 2 diabetes mellitus subjects on insulin.
3. Use of prescription sympathomimetics, ergot alkaloid derivatives, or anti-migraine medications, dopamine2 (D2)-like receptor antagonists (e.g. metoclopramide, domperidone) or systemic corticosteroids
4. Uncontrolled hypertension (systolic BP >160 or diastolic BP > 100 at screening) or a history of orthostatic hypotension
5. History of significant gastroparesis
6. Presence of diabetic retinopathy that is more severe than “background” level
7. Presence of clinically significant peripheral or autonomic neuropathy that is clearly of non-diabetic origin
8. Presence of renal impairment defined by serum creatinine > 1.4 mg/dl if female taking metformin, >1.5 mg/dl. if male taking metformin, and >1.6 mg/dl if not taking metformin
9. History of major macrovascular events such as myocardial infarction or cerebrovascular event such as stroke within the past 6 months. Other exclusions include coronary artery bypass graft or coronary angioplasty in the previous 3 months, unstable angina pectoris (chest pain at rest, worsening chest pain, or admission to the ER or hospital for chest pain) within the previous 3 months, or seizure disorders.
10. Active infection (e.g., HIV, hepatitis), or a history of severe infection during the 30 days prior to screening

11. Major surgical operation during the 30 days prior to screening
12. Cancer, other than non-melanoma skin or non-metastatic prostate cancer, within the past 5 years
13. Uncontrolled or untreated hypothyroidism as evidenced by TSH concentrations >4.8 uU/ml
14. Other serious medical conditions which, in the opinion of the investigator, would compromise the subject's participation in the study, including any concurrent illness, other than diabetes mellitus, not controlled by a stable therapeutic regimen, or conditions or abnormalities (e.g., blindness) that might interfere with interpretation of safety or efficacy data, or history of non-compliance
15. Clinically significant abnormalities on screening laboratory evaluation, unless approved by the Sponsor
16. Abnormalities of liver function defined as any liver enzymes (AST, ALT, SGPT, SGOT) greater than 3 times the upper limit of normal
17. History of NYHA Class III-IV congestive heart failure.
18. Concurrent participation in another clinical trial with use of an experimental drug or device within 30 days of study entry.
19. History (within 3 years) of alcohol or substance abuse, or dementia
20. Pregnant or lactating women. Women of childbearing potential must have a negative pregnancy test at screening. Women who become pregnant will be discontinued from the study.
21. Known hypersensitivity to any of the formulation components
22. Working rotating, varying or night shifts
23. Use of unapproved herbal supplements that may be associated with a risk of cardiovascular events (such as ephedra, yohimbe etc)
24. Subjects who have started therapy with an erectile dysfunction drug within 2 weeks prior to screening; subjects may not begin treatment with an erectile dysfunction drug during the study period; subjects currently taking erectile dysfunction drugs should do so only under medical supervision.
25. Donation of blood in the previous 30 days. Blood donation is also not allowed during the study or for 30 days after completion of the study.

5 STUDY TREATMENTS

5.1 DETAILS OF STUDY TREATMENTS

Bromocriptine-QR, 0.8 mg/day, with the dose increased by 0.8 mg/day every week to a maximum of 3.2 mg/day, or as tolerated to a minimum dose of 1.6 mg/day, or matching placebo, added on to usual diabetes therapy consisting of a stable anti-diabetes regimen of diet and/or metformin alone therapy or metformin plus an insulin secretion enhancer (sulfonylureas, DPP4 Inhibitors, GLP-1 analogs) therapy for a 60 day period prior to randomization.

5.2 SUPPLIES AND ACCOUNTABILITY

Bromocriptine-QR tablets and matching placebo will be provided by the Sponsor. Accountability will be performed at the study site at the end of the study.

5.3 COMPLIANCE

Subjects will be instructed to bring their current, used and unused (vials) of study drug to every visit.

5.4 RUN-IN MEDICATION

There is no run-in medication prior to enrollment. Subjects will remain on their current regimen of diabetes therapy.

6 PRIOR AND CONCOMITANT ILLNESSES AND TREATMENTS

6.1 PRIOR AND CONCOMITANT ILLNESSES

Additional illnesses present at the time informed consent is given are regarded as concomitant illnesses and must be documented in the case report form. Relevant past illnesses must also be documented in the case report form. Illnesses first occurring or detected during the study, and worsening of a concomitant illness during the study, are to be regarded as adverse events and must be documented as such in the case report form.

6.2 PRIOR AND CONCOMITANT TREATMENTS

All treatments being taken by the subjects on entry to the study or at any time during the study in addition to the study drug are regarded as concomitant treatments and must be documented on the appropriate pages of the case report form. Concomitant medications should be kept to a minimum during the study and adhere to the inclusion/exclusion criteria described above. However, if these are considered necessary for the subject's welfare and are unlikely to interfere with the investigational products, they may be given at the discretion of the investigator and recorded in the case report form. Subjects on systemic corticosteroids will be excluded.

7 OVERVIEW OF DATA COLLECTION AND STUDY PROCEDURES

7.1 STUDY PROCEDURES AND SCHEDULES

The study will consist of a screening visit, followed by visits at week 0 (baseline), week 0 + 1-3 days for study drug initiation, week 4, week 12 and week 24 or early termination. There will be a final telephone contact 30 days after discontinuation of study drug to assess for new adverse events or for resolution of ongoing adverse events.

7.2 DESCRIPTION OF STUDY DAYS

7.2.1 Screening - Visit 0

Screening of subjects will occur at Visit 0. Potential subjects will be instructed to arrive at the screening visit having fasted from midnight the night before. Consent form must be signed prior to conduct of any study procedures including screening tests for eligibility.

- Each subject will be assigned a study subject number*
- Assessment of inclusion/exclusion criteria will be performed*
- Medical history, including demographic and background assessments will be documented in the CRF*
- Routine physical examination will be performed*
- ECG test will be performed*
- Blood will be taken for the determination of HbA1c, clinical chemistry variables (and serum pregnancy test for women of child-bearing potential), hematology, (see study schedule).
- A C-peptide level (fasting or random) will be measured and must be > 2 ng/ml for continuation in the trial.*
- Total amount of blood drawn at this visit will be approximately 30 ml.

* These procedures may be performed at the same visit the consent form is signed or at Screening Visit 0.

7.2.2 Study Days – Treatment Period

7.2.2.1 Baseline – Visit 1 (Post Visit 0 + <30 days)

Subjects will be instructed to arrive having fasted from the night before this visit.

- Sudorimetry
  - Sudoscan
- Autonomic Function Tests
  - Heart Rate Variability (HRV)

- Blood will be taken for the determination of HbA1c, lipids, neuroendocrine factors, inflammatory markers and oxidative/nitrosative stress markers

- Urine will be obtained for measurement of microalbumin

- An OGTT will be administered and OGTT glucose and insulin levels will be obtained at 0, 30, 60, 90 and 120 minutes post-glucose challenge.
  -
- Skin Blood Flow (SkBF)
  - Continuous Laser Doppler
- Quantitative Sensory Tests (QST)

- Total amount of blood drawn at this visit will be approximately 50 ml.

- Subjects will be randomized per Eastern Virginia Medical School SOP II-805:

- Subjects will be provided with instructions on starting study drug the morning after this visit.

• Sub-investigator • Research coordinator	Randomize and blind an investigational drug by assigning each patient/subject a study number in the order they are recruited. The patients are randomized using a randomizing web site at (www.randomizer.org). The randomized patient numbers are recorded in the grid in Attachment A, Blinding/Randomization Code. This code is then placed in an envelope and sealed. Place a label (Attachment B, Blinding/Randomization Individual Subject Envelope Label) on the envelope. This envelope should remain sealed until the study is concluded.
• Sub-investigator • Research coordinator	Complete a blinding/randomization patient form, (Attachment C) for each patient and seal the individual envelopes. The outside of the envelopes should contain patient numbers for identification purposes. This allows the research coordinator to break the individual blind in emergency situations without compromising the rest of the blinding data. Document all circumstances appropriately and place in the study binder. Report all serious adverse events to the FDA and IRB. More general guidelines on adverse event reporting can be found in SM-404 and II-806 of these SOPs.
• Sub-investigator • Research coordinator	The randomization code and the individual patient forms should reside in the research coordinator's office in one large envelope labeled with the Blinding/Randomization Envelope Label (Attachment D).

7.2.2.2 Call 1 (Visit 1 + 7 days)

- Study site will contact the subject by telephone to remind them to increase the dose of bromocriptine-QR to 2 tablets and assess tolerance. Subjects will be instructed to contact the study site if they experience intolerable nausea or vomiting.

- Assessment for any AE/SAE will be completed

7.2.2.3 Call 2 (Call 1 + 7 days)

- Study site will contact the subject by telephone to remind them to increase the dose of bromocriptine-QR to 3 tablets and assess tolerance. Subjects will be instructed to contact the study site if they experience intolerable nausea or vomiting.

- Assessment for any AE/SAE will be completed

7.2.2.4 Call 3 (Call 2 + 7 days)

- Study site will contact the subject by telephone to remind them to increase the dose of bromocriptine-QR to 4 tablets and assess tolerance. Subjects will be instructed to contact the study site if they experience intolerable nausea or vomiting.

- If at any time a subject is not able tolerate the next higher dose of bromocriptine-QR, they may stay at the current dose, providing it is at least 2 tablets per day.

- Assessment for any AE/SAE will be completed

- Subjects will schedule an appointment for the week 4 follow up visit on day 28 ± 3 days

7.2.2.5 Visit 2 (Visit 1 + 4 weeks [± 3 days])

Subjects will be instructed to arrive having fasted from the night before this visit and having taken their morning dose of the study drug, with time taken noted.

- Assessment for any AE/SAE will be completed

- Medical history will be taken
- Sudorimetry
    - Sudoscan

- Autonomic Function Tests
    - Heart Rate Variability (HRV)

- Blood will be taken for the determination of HbA1c, HOMA-IR (FPG and insulin), neuroendocrine factors, inflammatory markers and oxidative/nitrosative stress (see study schedule).

- Total amount of blood drawn at this visit will be approximately 25 ml.

- Skin Blood Flow (SkBF)
    - Continuous Laser Doppler

- Quantitative Sensory Tests (QST)

7.2.2.6 Phone call 4 (Visit 2+ 4 weeks)

- AE/SAE Query
- Study Drug Compliance Assessment

7.2.2.7 Visit 3 (Visit 1plus 12weeks [± 3 days])

Subjects will be instructed to arrive having fasted from the night before this visit and having taken their morning dose of the study drug with time taken noted.

- Assessment for any AE/SAE will be completed
- Medical history will be taken
- Sudorimetry
    - Sudoscan
- Autonomic Function Tests
    - Heart Rate Variability (HRV)
- Blood will be taken for the determination of HbA1c, lipids, c-peptide, neuroendocrine factors, inflammatory markers and oxidative/nitrosative stress (see study schedule).
- Total amount of blood drawn at this visit will be approximately 55-60 ml.
- Urine will be obtained for measurement of microalbumin
- An OGTT will be administered and OGTT glucose and insulin levels will be obtained at 0, 30, 60, 90 and 120 minutes post-glucose challenge.
- Skin Blood Flow (SkBF)
    - Continuous Laser Doppler
- Quantitative Sensory Tests (QST)

7.2.2.8 Phone Call 5 (Visit 3 + 4 weeks [±3 days])

- AE/SAE Query
- Study Drug Compliance Assessment

7.2.2.9 Phone Call 6 (Phone Call 5 + 4 weeks [±3 days]))

- AE/SAE Query
- Study Drug Compliance Assessment

7.2.2.10 Visit 4 (Visit 1 plus 24 weeks [± 3 days] or Early Termination)

Subjects will be instructed to arrive having fasted from the night before this visit and having taken their morning dose of the study drug with time taken noted.

- Assessment for any AE/SAE will be completed
- Medical history will be taken
- Routine physical examination will be performed
- ECG test will be performed
- Study drug will be discontinued
- Sudorimetry
    - Sudoscan
- Autonomic Function Tests
    - Heart Rate Variability (HRV)
    -
- Blood will be taken for the determination of HbA1c, lipids, clinical chemistry variables, hematology, c-peptide, inflammatory markers and oxidative/nitrosative stress (see study schedule).
- Total amount of blood drawn at this visit will be approximately 55-60 ml.
- Urine will be obtained for measurement of microalbumin

- An OGTT will be administered and OGTT glucose and insulin levels will be obtained at 0, 30, 60, 90 and 120 minutes post-glucose challenge.

- Skin Blood Flow (SkBF)
    - Continuous Laser Doppler

- Quantitative Sensory Tests (QST)

7.2.3 End of Study Follow up Phone Call (Post Visit 4 + 27-30 days)

- Sites will contact all subjects to assess for new AE/SAE since study drug was discontinued or to resolve any AE/SAE that were ongoing at final visit.

7.3 STATISTICAL METHODS

Statistical analyses will be conducted on between group (bromocriptine-QR vs. placebo) differences in change from baseline of all primary and secondary endpoints. The treatment effects on all of the primary and secondary outcomes will also be assessed as a function of the duration of diabetes and other baseline demographics such as HbA1c, concomitant medications and metabolic status. Mean differences of glycemic control, various derivative autonomic function values, and inflammatory marker levels will be compared first using standard Analysis of Variance (ANOVA), with diagnostic group entered as the predictor variable. Within-group analysis for change in primary and secondary endpoints after 12 weeks of treatment will be done using repeated measures MANOVA (multivariate analysis of variance). For glycemic control analysis patients will be stratified according to post-prandial insulin response. If data are not normally distributed, the Wilcoxon signed-rank (within group) or Mann Whitney (between group) tests may be employed or Fisher's exact test in case of small sample sizes. The level of significance will be set at $p<0.05$. Relationships and/or trends between the effect of Cycloset on neurovascular function and diabetic neuropathy will be determined with Spearman's rank correlation.

A statistical analysis plan (SAP), providing details of the analyses and presentation structure of the results, will be developed and finalized before the database is locked.

A local laboratory will be utilized for collection and analysis of the laboratory tests associated with efficacy, and safety. Standardized guidelines for preparation, collection, and centralized analysis of blood specimens will provide uniformity of the data and will avoid potential inter-laboratory variability due to inconsistent methodology.

The analyses of plasma markers of inflammation, oxidative/nitrosative stress, neuroendocrine factors, and the adiponectin/leptin ratio will be conducted at VeroScience (Tiverton, RI).

7.3.1 Primary endpoint

The primary endpoint is the effect of bromocriptine-QR on changes in autonomic function measured by assessing sympathetic and parasympathetic function using conventional measures of autonomic function, including power spectral analysis of heart rate as well as peripheral autonomic function using sudorimetry and laser scanning of peripheral microvascular autonomic control.

7.3.2 Secondary endpoint

Secondary endpoints will be the evaluation of bromocriptine-QR's effects on inflammatory markers, neuroendocrine factors, markers of oxidative/nitrosative stress, the leptin/adiponectin system, and hormonal levels of renin-angiotensin system (RAS), aldosterone and cortisol. A co-secondary endpoint will be the impact of bromocriptine-QR vs Placebo on measures of insulin resistance and glycemic control (e.g., OGTT glucose and insulin, Matsuda index, HOMA-IR, HbA1c).

7.3.3 Safety Data

Safety will be monitored throughout the trial by active and passive collection of reports of AE/SAE.

Adverse Events

AE monitoring will be conducted at all study visits as well as all titration contacts. *(Please refer to Section 8 for additional information regarding AEs.)*

8 ADVERSE EVENTS

8.1 DEFINITIONS

8.1.1 Adverse Event

The term **adverse event** covers any unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the period of observation in the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be adverse events.

Worsening of a sign or symptom of the condition under treatment will normally be measured by efficacy parameters. However, if the outcome fulfills the definition of "serious adverse event", it must be recorded as such (see *Section 8.1.2*).

The adverse event may be:

- A new illness

- Worsening of a concomitant illness
- An effect of the study medication, including comparator
- A combination of two or more of these factors

No causal relationship with the study medication or with the clinical study itself is implied by the use of the term “adverse event”.

Adverse events fall into the categories “non-serious” and “serious” (see *Section 8.1.2*).

Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an adverse event, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the condition(s) leading to these measures are not adverse events, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.

8.1.2 Serious Adverse Event

A serious adverse event is one that at any dose (including overdose):

- Results in death
- Is life-threatening[1]
- Requires in subject hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity[2]
- Is a congenital anomaly or birth defect
- Is an important medical event[3]

[1]“Life-threatening” means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

[2]“Persistent or significant disability or incapacity” means that there is a substantial disruption of a person’s ability to carry out normal life functions.

[3]Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where no outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-subject hospitalization, or the development of drug dependency or drug abuse. A diagnosis of cancer during the course of a treatment should be considered as medically important.

The List of Critical Terms (1998 adaptation of WHO Adverse Reaction Terminology Critical Terms List, provided in the "Instructions for completing the 'Serious Adverse Event/Expedited Report from a Clinical Trial' form") should be used as guidance for adverse events that may be considered serious because they are medically important.

Clarification of the difference in meaning between "severe" and "serious"
The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.1.3 Alert Terms and Other Reasons for Expedited Reporting to Pharmacovigilance

No special events are subject to reporting as alert terms in this study.

However, cases in which a "significant overdose" of the investigational product was taken and a non-serious adverse event or no adverse event occurred are to be reported to the sponsor in an expedited manner on a "Serious Adverse Event/Expedited Report from a Clinical Trial" form.

In addition, any pregnancy diagnosed in a female subject or in the female partner of a male subject during treatment with the investigational product must be reported to the sponsor immediately. Information related to the pregnancy must be given on a "Drug Exposure via Parent – Data Collection" form that will be provided by the sponsor.

8.2 PERIOD OF OBSERVATION

For the purposes of this study, the period of observation for collection of adverse events extends from the time the subject gives informed consent until 30 days (± 3 days) after the last dose of study medication was given.

If the investigator detects a serious adverse event in a study subject after the end of the period of observation, and considers the event possibly related to prior study treatment, he or she should contact the sponsor to determine how the adverse event should be documented and reported.

8.3 DOCUMENTATION AND REPORTING OF ADVERSE EVENTS BY INVESTIGATOR

All adverse events that occur during the observation period set in this protocol (see *Section 8.2*) must be documented on the pages provided in the case report form in accordance with the instructions for the completion of adverse event reports in clinical studies. These instructions are provided in the investigator's study file and in the case report form itself.

The following approach will be taken for documentation:

- **All adverse events** (whether serious or non-serious, or considered as an alert term) must be documented on the "Adverse Event" page of the case report form.
- If the adverse event is serious (see *Section 8.1.2*), the investigator must complete that section of the Adverse Event case report form.
- **This form must be completed and faxed to the sponsor's Pharmacovigilance department within 24 hours.**
- If the adverse event is listed as an alert term (see Section 8.1.3) even if the "alert term" is non-serious, the investigator must complete, in addition to the "Adverse Event" page in the case report form, a "Serious Adverse Event/Expedited Report from a Clinical Trial" form at the time the adverse event is detected.
- **This form must be completed and faxed to the sponsor's Pharmacovigilance department within 24 hours.**
- When a "significant overdose" of the investigational product occurs without an adverse event or in other situations where the sponsor requires an expedited report without an adverse event (see *Section 8.1.3*), the investigator should only complete a "Serious Adverse Event/Expedited Report from a Clinical Trial" form. Instructions on where to send this form will be provided by the sponsor. In this case, there is no need to complete the "Adverse Event" page in the case report form.

Every attempt should be made to describe the adverse event in terms of a diagnosis. If a clear diagnosis has been made, individual signs and symptoms will not be recorded unless they represent atypical or extreme manifestations of the diagnosis, in which case they should be reported as separate events. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

All subjects who have adverse events, whether considered associated with the use of the investigational products or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed up according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

All questions on the completion and supply of adverse event report forms and any further forms issued to the investigator at a later date to clarify unresolved issues should be addressed to the sponsor.

8.4 IMMEDIATE REPORTING BY INVESTIGATOR TO SPONSOR

Serious adverse events and adverse events that fulfill a reason for expedited reporting to Pharmacovigilance (alert term and/or "significant overdose", as defined in *Section 8.1.3*) must be documented on the Adverse Event form.

- **This form must be completed and faxed to the sponsor's Pharmacovigilance department within 24 hours.**

The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s).

Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up Adverse Event form.

9 WITHDRAWALS

9.1 WITHDRAWAL OF SUBJECTS

Subjects may be withdrawn from the study (i.e. from any further study medication or study procedure) for the following reasons:

- At their own request or at the request of their legally authorized representative*
- If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being
- At the specific request of the sponsor

* “Legally authorized representative” means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject’s participation in the procedure(s) involved in the research.

Subjects must be withdrawn from the investigational product under the following circumstances:

- Women who become pregnant
- Women of childbearing potential who discontinue contraception with the intention of becoming pregnant.

In all cases, the reason for and date of withdrawal must be recorded in the case report form and in the subject's medical records. The subject must be followed up to establish whether the reason was an adverse event, and, if so, this must be reported in accordance with the procedures in *Section 8*.

The investigator must make every effort to contact subjects lost to follow-up. Attempts to contact such subjects must be documented in the subject’s records (e.g., times and dates of attempted telephone contact, receipt for sending a registered letter).

Subject withdrawal due to deterioration of glycemic control

If a subject has a HbA1c of > 12% or fasting glucose readings of greater than 275 mg/dl on two consecutive days, then the subject’s regimen must be adjusted to include medications offering stricter glycemic control. As per the exclusion criteria, subjects requiring insulin must be withdrawn from the study. If the subject's HbA1c remains > 12%, he/she should cease study treatment to be stabilized on alternative medications. Subjects that are withdrawn will be referred to their primary care physician or endocrinologist for further diabetes medication adjustments as needed.

Replacement of subjects

Subjects that are withdrawn within 4 weeks following completion of study drug titration will be replaced. As far as possible, for subjects who are withdrawn from the study, all safety examinations scheduled for the final study day will be performed prior to withdrawal.

9.2 EMERGENCY SPONSOR CONTACT

In emergency situations, the investigator should contact the sponsor by telephone at the number given on the title page of the protocol.

9.3 EMERGENCY IDENTIFICATION OF INVESTIGATIONAL PRODUCTS

If during the course of the study, it becomes vital for the safety of the study subject to have her/his randomization code broken then the following Eastern Virginia Medical School SOP II-805 is to be followed.

• Sub-investigator • Research coordinator	Randomize and blind an investigational drug by assigning each patient/subject a study number in the order they are recruited. The patients are randomized using a randomizing web site at (www.randomizer.org). The randomized patient numbers are recorded in the grid in Attachment A, Blinding/Randomization Code. This code is then placed in an envelope and sealed. Place a label (Attachment B, Blinding/Randomization Individual Subject Envelope Label) on the envelope. This envelope should remain sealed until the study is concluded.
• Sub-investigator • Research coordinator	Complete a blinding/randomization patient form, (Attachment C) for each patient and seal the individual envelopes. The outside of the envelopes should contain patient numbers for identification purposes. This allows the research coordinator to break the individual blind in emergency situations without compromising the rest of the blinding data. Document all circumstances appropriately and place in the study binder. Report all serious adverse events to the FDA and IRB. More general guidelines on adverse event reporting can be found in SM-404 and II-806 of these SOPs.
• Sub-investigator • Research coordinator	The randomization code and the individual patient forms should reside in the research coordinator's office in one large envelope labeled with the Blinding/Randomization Envelope Label (Attachment D).

9.4 EMERGENCY TREATMENT

During and after a subject's participation in the trial, the investigator and/or institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial. The investigator and/or institution should inform a subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware.

9.5 ANALYSIS VARIABLES

A statistical analysis plan (SAP), providing details of the analyses and presentation structure of the results, will be developed and finalized before the database is locked.

9.6 ANALYSIS POPULATIONS

The primary analysis of the study will be the assessment of the primary and secondary study endpoints after 12 weeks of study drug treatment. The secondary analysis of the study will be the assessment of the primary and secondary study endpoints after 24 weeks of study drug treatment.

Dropouts will be replaced as described in section 9.1.

9.7 SAMPLE SIZE

We propose that an effect size of 10% mean change from baseline in treated groups represents a clinically meaningful difference, and we propose that sample size reflects the ability to detect a 10% change due to intervention factor.

We intend to use the parametric statistical procedures ANOVA and MANOVA in these analyses, based on our previous experience that the data from these tests is normally distributed in raw form or in some cases after simple log-transformations. If data are not normally distributed, the Wilcoxon signed-rank (within group) or Mann Whitney (between group) tests may be employed or Fisher's exact test in case of small sample sizes. The level of significance will be set at $p<0.05$. Relationships and/or trends between the effect of Cycloset on neurovascular function and diabetic neuropathy will be determined with Spearman's rank correlation.

Based on these considerations, we calculate a total of 80 participants will result in a power greater than 0.80 for observing statistical significance at the $p < 0.05$ level. Specifically, we plan to recruit approximately 40 in the placebo group and approximately 40 in the treatment group. In total, approximately 120 people will be screened in order to enroll approximately 80 participants in this study. JMP statistical Software version 9.3 will be used to perform all statistical analyses.

10 REGULATORY REQUIREMENTS

10.1 GOOD CLINICAL PRACTICE

This study is to be conducted according to globally accepted standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice, 1 May 1996), in agreement with the Declaration of Helsinki and in keeping with local regulations.

10.2 DELEGATION OF INVESTIGATOR DUTIES

The investigator will ensure that all persons assisting with the trial are adequately qualified, informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions.

The investigator will maintain a list of sub investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

10.3 SUBJECT INFORMATION AND INFORMED CONSENT

Before being enrolled in the clinical study, subjects must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to them.

An informed consent document that includes information about the study will be prepared and given to the subject. This document will contain all the elements required by the ICH E6 Guideline for Good Clinical Practice and any additional elements required by local regulations. The document must be in a language understandable to the subject and must specify who informed the subject. Where required by local law, the person who informs the subject must be a physician.

After reading the informed consent document, the subject must give consent in writing. The subject's consent must be confirmed at the time of consent by the personally dated signature of the subject and by the personally dated signature of the person conducting the informed consent discussions.

If the subject is unable to read, oral presentation and explanation of the written informed consent form and information to be supplied to subjects must take place in the presence of an impartial witness. Consent must be confirmed at the time of consent orally and by the personally dated signature of the subject or by a local legally recognized alternative (e.g., the subject's thumbprint or mark). The witness and the person conducting the informed consent discussions must also sign and personally date the consent document.

A copy of the signed consent document must be given to the subject. The original signed consent document will be retained by the investigator.

"Legally authorized representative" means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the research.

The investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

It is recommended that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

10.4 CONFIDENTIALITY

Subject names will not be supplied to the sponsor. Only the subject number and subject initials will be recorded in the case report form, and if the subject name appears on any other document (e.g., laboratory report), it must be obliterated on the copy of the document to be supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. The subjects will be informed that representatives of the sponsor, independent ethics committee (IEC)/ institutional review board (IRB), or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The investigator will maintain a personal subject identification list (subject numbers with the corresponding subject names) to enable records to be identified.

10.5 PROTOCOL AMENDMENTS

Neither the investigator nor the sponsor will alter this clinical study protocol without obtaining the written agreement of the other. Once the study has started, amendments should be made only in exceptional cases. The changes then become part of the clinical study protocol.

10.6 APPROVAL OF THE CLINICAL STUDY PROTOCOL AND AMENDMENTS

Before the start of the study, the clinical study protocol, informed consent document, and any other appropriate documents will be submitted to the IEC/IRB with a cover letter or a form listing the documents submitted, their dates of issue, and the site (or region or area of jurisdiction, as applicable) for which approval is sought. If applicable, the documents will also be submitted to the authorities, in accordance with local legal requirements.

Investigational products can only be supplied to the investigator after documentation on **all** ethical and legal requirements for starting the study has been received by the sponsor. This documentation must also include a list of the members of the IEC/IRB and their occupation and qualifications. If the IEC/IRB will not disclose the names of the committee members, it should be asked to issue a statement confirming that the composition of the committee is in accordance with GCP. Formal approval by the IEC/IRB should preferably mention the study title, study code, study site (or region

or area of jurisdiction, as applicable), amendment number where applicable, and any other documents reviewed. It must mention the date on which the decision was made and must be officially signed by a committee member.

Before the first subject is enrolled in the study, all ethical and legal requirements must be met.

The IEC/IRB and, if applicable, the authorities must be informed of all subsequent protocol amendments and administrative changes, in accordance with local legal requirements. Amendments must be evaluated to determine whether formal approval must be sought and whether the informed consent document should also be revised.

The investigator must keep a record of all communication with the IEC/IRB and, if applicable, between a coordinating investigator and the IEC/IRB. This also applies to any communication between the investigator (or coordinating investigator, if applicable) and the authorities.

10.7 ONGOING INFORMATION FOR INDEPENDENT ETHICS COMMITTEE/ INSTITUTIONAL REVIEW BOARD

Unless otherwise instructed by the IEC/IRB, the investigator must submit to the IEC/IRB:

- Information on serious or unexpected adverse events from the investigator's site, as soon as possible
- Expedited safety reports from the sponsor, as soon as possible
- Periodic reports on the progress of the study

10.8 CLOSURE OF THE STUDY

The study must be closed at the site on completion. Furthermore, the sponsor or the investigator has the right to close this study site at any time. As far as possible, premature closure should occur after mutual consultation. Depending on local legislation, it may be necessary to inform IEC/IRB and the regulatory authorities when the study site is closed.

Study materials must be returned, disposed of or retained as directed by the sponsor.

10.9 RECORD RETENTION

The investigator must obtain approval in writing from the sponsor before destruction of any records.

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, because of international regulatory requirements, the sponsor may request retention for a longer period.

Essential documents include:

- Signed informed consent documents for all subjects

- Subject identification code list, screening log (if applicable) and enrollment log
- Record of all communications between the investigator and the IEC/IRB
- Composition of the IEC/IRB (or other applicable statement as described in *Section 12.6*)
- Record of all communications between the investigator and sponsor (or CRO)
- List of subinvestigators and other appropriately qualified persons to whom the investigator has delegated significant trial-related duties, together with their roles in the study and their signatures
- Copies of case report forms and of documentation of corrections for all subjects
- Investigational product accountability records
- Record of any body fluids or tissue samples retained
- All other source documents (subject medical records, hospital records, laboratory records, etc.)
- All other documents as listed in section 8 of the ICH E6 Guideline for Good Clinical Practice (Essential Documents for the Conduct of a Clinical Trial)

Normally, these records will be held in the investigator's archives. If the investigator is unable to meet this obligation, he or she must ask the sponsor for permission to make alternative arrangements. Details of these arrangements should be documented.

10.10 LIABILITY AND INSURANCE

Liability and insurance provisions for this study are given in separate agreements.

10.11 FINANCIAL DISCLOSURE

Before the start of the study, the investigator will disclose to the sponsor any proprietary or financial interests he or she might hold in the investigational products or the sponsor company as outlined in the financial disclosure form provided by the sponsor. The investigator agrees to update this information in case of significant changes during the study or within one year of its completion. The investigator also agrees that, where required by law or regulation, the sponsor may submit this financial information to domestic or foreign regulatory authorities in applications for marketing authorizations.

Similar information will be provided by each sub-investigator to whom the investigator delegates significant study related responsibilities.

11 STUDY MONITORING AND AUDITING

Monitoring and auditing procedures will be followed according to study site policy in order to comply with GCP guidelines.

11.1 STUDY MONITORING AND SOURCE DATA VERIFICATION

Monitoring will be done by personal visits from a representative of the sponsor (study monitor) that will check the case report forms for completeness and clarity, and crosscheck them with source documents. In addition to the monitoring visits, frequent communications (letter, telephone, and fax), by the study monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements.

Study close-out will be performed by the study monitor upon completion of the study.

11.2 ON-SITE AUDITS

Domestic and foreign regulatory authorities, the IEC/IRB, and an auditor authorized by the sponsor may request access to all source documents, case report forms, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities. Medical records and other study documents may be copied during audit or inspection provided that subject names are obliterated on the copies to ensure confidentiality.

12 DOCUMENTATION AND USE OF STUDY FINDINGS

12.1 DOCUMENTATION OF STUDY FINDINGS

A case report form will be provided for each subject.

All protocol-required information collected during the study must be entered by the investigator, or designated representative, in the case report form. Details of case report form completion and correction will be explained to the investigator. If the investigator authorizes other persons to make entries in the case report form, the names, positions, signatures, and initials of these persons must be supplied to the sponsor.

The investigator, or designated representative, should complete the case report form pages as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

A source data location list will be prepared prior to study start. This list will be filed in both the trial master file and the investigator study file and updated as necessary.

The completed case report form must be reviewed and signed by the investigator named in the clinical study protocol or by a designated sub-investigator.

The sponsor will retain the originals of all case report forms. The investigator will retain a copy of all completed case report form pages.

12.2 USE OF STUDY FINDINGS

All information concerning the product as well as any matter concerning the operation of the sponsor, such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by the sponsor and are unpublished, are confidential and must remain the sole property of the sponsor. The investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission from the sponsor is obtained.

The sponsor has full ownership of the original case report forms completed as part of the study.

By signing the clinical study protocol, the investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. The authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

The sponsor will ensure that a final report on the study is prepared.

The investigator (or coordinating investigator) will be required to sign a statement that he or she confirms that, to the best of his or her knowledge, it accurately describes the conduct and results of the study.

All materials, documents and information supplied by the sponsor to the investigator, and all materials, documents and information prepared or developed in the course of the study to be performed under this protocol, shall be the sole and exclusive property of the sponsor. Subject to obligations of confidentiality, the investigator reserves the right to publish only the results of the work performed pursuant to this protocol, provided, however, that the investigator provides an authorized representative of the sponsor with a copy of any proposed publication for review and comment at least 45 days in advance of its submission for publication. In addition, if requested, the investigator will withhold publication an additional 90 days to allow for filing a patent application or taking such other measures as sponsor deems appropriate to establish and preserve its proprietary rights.

It is agreed that, consistent with scientific standards, publication of the results of the study shall be made only as part of a publication of the results obtained by the investigator performing the protocol.

13 DECLARATIONS OF SPONSOR AND INVESTIGATOR

13.1 DECLARATION OF SPONSOR

This clinical study protocol was subject to critical review and has been approved by the sponsor. The information it contains is consistent with:

- The current risk-benefit evaluation of the investigational product
- The moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the principles of GCP as described in the US Code of Federal Regulations, part 50, 54, 56, and 312, as well as in the ICH Guidelines, May 9, 1997.
- The investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

Sponsor Representative

Date: _____ Signature: _____

Name (block letters): _____

13.2 DECLARATION OF INVESTIGATOR

I confirm that I have read the above protocol. I understand it, and I will work according to the principles of GCP as described in 21 CFR parts 50, 54, 56, and 312 and according to applicable local requirements.

Investigator

Date: _____ Signature:_____

Name (block letters): _____