Title Page

Cardio-Protective Effect of Saxagliptin and Dapagliflozin Combination on Endothelial Progenitor Cells in Patients with Type 2 Diabetes

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PROTOCOL SYNOPSIS

Protocol Title:	Cardio-Protective Effect of Saxagliptin and Dapagliflozin Combination on Endothelial Progenitor Cells in Patients with Type 2 Diabetes
Site Numbers & Names	GWU Medical Faculty Associates
Research Hypothesis:	We hypothesize that Dapagliflozin will improve EPC number and function AND Saxagliptin in addition to Dapagliflozin (additive effect) may improve EPC number and function even more than Dapa alone, compared to placebo.
Study Schema: Drugs / Doses / Length of Treatment	We propose a 3-arm randomized, parallel group, longitudinal study of 16-week intervention duration. Patients will be randomized to 3 groups: Group A: Dapa (10 mg) + Saxa Placebo, Enroll n=15, retain n=12 Group B: Dapa (10 mg) + Saxa (5 mg), Enroll n=15, retain n=12 Group C: Dapa Placebo + Saxa Placebo, Enroll n=15, retain n=12 **Total enroll 45, retain total 36**

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Study Objectives: • **Primary** • **Secondary** • **Tertiary**	**Primary Outcome: Cellular Biomarker of Endothelium** The primary objective is to ascertain if 16 weeks of Dapa or Dapa+Saxa Combo therapy will improve CD34+ cell number, function and gene expression in type 2 diabetes with CVD. **Secondary Outcome: Arterial Stiffness and Renal Function, Non-Cellular Markers of Endothelium** The secondary objective is to determine whether use of Dapa or Dapa+Saxa Combo alters markers of endothelial function such as: arterial stiffness, blood biochemical measures pertaining to endothelial, renal function, and urine exosomes. **Tertiary Outcome: Metabolism Markers** The tertiary objective is to determine whether use of Dapa or Dapa+Saxa Combo alters body composition, fasting lipid profile, and levels of insulin, glucose, and appetite controlling hormones.
Study Design:	Prospective, double-blind, randomized placebo-controlled trial
Accrual Goal (Total number of subjects):	N = 45
Accrual Rate (Number of subjects expected per month):	Recruit 45 patients in approximately 20 months
Estimated Timeline:	FPFV: 06/01/2018 LPLV: 02/28/2020
Co-relative studies (PK/PD, etc.):	N/A

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1 BACKGROUND

INTRODUCTION

Diabetes affects more than 11% of adults in the United States and this is projected to nearly double by 2025.[1] In addition, 2 out of 3 Americans are overweight, and many diabetic patients are either overweight or obese with vascular complications.[1,2] Both diabetes and obesity are associated with endothelial dysfunction, oxidative stress, endothelial cell inflammation, cardiovascular pro-thrombotic states and kidney disease.[1,3]

While there are many anti-diabetic medications on the market, two of the most popular classes are sodium-glucose linked transporter type 2 (SGLT-2) inhibitors, and dipeptidyl peptidase-4 (DPP4) inhibitors. SGLT-2 inhibitors have shown promise in improving glycemic control, weight reduction, hypertension, arterial stiffness and even changes in circulating Renin-angiotensin-aldosterone system (RAAS) and nitric oxide (NO).[4,5,6,7] Recently Empagliflozin, a SGLT-2 inhibitor, has been shown to have positive effect on cardiovascular disease (CVD) risk modification and metabolic responses, however, the mechanism for the benefit is unclear.[8,9,10] Concerns with certain SGLT2 inhibitors has been raised on bone metabolism and again the mechanism is unclear.[11]

DPP-IV inhibitors have been in use longer and have been shown to achieve better glycemic control by lowering HbA1C, without causing hypoglycemia, and is considered weight neutral.[1] We have recently completed a study looking at effect of Saxagliptin on CD34+ cells (an Investigator Initiated Trial) and we are currently investigating into the role of Linagliptin (an Investigator Initiated Trial) towards improving CD34+ cell and endothelial function, halting progressive deterioration of renal function in a type 2 diabetes population with chronic kidney disease (CKD). In our studies we are using CD34+ endothelial progenitor cells as a cellular surrogate of future endothelium to discern improvement in endothelial function.

We, and others, have previously shown that EPCs can act as a cellular biomarker and that it is more reliable than serum based markers for CVD and even CKD risk estimation[12,13,14,15] CD34+ cells has been used extensively as a vascular regenerative tool.[16,17,18,19,20] While serum based inflammatory markers may take several months to change and give no preventive and predictable information as to whether a particular medication may improve future endothelium and reduce vascular complications of diabetes, the CD34+ response to an intervention is much faster particularly the gene expression changes which has to precede protein or inflammatory molecule changes.[3,13,21]

We, and others, have shown EPCs are impaired in number, function and gene expression in hyperglycemia and diabetes and related complications.[12,14,15,18,22,23,24] We have demonstrated that gene expression in EPCs, changes within two weeks of an intervention such as aerobic exercise and can be used as a reliable non serum based cellular bio-marker.[13,24] Also, elevated serum based biomarkers such as endothelium based inflammatory markers is a relatively poor bio-marker of endothelial function and may indicate that the endothelium is already damaged and inflamed.[3]

We recently completed a study using Saxagliptin (Saxa) and our results on CD34+ cells are promising (results shown below in Preliminary Data Section).
Dapagliflozin (Dapa), a SGLT2 inhibitor, a newer diabetes medication, can work synergistically with Saxa to augment CD34+ cell function. Literature has shown positive effect of DPP4

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inhibitors (like Saxa) on CD34+ cell migration.[25] This is likely via its role in increasing SDF1alpha bio-availability in a setting of type 2 diabetes and type 2 diabetes with complications such as diabetic chronic kidney disease.[26,25,23] We hypothesize that Saxa and Dapa can work synergistically to improve CD34+ cell and endothelial function

The EMPA-REG trial where Empagliflozin (a medication in the SGLT2 inhibitor class, similar to Dapa) was added to other anti-diabetic medications, has shown remarkable positive effect on cardiovascular outcome measures, and alludes to a mechanism beyond glucose lowering for SGLT2 inhibitors like Dapa.[7,8,9] It is likely that the mechanism could be related to the fact that Empa or Dapa may have direct positive effect on endothelium, and it is possible that Empa or Dapa can reverse endothelial dysfunction in diabetes by inhibiting glucose transporters (such as SGLTs) on CD34+ cells. This would thereby reduce intracellular excess glucose entry and prevent gluco-toxicity. It can also improve arterial stiffness, cardiovascular fluid volume along with positive effect on mature endothelium or endothelial progenitor cells.[5,6]

As mentioned before, DPP4 inhibitors improve CD34+ cell migration by increasing SDF1 alpha bio-availability (endogenous DPP4 enzyme de-activates SDF1a). Therefore Saxa and Dapa are two different diabetes medications that may improve hematopoetic stem cells in two different pathways and can be hypothesized to work synergistically to maintain healthy, functional and chemotactic factor responsive CD34+ EPCs. This should help endothelial regeneration and limit progression of vascular complications of diabetes such as CKD.[18,19,23] In a type 2 diabetes population, where oral therapy is limited to metformin, DPP4 inhibitors, SGLT2 inhibitors and combination of the latter two, added at the same time to metformin or insulin may help reverse endothelial dysfunction and renal impairment.[1,2,3]

Our rationale to use EPCs as an endothelial function bio-marker is because EPCs have been shown to mature into endothelial cells if the environment is conducive and are therefore considered the future endothelium.[16,21,24] Therefore studying EPCs will help us to understand the effect of an intervention (such as Dapa alone or Dapagliflozin + Saxagliptin combination) on the future endothelial health with good disease predictability.

Role of glucose as an energy substrate and glucose entry inside a cell (either stem or mature) is important. Primarily, glucose utilizes three transporters to enter a cell, they are: sodium-glucose transporters (SGLTs), glucose transporters (GLUTs) and taste receptors such TAS1R2/TAS1R3.[27] In type 2 diabetes there is increased intra-cellular glucose accumulation (even gluco-toxicity), with up-regulation of glucose transporters particularly SGLT transporters leading to increased intra-cellular glucose thereby upregulating pro-inflammatory molecules and accumulation of increased amounts of ROS (reactive oxygen species) intracellularly.[3,27] Increased presence of intra-cellular ROS leads to cellular inflammation and apoptosis, a process that chronically depletes the EPC population. It seems this process affects Endothelial Progenitor Cells (EPCs) rather than mature endothelium.[24] The relative lack of EPCs secondary to apoptosis, will lead to a scenario where damaged and inflamed mature endothelium is not replaced or replenished.

PRELIMINARY RESULTS

Regarding Saxagliptin effect on CD34+ cells, serum biochemistry and arterial stiffness, our recently concluded results showed: Saxa significantly increased migration (using SDF1α 100ng/ml, $p=0.04$) with a trend of higher CFU count ($p=0.07$). There was an significant ($p<0.001$) increase in mean %CD34+ cells, Flow on MNCs showed more cells with double-

positive CD34 & CXCR4 (p=0.008), and CD31 & CXCR4 (p=0.036). [CXCR4 is a SDF1a receptor]. mRNA gene expression of CD34+ cells showed decreased inflammatory markers (Endothelin-1, p=<0.0001), and a similar declining trend in apoptosis marker, p53 (p=0.026). For AS (arterial stiffness), Pulse Wave Analysis (PWA) increased after visit 1 in the placebo arm unlike Saxa. Pulse Wave Augmentation Index @HR75 (A1x75), and radial Systolic BP declined (A1x75, p=0.037 and SBP,p=0.06) with Saxa vs placebo. Our conclusions from the study were: when Saxa was added to diabetes subjects with no known CVD, we noted improved number and migratory function of EPCs with increased SDF1a receptor, CXCR4, positivity. Gene expression analysis showed a reduced inflammatory and apoptotic state with improved AS. Our findings indicate how Saxa may help improve endothelial cell dysfunction, when added to Metformin, in early stages of diabetes, before macro-vascular complications appear.

Our results and literature indicates that the stem-cell "homing process" is based on the ability of DPP4 inhibitors to inhibit the degradation of SDF-1 and of its receptor CXCR-4, a pathway of importance for progenitor cell recruitment to ischemic tissue (myocardial or otherwise)

As mentioned earlier, we noted that CD34+ cells show mRNA expression of both SGLT1 and SGLT2 receptors with SGLT1 expression being higher than SGLT2. The expression of SGLT2 mRNA increased once the CD34+ cells were exposed to hyperglycemia (20mM glucose). Similarly, SGLT2 mRNA expression was higher in CD34+ cells from diabetic subjects versus non diabetic subjects. Increased expression of SGLT2 in presence of hyperglycemia would allow more glucose to enter the endothelial progenitor cells, which may lead to intra-cellular gluco-toxicity, which subsequently will lead to increased ROS formation, inflammation and cellular apoptosis.[24]

Interestingly, cultured commercially obtained mature human endothelium cells (derived from human umbilical vein, HUVEC) do not show any SGLT2 mRNA expression, even when the mature endothelial cells were exposed to high glucose. The explanation could be SGLT2 mRNA expression decreases as the cell transitions from endothelial progenitor stage cell (marked by CD34+) to a mature endothelium cell such as HUVEC, (marked by CD31).

The presence of SGLT2 transporter expression in progenitor cells such as CD34+ (as per our preliminary data) and not in mature HUVECs makes CD34+ cells even more interesting as a cellular bio-marker entity, more so than mature endothelial cells in a context of SGLT2 mRNA expression and relevance of SGLT2 inhibitor.

Use of Dapa, a SGLT2 inhibitor, will lead to reduced intracellular glucose accumulation in diabetes, which will prevent CD34+ endothelial progenitors undergo less cellular apoptosis and function better. Prevention of intracellular glucotoxicity in non CD34+ stem cells such as Mesenchymal Stem Cells has been shown to prevent intracellular ROS (reactive oxygen species) formation and accumulation, which may lead to less cellular inflammation.[28] Secondary to a similar mechanism, use of SGLT2 inhibitor such as Dapa could lead to increased number of CD34+ cells (due to less apoptosis and improved cell survival) and better colony formation

(colony formation unit, CFU). Dapa may also improve *non cellular parameters* of endothelial function such as arterial stiffness and biochemistry.[5,6,7,8,9] We and others have demonstrated that arterial stiffness predicts endothelial function and has high degree of correlation with biophysical parameters and plasma biochemistry in type 2 diabetes subjects.[29,30]

We believe that Dapagliflozin, by improving EPC number and function, will have positive effect on endothelium throughout the body, including vasculature in kidneys, and possibly reverse endothelial dysfunction in diabetes. We know that, Dapagliflozin, in comparison to others in its class, has preferential activity on SGLT2 transporters over SGLT1.[5,6] If the positive endothelial function effect of this class of medication is mediated via SGLT2 transporters that are present on CD34+ cells, then the SGLT2 transporter inhibitor specificity increases the importance of studying CD34+ cells in diabetic subjects exposed to Dapagliflozin.

As mentioned before, we just finished a study using Saxagliptin in middle aged diabetic subjects and we are currently studying the role of Linagliptin, a dipeptidyl peptidase-4 (DPP4) inhibitor, on CD34+ cell number and function (IIS-Study) in a diabetic-CKD population. DPP4 is an enzyme that degrades not only GLP1 and GIP but also degrades a prime ischemic tissue chemotactic factor such as SDF1 alpha. The stem-cell "homing process" is based on the ability of DPP4 inhibitors to inhibit the degradation of SDF-1 and of its receptor CXCR-4, a pathway of importance for progenitor cell recruitment to ischemic tissue (myocardial or otherwise).[25,26] Our study confirms that Saxagliptin will improve stem cell function particularly migration and "homing-in" of EPCs to damaged endothelial lining in type 2 diabetes subjects.

As mentioned before the presumed pathways of how Saxagliptin and Dapagliflozin would impact CD34 + cells are different. The former should improve migration and the latter should improve the progenitor stem cell itself by preventing increased intracellular glucose accumulation.

Both Dapa and Saxa are glucose lowering medications and glucose lowering per se can also improve EPC health as shown before.[12,14,24,31] Clinically, this is best studied in a context of type 1 diabetes, where hyperglycemia is the principal agent that can influence cellular apoptosis.[34] In these quoted studies, the glucose lowering effect that showed discernable EPC improvement was in the range of 3-4% of HbA1C.[31] This is way more than what is afforded by Dapagliflozin, or Saxa or combination, which is a reduction of 0.8-1.2% in HbA1C or approximately 30mg/dl of glucose.[1,6]

In our laboratory, in-vitro, we have shown EPC health to be affect when glucose levels increase to 360mg/dl from 100mg/dl with some cellular deleterious effect noted at 200mg/dl which is more pronounced at 360mg/dl.[24]

We believe Dapagliflozin's positive effect on progenitor cells may be more than simply a reduction in serum glucose values, similar to the effect seen by exercise intervention effect on CD34+ cells from prediabetes subjects in our human in-vivo experiments.[13]

Also, while recent findings from EMPA-REG study has confirmed the positive effect of Empagliflozin on cardiovascular outcomes, the mechanism behind the findings is unclear.[8,9,10] Improved CD34+ cell function may the mechanism or contributory factor. Along with Dapa we want to investigate if there is added endothelial function improvement on addition of Saxagliptin. As we propose to study a cohort similar to EMPA-REG study, we plan to recruit subjects with known CVD.

Though some studies on combination of DPP4 and SGLT2 inhibitor do exist primarily to show HbA1C reduction its role on endothelium or endothelial progenitors is unknown.[33,32] In this proposal we are planning to study effect of Dapagliflozin on CD34+ cell function, particularly migration and colony aggregation/formation via its SGLT transporter inhibitor effect which is independent of EPC migration effect mediated through SDF1 alpha and its receptors (relevant for Saxagliptin) on its own and in combination with Saxagliptin.

SGLT2 inhibitors are associated Euglycemic ketosis and also associated with weight loss. Exact mechanism for this weight loss is still unclear. We want to investigate if the weight loss, if there is any at all, is associated with ketosis or with hyperglycemia. If weight loss is seen in the Group A and Group B, which has SGLT2 inhibitors, and no weight loss seen in placebo group we can gather data for preliminary correlation and opens up basis for further investigation on this topic. We will do a blood test for β-Hydroxybytyrate to determine if there is any elevation of blood ketones and correlate that with any changes in weight.

Bone fracture is another reported side effect for SGLT2 inhibitors under investigation. SGLT2 inhibitors increase serum phosphate by promoting renal tubular phosphate absorption[42], and elevated phosphate increases PTH. Both PTH and FGF23 promote phosphaturia by decreasing renal tubular reabsorption of phosphate[43]. In contrast, the two hormones exert opposite effects upon 1α-hydroxylation of 25-hydroxyvitamin D – with PTH increasing and FGF23 decreasing 1,25-dihydroxyvitamin D formation[43]. Sustained increases in PTH enhance bone resorption, and increase the risk of bone fractures. Similarly, increased levels of FGF23 have been associated with bone disease.[8] Finally, decreased levels of 1,25-dihydroxyvitamin D may decrease absorption of Ca^{+2} from the GI tract, and impair bone calcification[43]. Thus increase in FGF23 might cause bone disease. We will get levels of Ca, Phosphate, PTH values to investigate the link. This mechanistic understanding will allow us to identify patients who are at risk and deduce treatment approach to minimize the risk.

2 RESEARCH HYPOTHESIS

We hypothesize that Dapagliflozin will improve EPC number and function AND Saxagliptin in addition to Dapagliflozin may have an additive effect to improve EPC number and function even more than Dapa alone, compared to placebo.

In this proposal we plan to conduct a placebo matched study with type 2 diabetes subjects on any doses of metformin, Insulin, Sulfonylureas (including other insulin secretagogue like

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meglitinides) or any combination above and has no history of DPP4 inhibitor, incretin mimetic or SGLT2 inhibitor intake history. Subjects will have known macrovascular complications (such as CVD, CVA, and PVD).

3 STUDY OBJECTIVES

PRIMARY OBJECTIVE: CELLULAR BIOMARKER OF ENDOTHELIUM

The primary objective is to ascertain if 16 weeks of Dapa or Dapa+Saxa Combo therapy will improve :
CD34+ cell number,
CD34+ migratory function and
CD34+ gene expression in type 2 diabetes with CVD.

Please see appendix A for the primary objective laboratory protocol.

SECONDARY OBJECTIVE: ARTERIAL STIFFNESS AND RENAL FUNCTION, NON-CELLULAR MARKERS OF ENDOTHELIUM

To determine whether use of Dapa or Dapa+Saxa Combo alters markers of endothelial function such as: arterial stiffness measures (via tonometry), biochemical measures derived from plasma, pertaining to endothelial function (hs-CRP, IL-6, TNF-alpha), renal function such as proteinuria (microalbumin/creatinine ratio) and urine exosome study to determine podocyte health. The secondary measures are indirect measures of endothelial inflammation in early type 2 diabetes patients.[27,33,34]

Effect on Arterial Stiffness:

I. We will acquire Pulse Wave Analysis and Vascular Flow using SphygmoCor CP system from ATCOR as a measure of central arterial pressure and arterial stiffness. [34]
II. Vessel health will be assessed by degree of arterial stiffness, using arterial tonometry.
III. The central and the aortic pressure is assessed by pulse wave analysis (PWA) and pulse wave velocity (PWV).

Effect on Blood Biochemistry:

While we believe cell based biomarkers are superior to traditional serum and plasma biomarkers, we believe the outcome report will be stronger if one can show positive co-relation between the two outcome measures. We therefore will be looking at:

I. Inflammation, apoptosis and anti-oxidant protein levels: Highly selective C-reactive protein (hs-CRP), IL-6, TNF-alpha.
II. Plasma SDF1 alpha (ELISA) and GLP-1 and Ghrelin (ELISA) will be estimated to assess endothelial health and factors that may influence CD34+ cell chemotaxis
III. Podocyte health via urine exosome analysis. Please see Appendix B for protocol.
IV. The glomerular filtration rate (GFR) will be estimated by MDRD equation.
    a. GFR = 141 X min (Scr/κ,1)α X max(Scr/κ,1)-1.209 X 0.993Age X 1.018 [if female] X 1.159 [if African American]; where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.[32]

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TERTIARY OBJECTIVE: METABOLISM MARKERS

The tertiary objective is to determine whether use of Dapa or Dapa+Saxa Combo alters body composition, fasting lipid profile, and levels of insulin, glucose, and appetite controlling hormones.

Effect on Blood Biochemistry:

While we believe cell based biomarkers are superior to traditional serum and plasma biomarkers, we believe the outcome report will be stronger if one can show positive co-relation between the two outcome measures. We therefore will be looking at:

Effect of Dapa and Dapa+Saxa Combo on Serum Biochemistry:

While we believe cell based biomarkers are superior to traditional serum biomarkers, we believe the outcome report will be stronger if one can show positive co-relation between the two outcome measures. We therefore will be looking at:

I. Fasting glucose, and insulin.
    a. Glycemic control will be evaluated by measuring fasting blood glucose, insulin levels and HbA1c. Fasting blood glucose, insulin and lipid profile will be used to assess insulin resistance.[28,31]
II. Lipid profile
III. Appetite controlling hormones via LabCorp: Leptin, Adiponectin
IV. Appetite controlling hormones, via ELISA: GLP1, Ghrelin
V. Blood Ketones, via Acetone and β-Hydroxybutyrate
VI. Calcium, Phosphate, PTH.

Effect of Dapa and Dapa+Saxa Combo on Body Habitus (Determination of body composition and visceral fat)

As we plan to study cardio-metabolic effect of Dapa and Dapa+Saxa Combo, we believe the body composition data is useful.

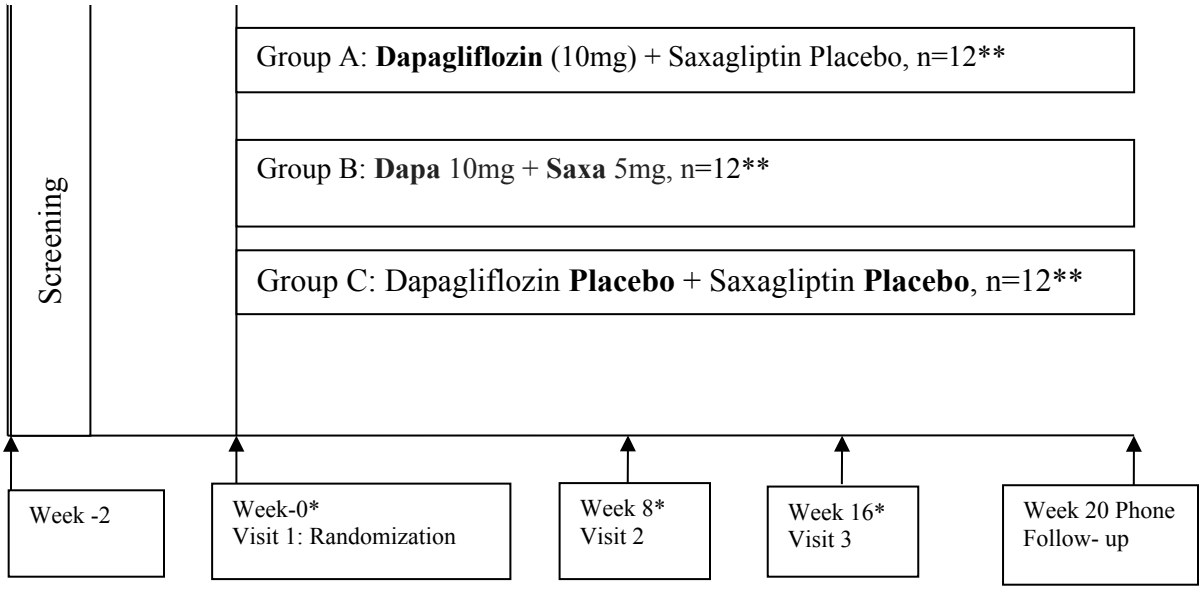
I. Using body composition scale:
    a. Height and weight will be measured and the body mass index (BMI=kgm2) used as an indicator of relative weight.
    b. The body composition scale calculates body fat%, total body water%, fat free mass, etc., in addition to BMI.

The secondary outcome markers (arterial stiffness and renal outcome measures) and tertiary outcome markers (serum biochemistry) are crucial in order to corroborate our cellular findings with currently accepted [21] clinical efficacy outcome measures such as arterial stiffness [29] and serum biochemistry. This design is similar to our recently published manuscript on Saxagliptin and cellular outcome measures [41].

4 INVESTIGATIONAL PLAN

STUDY DESIGN AND DURATION

Enroll n=45 (15/group) for evaluable n=36 (12/group)



+/- 6 day window for visits
*Assessed at week 0, 8 and 16: Primary, Secondary & Tertiary Outcomes.

Week 20: A telephone call to subjects will be made 4 weeks after last dose of study medication to determine if there have been any adverse events.

5 STUDY POPULATION

Inclusion Criteria:

1. Able to understand the study, and provide a signed & dated informed consent.
2. Diagnosis of Type 2 diabetes mellitus using criteria of the American Diabetes Association.
3. 30-70 years old.
4. HbA1C 7 to 10%, both inclusive
5. BMI of 25 - 39.9 kg/m^2 both inclusive.
6. Taking a stable dose (for 12 weeks) of Metformin (any dosage), Insulin (any dosage), sulfanylureas (any dose) or meglinitide (any dose) for the treatment of T2DM
7. Patients with current Cardiovascular Disease (CVD) in tye 2 diabetes patients, defined by ≥ 1 of the following:
    a. MI >2 months prior
    b. Multivessel CAD
    c. Angina (intermittent or chronic)
    d. Single vessel CAD with positive stress test or UA hospitalization in prior year
    e. UA >2 months prior and evidence of CAD
    f. Stroke >2 months prior
    g. Occlusive PAD
    h. Protenuria of more than 30mg/dl

Exclusion Criteria:

Past Medical History

1. Planned CV surgery or angioplasty in 1 month
2. Prior surgery with chronic malabsorption (eg, bariatric) in prior 1 year
3. Diagnosis of Type 1 diabetes mellitus
4. History of GAD antibody positive status
5. Uncontrolled Inflammatory Disease/Inflammatory drug use. ***Evaluated by PI on case-by-case basis***
6. Recent history of diabetic keto-acidosis in the past 3months, or recurrent history of diabetic ketoacidosis (≥ 3 times)
7. Active bladder cancer
8. Active wounds (e.g. Diabetic ulcers) or recent surgery within 1 month
9. Untreated hyper/hypothyroidism
10. Women of child bearing potential who are not willing to use a contraceptive method to avoid pregnancy for the 16 weeks of study duration
11. Women who are pregnant or breastfeeding
12. Implanted devices (eg. Pacemaker) that may interact with Tanita scale
13. Any other clinical condition that would jeopardize patients safety while participating in this clinical trial

Concomitant Medications

14. Taking any other oral anti-diabetic agent other than Metformin, Insulin, Sulfanylureas and Meglinitide for their treatment of T2DM.

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15. Beginning statin medications in the past 1 month or change in statin dose in the past 1 month
16. Use of consistent long-term steroid medication (oral, inhaled, injected) within the last 1 month
17. Treatment with a strong cytochrome P450 3A4 (CYP34A) or P-gp inducer (ie. Rifampin)
18. Subjects with a history of any serious hypersensitivity reaction to Dapagliflozin / Saxagliptin or another SGLT-2 inhibitor/ DPP4 inhibitor

Laboratory Findings

19. Uncontrolled hyperglycemia, defined as a fasting glucose >240 mg/dL (>13.3 mmol/L) at screening.
20. Liver disease with ALT, AST or ALP x3 ULN
21. eGFR < 60 mL/min/1.73 m^2 by MDRD equation in the past 3 months
22. Clinically significant RBC disorders such as hemoglobinopathies
23. Serum creatinine levels ≥1.8 mg/dL with estimated eGFP < 60 mL/min
24. Triglycerides > 450 mg/dL
25. Baseline Hematuria (judged by a urinalysis dipstick at screening)

Social History

26. Active smokers
27. Chronic or persistent alcohol or drug abuse
28. Prisoners or subjects who are involuntarily incarcerated
29. Subjects who are compulsorily detained for treatment of either a psychiatric or physical (eg. infectious disease) illness
30. Participation in another trial with an investigational drug within 30 days prior to informed consent

6 TREATMENT

STUDY TREATMENT: DAPAGLIFLOZIN AND DAPAGLIFLOZIN + SAXAGLIPTIN COMBO

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

This study is being conducted under FDA mandated guideline for use in type 2 diabetes. Though in this particular study Dapaglilfozin and Saxagliptin are referred to as the investigational drug, they are not novel drugs and we should not need a separate IND for execution of this study.

Definition of Non-Investigational Product: Other medications used in the study as support or escape medication for preventative, diagnostic, or therapeutic reasons as components of a given standard of care. In this protocol, the non-investigational products are Metformin, Insulin, Sulfonylurea and Meglitinides. Patients will continue on their anti-diabetic therapy per their study entry dose. Doses may be altered if laboratory results indicate this is necessary.

METHOD OF ASSIGNING SUBJECTS TO A TREATMENT

Subjects, as per selection criteria below will be randomized to treatments using a permuted block design, developed by the Epidemiology & Biostatistics Research Core. This approach ensures groups will be approximately balanced at any time during the study and at study completion. Randomization will be performed by the MFA IDS Pharmacy staff who never come in contact with subjects.

The recommended dose of Dapagliflozin is 10 mg PO will be given once daily in the Dapa group. The recommended dose of Saxagliptin 5mg along with Dapagliflozin 10 mg (one of each) will be given PO once daily in the Dapa+Saxa Combo group. Saxagliptin should be administered once daily with or without food, at any time of day. Dapa should be administered once daily with food, **in the morning**.
*Because of this, all subjects will be instructed to take their medication **once daily in the morning with food**, as we will not know which subjects will be in the Dapa, Dapa+Saxa Combo, or placebo group.*

DRUG DISPENSATION PLAN

In order to maintain the blind, all subjects, regardless of which study group they are assigned to, will be taking a total of 2 pills orally each morning. Each pill will come from a separate bottle. Subjects in their respective groups will take the following each morning:

- **Dapagliflozin Group:** 1 active Dapagliflozin pill (Bottle A) ***AND*** 1 blinded Saxagliptin placebo pill (Bottle B)
- **Dapagliflozin + Saxagliptin Combo Group:** 1 active Dapagliflozin pill (Bottle A) ***AND*** 1 active Saxagliptin pill (Bottle B)
- **Placebo Group:** 1 blinded Dapagliflozin placebo pill (Bottle A) ***AND*** 1 blinded Saxagliptin placebo pill (Bottle B)

In order to help subjects remain compliant, and remember which bottle the took a pill from, they will be instructed to always take from the Dapagliflozin (or Dapa placebo) bottle, Bottle A, first. Both bottles will be labeled with some feature to clearly distinguish the two (ie. Colored labels, highlighting on the bottles, etc.)

DOSE MODIFICATIONS

No dose modification is necessary for prior to CKD stage 3 (eGFR ≥ 60 mL/min/1.73 m²). Treatment should not be initiated with an eGFR <60 mL/min/1.73 m². If the eGFR declines persistently between 30 to <60 mL/min/1.73 m² then treatment should be halted.

STUDY MATERIALS

AstraZeneca (AZ) will provide the site with Dapagliflozin, Saxagliptin, a Dapagliflozin matching placebo, and a Saxagliptin matching placebo at no cost for this study. The placebo will be indistinguishable from its corresponding active drug, and will only be unblinded only to the site's research pharmacy.

MAINTAINING THE BLIND

Blinding is critical to the integrity of this clinical study. However, in the event of a medical emergency or pregnancy in a subject, in which knowledge of the investigational product is critical to the subject's management, the blind for that subject may be broken.
Before breaking the blind of an individual subject's treatment, the investigator should have determined that the information is necessary, i.e., that it will alter the subject's immediate management. In many cases, particularly when the emergency is not investigational product-related, the problem may be properly managed by assuming that the subject is receiving active product without the need for un-blinding.

END OF STUDY UN-BLINDING PROTOCOL
Upon completion of the research study, we will undergo the following protocol in order to unblind the research data.

Unblinding is the process by which the allocation code is broken so that the investigator, clinical staff and the trial statistician becomes aware of which intervention each subject enrolled in the research study was taking.
Unblinding at the end of the study is required in order to make unmasked analysis in accordance with the study analysis plan. It is also conducted in order to inform the participants of which investigational product they were assigned to.

Time to unblind:
Unblinding shall be conducted when all subjects enrolled in the research study have finished treatment, and all follow up visits. There must be no plan to recruit any more subjects in the research study. Additionally, all data points and outcome measures for each research subject must have been collected, and ideally compiled. Prior to unblinding there will be a data lock on clinical outcome measures and basic side outcome measures and associated research data collected for the study.

Procedure to unblind
Once data (from both the clinical and the basic science side) and has been compiled and is data locked, the investigator can choose to unblind. The Principal Investigator must contact the study sponsor, and receive permission to un-blind. If, for unforeseen reasons, at the pre-determined date for full study unblinding the data analysis on the cellular or basic aspects of the study is lagging behind the clinical data outcome measures (though the data has been acquired) the Principal Investigator in consultation with the study sponsor may choose to un-blind the clinical outcome measures before the basic side data has been analyzed but compiled. Upon confirmation from the study sponsor, the principle investigator must make a written request to the designated party to unblind, hereto referred at the "unblinder". The unblinder is the bio-statistician of the MFA, **Dr. Richard Amdur**. Upon receipt of an instruction to unblind, the unblinder will sign the request form, indicating their agreement to unblind. This form will then be taken to the pharmacy, **MFA's IDS**, where a member of the IDS staff will take the form, and will give the unblinding study binder to the unblinder. The pharmacy will sign to indicate their release of the binder, and the unblinder will sign to indicate receipt of the binder. At this point in time the chain of custody of the pharmacy unblended binder has been transferred to the designated party to unblind. The form with all of the signatures will be provided to the study coordinator to be kept in the regulatory binder.

DISCONTINUATION OF SUBJECTS FROM TREATMENT

Subjects **MUST** discontinue from the investigational product (and non-investigational product at the discretion of the investigator) for any of the following reasons:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Any clinical adverse event, laboratory abnormality, or inter-current illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject.
- Pregnancy
    - Pregnant patients will be advised to contact the investigator or study staff immediately if they suspect they might be pregnant (e.g. missed or late menstrual period) at any time during study participation.
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical illness (e.g. infectious disease).
- If ketoacidosis is suspected, promptly discontinue treatment.

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

7 ADVERSE EVENTS: DEFINITION + REPORTING

The Principal Investigator is responsible for ensuring that all staff involved in the study are familiar with the content of this section.

Adverse Event (AE)

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

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

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

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

Laboratory Test Abnormalities as AEs:

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

The following laboratory abnormalities should be documented and reported appropriately:

- Any laboratory abnormality that required the subject to have study drug discontinued or interrupted
- Any laboratory abnormality that required the subject to receive specific corrective therapy.

Serious Adverse Event (SAE)

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

- Results in death
- Is immediately life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above. Examples of such events include but are not limited to intensive treatment in an emergency department or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization. Potential drug induced liver injury (DILI) us also considered an important medical event (see Section 7.6 for the definition of potential DILI)
- Suspected transmission of an infectious agent (e.g., any organism, virus or infectious particle, pathogenic or non-pathogenic) via the study drug is an SAE.
- Pregnancy, only where there is a suspicion that the investigational medicinal product may have interfered with the effectiveness of the contraceptive medication.
- Congenital abnormalities/ birth defects and spontaneous miscarriages.

NOTE: The following hospitalizations are **not** considered SAEs:

- A visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event)
- Elective surgery planned before signing consent
- admissions as per protocol for a planned medical/surgical procedure

- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

Pregnancy

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

If any pregnancy occurs in the course of the study, then the Investigator or other site personnel should inform the appropriate AstraZeneca representatives within 1 day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

Following initial AstraZeneca notification, the PI will ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site within 1 to 5 calendar days for SAEs and within 30 days all non SAE pregnancies.

The same timelines apply when outcome information is available.

Potential Drug-Induced Livery Injury

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

Potential drug induced liver injury is defined as

1. AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)
   AND
2. Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),
   AND
3. No other immediately apparent possible causes of AT elevation and hyper-bilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these

procedures are required by the protocol, should also be recorded as a AEs or SAEs (provided they meet the criteria for SAEs), as appropriate, and reported accordingly.

Adverse Event Collecting and Reporting

The collection of adverse event (AEs / SAEs) information will begin after the signing of the informed consent. Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. To prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events. AEs will be followed to completion of the study, if considered not related, or until resolution or stabilization if considered related. They will be reported as SAEs if they become serious.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: **onset, duration, intensity, seriousness, investigator causality to investigational product (yes or no), action taken, treatment required, and outcome.** If treatment for the event was administered, it should be recorded in the medical record. The investigator will report these to the IRB upon continuing review. The investigator must supply AstraZeneca and the IRB with any additional information requested.

Serious Adverse Event Collecting and Reporting

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, will be collected, including those thought to be associated with protocol-specified procedures. All SAEs will be collected that occur within 30 days of discontinuation of dosing or within 30 days of the last visit for screen failures. If applicable, SAEs will be collected that relate to any later protocol-specified procedure (e.g., a follow-up skin biopsy). The investigator will report any SAE occurring after these time periods that is believed to be related to study drug or protocol-specified procedure at 90 days and 180 days period. An SAE report will be completed for any event where doubt exists regarding its status of seriousness.

The following variables (if applicable) are recommended to be collected for SAEs: date AE met criteria for SAE, date PI became aware of SAE, seriousness criteria fulfilled, date of hospitalization, date of discharge, probable cause of death, date of death, whether an autopsy was performed, causality assessment in relation to study procedures, causality assessment in relation to other study medication, and description of SAE.

If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship will be specified in the narrative section of the SAE Report Form. All SAEs, whether related or unrelated to Dapa or Saxagliptin, and all pregnancies will be reported to AZ (by the investigator or designee) **within 24 hours**.

The investigator must inform the FDA, via a MedWatch form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32 , **and will concurrently forward all such reports to AstraZeneca.** A copy of the MedWatch report must be emailed to AstraZeneca (TCS vendor) at the time the event is reported to the FDA. It is

the **responsibility of the Investigator** to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

NOTE TO THE MEDWATCH REPORT AUTHOR:
A ***cover page*** should accompany the ***MedWatch*** form indicating the following:

- "Notification from an Investigator Sponsored Study"
- The investigator's name and address
- The trial name/title and AstraZeneca ISS reference number (ESR 17-13131)

**** Investigator (sponsor)*** must also indicate, either in the SAE report or the cover page, the ***causality*** of events ***in relation to all study medications*** and if the SAE is ***related to disease progression***, as determined by the principal investigator.

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

SAE Email Address: AEMailboxClinicalTrialTCS@astrazeneca.com
SAE Fax Number: 1-302-886-4114

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca and the FDA.

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

MedWatch SAE forms should be sent to the FDA at:

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

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

DSMB

There will be no DSMB for this study.

Dissemination of Safety Information from Astra Zeneca to Institution/Sponsor Investigator

Astra Zeneca agrees to provide to the Sponsor Investigator SUSAR reports for the Study Product as they become available until all subjects in the Protocol have completed their last Study visit according to the Protocol (i.e. Last Subject Last Visit has occurred).

8 STUDY SCHEMATIC: TIME AND EVENTS

X CHART
See Appendix C

RESEARCH DESIGN AND METHODS
Recruitment:
A data search will be requested from MFA IT to generate a list of potentially eligible patients, and research staff will assess if patients meets basic eligibility requirements. Potentially eligible subjects may also be referred from the local clinics or providers. Phone numbers, (and potentially) home addresses will be obtained, in order to contact subjects about the research study, and see if they would be interested in participating. This contact will done by calling subjects with an IRB approved phone recruitment script. When calling the subject for recruitment, a voicemail will not be left. When speaking to the subject over the phone a two-step verification process will be utilized, with subject's full name and date of birth to validate the person who picked up the phone is in fact the intended subject. Alternatively, subjects will be approached by the research staff in the MFA clinics, during a regularly scheduled visit with their provider. Any subjects who are interested will be asked a series of pre-screening questions either in person or over the phone to see if it suitable to schedule a screening visit.

Alternative methods (all IRB Approved) for recruitment include: Study recruitment flyers posted in the MFA buildings, and in local clinics in the DMV area. Newspapers Ad will be placed in the Washington Post. The study recruitment flyer may be given to potentially eligible patients during their visit. Electronic bulletin board advertisement in the MFA clinics.

We will attempt to recruit patients from across all genders, races and ethnicities as much as possible to minimize these factors affecting results.

Study Design Overview:
We propose a 3-arm randomized, double-blind, placebo-controlled, parallel group, longitudinal study of 16-weeks duration. (See study design below), recruited over 20 months.
Patients will be randomized to one of 3 groups:

Group A: **Dapa (10 mg)** + matched placebo for Saxa, Enroll n=15, retain n=10
Group B: **Dapa (10) + Saxa (5mg),** Enroll n=15, retain n=10
Group C: matched **placebo** for Dapa + matched **placebo** for Saxa, Enroll n=15, retain n=10

This proposed study is based on patients with type 2 diabetes and VD, who are taking metformin and/or insulin for anti-diabetic treatment (See section 5 for full inclusion/exclusion criteria).

Though our study is over 16 weeks, we do not anticipate significant drop in HbA1C in between the three groups in this relatively short time period to act as a confounding factor on CD34+ cell number, function and gene expression.

Subjects will be taking 10 mg of Dapa, Dapa+Saxa combo (10 mg Dapa and 5 mg Saxa), or placebo. Dose titration of metformin, will not be allowed. Dose titration of insulin will be allowed to avoid hypoglycemia while maintaining HbA1C under 10%, preferably below 7% as that is the treatment guideline as per ADA. If the subject is only on metformin, Insulin will be started if the patient's glycemic control warrants it, as it can be titrated while the patient is on the study medications.

Visits 1, 2, and 3 with outcome measurements will be scheduled for the morning while overnight fasting (except water) to reduce diurnal variability, and clinical parameters will be obtained at scheduled office visits as depicted in the study schema and will be measured at each outcome visit as follows:

Before Week -2 (Pre-screening): Subjects will be pre-screened in person or over the phone to determine initial eligibility. If the pre-screening indicates possible eligibility, then the subjects will come to an in-person screening visit when next available.

Week -2 (In-person screening): Subjects will be screened and eligibility will be verified based on physical and laboratory tests. Equal numbers of male and female patients will be recruited.

Week 0 (Visit 1): At this stage patients will be randomized into the study. Subjects will have the first set of measurements. We will make sure that patients still meet the inclusion criteria. They will be started on one of the three study arms.

Weeks 0, 8 and 16 (Visits 1-3): Subjects will all have the same measurements and will continue on study medication or placebo. All primary and secondary measures as outlined before will be carried out.

Outcomes (For full outcomes please see section 2)

Primary Outcome: Cellular Biomarker of Endothelium

The primary objective is to ascertain if 16 weeks of Dapa or Dapa+Saxa Combo therapy will improve CD34+ cell number, function and gene expression in type 2 diabetes with CVD.

Secondary Outcome: Arterial Stiffness and Renal Function, Non-Cellular Markers of Endothelium

The secondary objective is to determine whether use of Dapa or Dapa+Saxa Combo alters markers of endothelial function such as: arterial stiffness, blood biochemical measures pertaining to endothelial, renal function, and urine exosomes.

Tertiary Outcome: Metabolism Markers

The tertiary objective is to determine whether use of Dapa or Dapa+Saxa Combo alters body composition, fasting lipid profile, and levels of insulin, glucose, and appetite controlling hormones.

PRIVACY PROTECTION

All data collected for the research study will be collected under a random subject ID.

- All samples being sent to LabCorp for standard of care labs will be labeled with the subject ID, DOB, sex, Date of visit, time of collection, and Visit number. These samples will not be stored as they will be sent down to LabCorp immediately.
- All biological samples (blood + urine) being used for ELISA and CD34+ analysis will be labeled with the subject ID, Date of visit, and Visit number. No additional data will be provided with the samples. These samples be stored at either the MFA or GW, and will be analyzed The George Washington University Ross Hall in Dr. Sen's Laboratory

All de-identified study data will be stored electronically on RedCap. No PHI will be logged. All other patient files, including ICFs will be physically stored as patients files locked in the Department of Medicine in a member of the research staff's office. The only individuals who will have access to the study data will be the members of the research staff team, as indicated in the GW IRB Form "Research Team Personnel Form (HRP-201)".

9 STATISTICAL & DATA ANALYSIS

STATISTICAL ANALYSIS

Sandri et al. (2005, Study A, patients with peripheral arterial occlusive disease) found that in the control group, CD34+ cells increased from 372 cells per mL blood at baseline (SD 156) to 402 (SD 183) at 4 weeks.[35] In their exercise training group, the increase was from 458 (SD 252) at baseline to 2977 (SD 852) at 4-weeks. We created a simulated data set with these parameters in order to calculate the percent of CD34+ cell variation that was explained by the group x time interaction. We tested two random effects mixed models, one without and the other with, a group x time interaction (group is treatment vs control; time is pre vs. 4-weeks post). The percent variance explained = (Vno-int – Vint)/Vno-int, where Vno-int is the residual covariance parameter estimate without a group x time interaction term in the model, and Vint is the residual covariance parameter estimate with a group x time interaction term in the model. We found that based on the baseline and 4-week mean and standard deviation of CD34+ cell concentrations reported by Sandri et al., the interaction explained 84.5% of the variance in CD34+ cell concentration (a very large effect size).[35] The correlation among repeated measures was r=.62. Using these parameters in G-Power3 (version 3.1.3), with 2 groups measured at 2 time points, in order to achieve power >.95 would require a total sample size of 6 subjects (3 per group) each measured at 2 time points. If the group x time interaction explained only 50% of the variance in CD34+ cell count (still a large effect), we would need sample size of 8 (4 per group) in order to achieve power >.95, if only two groups are present.

In another study, Sen et al., in a sample of patients with pre-diabetes (n=11), CD34+ cell number increased from 0.8 (SEM 0.1) before exercise, to 1.4 (SEM 0.2) after exercise, a pre-post effect size (Cohen's d) of 1.81.[13]

Taking multiple study observation factors, in order to detect an effect of this size using a 2-tailed paired t-test, with alpha=.05, for power >.95, the number of subjects required would be 7-8, per group.[36,37,38]

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However, these two quoted studies are different from our proposed study on patients with diabetes. It is well known that diabetes can affect CD34+ number and function, therefore in our proposed study the number of CD34+ cells available from the subjects may be a limiting factor. *Therefore, we propose to enroll 45 and retain 36 patients (10 subjects per group), in order to safely determine the effect of two individual groups of Dapa alone and Dapa+Saxagliptin combination with adequate number of CD34+ cells available for all proposed cellular outcome measures.*

Power calculation based on Sandri et al emphasizes CD34+ cell number and our recent study which looked at one of the medications being tested in this current combination protocol [41] takes into consideration CD34+ cell function and gene expression which verified our current statistical calculation. Our study actually demonstrated the validity of CD34 function and gene expression measures which were statistically significant.

However, this current study protocol describes a different cohort than Dore/Domingues et al [41] and similar to cohort described in Sandri et al (that is subjects with CVD). Currently there is no published study on progenitor cells looking at SGLT-2 inhibitor or in combination with a DPP4 inhibitor that we can base our calculations on and the closest will be our published study. [41].

DATA ANALYSIS

We will use generalized estimating equations (GEE) with robust standard error estimates to model within-subject and between-group differences over time. First we will investigate whether an unstructured correlation matrix is best fitting for the error term based on examination of the working correlation matrix output. If an exchangeable structure fits the data, we will consider using a random-effects mixed model with restricted maximum likelihood, due to its weaker assumption for missing data on the response variable. Using either approach, of primary interest will be the group (drug treatment) by time interaction, which will tell us whether the change in the dependent variable over time differs between groups. We will include as covariates any pre-treatment variables (e.g., demographics, baseline levels of the dependent variable, comorbidities, laboratory test values) that differ between groups even at a trend level of significance ($p<.10$).

10 APPENDICES

APPENDIX A: PRIMARY OUTCOME LABORATORY PROTOCOL

Protocol for obtaining CD34+ cells: We will obtain a total of approximately 95 mL of peripheral blood per visit. Of these 95 mL, 60-70 mL will be used to obtain CD34+ cells from mononuclear cell (MNC) population and 25-35 mL for biochemistry and plasma ELISA assays. No samples will be stored for future research. MNC will be obtained from whole blood similar to protocols described before.

MNCs will be put through CD34 magnetic bead column to obtain CD34+ cells (Miltenyi Biotec). Purity of CD34+ cells, post sort, usually is above 90%, to be verified by FACS analysis.

A. FACS Analysis, 1 million cells in each tube/assay column. 12 tubes-- total 12 million. (All labelling antibodies are obtained from Miltenyi Biotec except Sytox blue-Invitrogen and anti-VEGEFR2/KDR-R&D systems)

1. Unlabelled
2. CD34
3. CD309 (VEGFR2-KDR)
4. CD184 (CXCR4), receptor substrate for SDF1 alpha
5. CD31 (PECAM-1, a mature circulating endothelial cell marker)
6. CD133 (a progenitor marker)
7. CD144 (Vascular Endothelial cadherin)
8. Sytox Blue OR Propidium Iodide staining (to detect apoptotic cells)
9. Combinations of 2+3+4+8, to assay percentage of double or triple positive cells
10. (3+4+5+8), to assay percentage of double or triple positive cells
11. 500,000 CD34+ cells, post column will be stained with – PE labelled + Sytox Blue (as a quality control measure, post column)

B. From Mononuclear (MNC) population (pre CD34+ magnetic column sorting): MNC cells (prior to CD34 magnetic bead sorting) will be also used to assay CFU Hill Colony forming unit (Stem Cell Technologies- Cat#05900) media for Colony Forming unit (CFU) Assay. CFU will be counted on 5-7 days post plating (from initial plating).

C. From CD 34+ cell population (Post Sorting):
   a. Migration Assay using SDF1Alpha concentrations of 0, 10, 100ng/ml and VEGF-A concentrations of 20 and 50 ng/ml, using 150,000 cells in 300ul serum free cell suspension media per insert.
   b. Gene expression studies. Genes to be assessed on sorted CD34 positive cells:
      i. Endothelial lineage cell surface markers: CD34, VEGFR2 (KDR), CD31, CD144
      ii. For Anti-oxidant gene expression: Superoxide dismutase (SOD) 1, 2 and 3, catalase, glutathione- peroxidase.
      iii. Apoptosis pathway: p53, p21, Bcl2, caspase-3
      iv. Endothelial Function Assay Gene: endothelial Nitric Oxide Synthase, von-Willebrand's Factor
      v. Genes associated with progenitor cell chemotaxis: VEGF-A, SDF1 alpha, CXCR4.

APPENDIX B:
URINE EXOSOME LABORATORY PROTOCOL

Purpose: **Exosomes** are cell-derived vesicles that are present in many and perhaps all biological fluids, including blood, urine, and cultured medium of cell cultures. The reported diameter of **exosomes** is between 30 and 100 nm, which is larger than LDL, but much smaller than for example, red blood cells. Exosomes are either released from the cell when multivesicular bodies fuse with the plasma membrane or they are released directly from the plasma membrane.[39] Evidence is accumulating that exosomes have specialized functions and play a key role in, for example, coagulation, intercellular signaling, and waste management.[40] Consequently, there is a growing interest in the clinical applications of exosomes. Exosomes can potentially be used for prognosis, therapy, and biomarkers for health and disease. In this study we are particularly interested in exosomes released from podocytes as a marker of podocyte function and podocyte inflammation. Podocytes are often considered a modified endothelium and one of its more commonly used parameter is microalbuminuria.
In this study we are using urine exosomes as an added parameter of kidney function other than microalbuminuria and GFR calculation.

The procedure for this study is as follows:
10-50 mL of the fresh spot urine is placed into a urine cup containing 1 tab of cOmplete Ultra - protease inhibitor cocktail (Roche) for exosome analysis.
The specimen can be refrigerated at 4ºC for ~ 1 week, frozen at -80C, or processed immediately. The urine will be processed for exosomes as follows:

The supernatant may be subjected to ultracentrifugation, with final centrifugation at 200,000g for 2 hr at 4ºC to obtain the urinary exosome pellet. The pellet would then be tested for exosomes markers, namely CD63, CD9, CD81, Hsp70, and WT-1, nephrin and podocalyxin, using one of two methods. Preferably mRNA will be extracted and then RT-PCR will be run. Alternative analysis will be done using gel electrophoresis followed by Western blot techniques.

Method 1: Urine exosome mRNA extraction
mRNA extraction from exosomes will be accomplished by using commercial kits (Norgen Biotek Corp., Catalog # 47200). Thereafter, a pre-amplification step will take place, this is a necessary step to measure mRNA in exosomes and will be followed by quantification of specific endothelium and podocyte proteins such as CD9, WT-1, nephrin and podocalyxin by RT-PCR.

Total urinary protein content, albumin and creatinine will be measured via Lapcorp for the same urine samples that have been collected.

Method 2: Gel electrophoresis and Western blot
Exosome pellet will be dissolved in Laemli buffer and proteins will be resolved by gel electrophoresis (4-12% polyacrylamide gel) under denaturing conditions. Subsequently, proteins will be transferred to a PVDF membrane by Western blotting using a dry blotting method (iBlot). The blot will be incubated with blocking solution for 1h, and subsequently exposed to a solution with the first antibody overnight, at 4ºC. After washing (5 x 5 min), the membrane will be

exposed to a secondary antibody (horseradish peroxidase [HRP]-conjugated) for 1h at room temperature. Finally, the membrane will be incubated with an HRP substrate reagent to reveal the proteins bound to the antibodies tested. Quantitative analysis will be performed by densitometry readings of the positive signals corrected to creatinine concentration of the urine aliquot equivalent to each exosome sample.

End-point: Exploratory but in conjunction with urine albumin/creatinine ratio will help to establish impact of Dapa and Dapa+Saxa Combo on renal function in diabetes related CKD population.

APPENDIX C: X CHART

	Phone Screening (Before week -2)	Screening Visit (Week -2)	Baseline/ Randomization Visit 1 (Week 0)	During Treatment Visit 2 (Week 8)	End-of-Treatment Visit 3 (Week 16)	Follow up phone call (Week 20)
Informed Consent		X				
Inclusion / Exclusion Criteria	X	X				
Medical History		X				
Physical Examination		X				
Targeted Physical Examination (as needed)			X	X	X	
Vital Signs		X	X	X	X	
Assessment of Signs and Symptoms		X	X	X	X	
Adverse Events Assessment			X	X	X	X
Laboratory Tests (Biochemical + Ketones)		Screening	X	X	X	
Laboratory Tests (Bone Parameters)			X		X	
Urine Pregnancy Test (As needed)		X	X	X	X	
Spot Urine Sample			X	X	X	
Waist/Hip Measurements			X	X	X	
Body Composition Scale			X	X	X	
Primary Outcome Measures			X	X	X	
Secondary & Tertiary Outcome Measures			X	X	X	
Randomize			X			
Dispense Study Treatment			X	X		

INVESTIGATIONAL PRODUCTS AND OTHER TREATMENTS

Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Dapagliflozin	Green, plain, diamond shaped, film coated 10 mg tablet (orally).	AstraZeneca
Matching placebo for dapagliflozin	Green, plain, diamond shaped, film coated tablet (orally). Does not contain active ingredient	AstraZeneca
Saxagliptin	Yellow, plain, biconvex, round, film coated 5 mg tablet (orally).	AstraZeneca
Matching placebo for saxagliptin	Yellow, plain, biconvex, round, film coated tablet (orally). Does not contain active ingredient	AstraZeneca

Primary packaging of the Investigational Medicinal product (IMP) will be carried out by AstraZeneca or their designee in accordance with Good Manufacturing Practice (GMP). Unlabeled bottles (identical) containing 35 tablets of each IMP will be provided by AstraZeneca. The tablets contain lactose, which may cause discomfort in lactose-intolerant individuals. Label, storage and distribution are a sponsor's responsibility.

Preparation and labelling of Investigational Medicinal Product

The sponsor or their designee (will issue labels, label the bottles and release package study drug/IMP) will label the IMP in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines for labelling. (The labels will fulfill GMP Annex 13 requirements for labelling.)

Storage
All study drugs should be kept in a secure place under appropriate storage conditions. The IMP label on the bottle specifies the appropriate storage.

11 REFERENCES

[1] American Diabetes Association, "Standards of medical care in diabetes--2014," Diabetes Care, vol. 37 Suppl 1, pp. S14–80, Jan. 2014.

[2] M. Afkarian, M. C. Sachs, B. Kestenbaum, I. B. Hirsch, K. R. Tuttle, J. Himmelfarb, and I. H. de Boer, "Kidney disease and increased mortality risk in type 2 diabetes," J. Am. Soc. Nephrol. JASN, vol. 24, no. 2, pp. 302–308, Feb. 2013.

[3] C. Rask-Madsen and G. L. King, "Mechanisms of Disease: endothelial dysfunction in insulin resistance and diabetes," Nat. Clin. Pract. Endocrinol. Metab., vol. 3, no. 1, pp. 46–56, Jan. 2007.

[4] R. C. Stanton, "Sodium glucose transport 2 (SGLT2) inhibition decreases glomerular hyperfiltration: is there a role for SGLT2 inhibitors in diabetic kidney disease?," Circulation, vol. 129, no. 5, pp. 542–544, Feb. 2014.

[5] D. Z. Cherney, B. A. Perkins, N. Soleymanlou, R. Har, N. Fagan, O. E. Johansen, H.-J. Woerle, M. von Eynatten, and U. C. Broedl, "The effect of empagliflozin on arterial stiffness and heart rate variability in subjects with uncomplicated type 1 diabetes mellitus," Cardiovasc. Diabetol., vol. 13, p. 28, 2014.

[6] M. A. Nauck, "Update on developments with SGLT2 inhibitors in the management of type 2 diabetes," Drug Des. Devel. Ther., vol. 8, pp. 1335–1380, 2014.

[7] I. Tikkanen, K. Narko, C. Zeller, A. Green, A. Salsali, U. C. Broedl, H. J. Woerle, and EMPA-REG BP Investigators, "Empagliflozin reduces blood pressure in patients with type 2 diabetes and hypertension," Diabetes Care, vol. 38, no. 3, pp. 420–428, Mar. 2015.

[8] J. Rosenstock, A. Jelaska, G. Frappin, A. Salsali, G. Kim, H. J. Woerle, U. C. Broedl, and EMPA-REG MDI Trial Investigators, "Improved glucose control with weight loss, lower insulin doses, and no increased hypoglycemia with empagliflozin added to titrated multiple daily injections of insulin in obese inadequately controlled type 2 diabetes," Diabetes Care, vol. 37, no. 7, pp. 1815–1823, Jul. 2014.

[9] A. H. Barnett, A. Mithal, J. Manassie, R. Jones, H. Rattunde, H. J. Woerle, U. C. Broedl, and EMPA-REG RENAL trial investigators, "Efficacy and safety of empagliflozin added to existing antidiabetes treatment in patients with type 2 diabetes and chronic kidney disease: a randomised, double-blind, placebo-controlled trial," Lancet Diabetes Endocrinol., vol. 2, no. 5, pp. 369–384, May 2014.

[10] E. Ferrannini, E. Muscelli, S. Frascerra, S. Baldi, A. Mari, T. Heise, U. C. Broedl, and H.-J. Woerle, "Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients," J. Clin. Invest., vol. 124, no. 2, pp. 499–508, Feb. 2014.

[11] S. I. Taylor, J. E. Blau, and K. I. Rother, "Possible adverse effects of SGLT2 inhibitors on bone," Lancet Diabetes Endocrinol., vol. 3, no. 1, pp. 8–10, Jan. 2015.

[12] N. Werner, S. Kosiol, T. Schiegl, P. Ahlers, K. Walenta, A. Link, M. Böhm, and G. Nickenig, "Circulating endothelial progenitor cells and cardiovascular outcomes," N. Engl. J. Med., vol. 353, no. 10, pp. 999–1007, Sep. 2005.

[13] **Sabyasachi Sen**, Sarah Witkowski, Ann Lagoy, Ashequl M. Islam: A six-week home exercise program improves endothelial function and CD34+ circulating progenitor cells in patients with pre-diabetes. J Endocrinol Metab.2015; 5 (1-2):163-171.

[14] J. M. Hill, G. Zalos, J. P. J. Halcox, W. H. Schenke, M. A. Waclawiw, A. A. Quyyumi, and T. Finkel, "Circulating endothelial progenitor cells, vascular function, and cardiovascular risk," N. Engl. J. Med., vol. 348, no. 7, pp. 593–600, Feb. 2003.

[15] G. Krenning, P. Y. W. Dankers, J. W. Drouven, F. Waanders, C. F. M. Franssen, M. J. A. van Luyn, M. C. Harmsen, and E. R. Popa, "Endothelial progenitor cell dysfunction in patients with progressive chronic kidney disease," Am. J. Physiol. Renal Physiol., vol. 296, no. 6, pp. F1314–1322, Jun. 2009.

[16] D. W. Losordo, R. A. Schatz, C. J. White, J. E. Udelson, V. Veereshwarayya, M. Durgin, K. K. Poh, R. Weinstein, M. Kearney, M. Chaudhry, A. Burg, L. Eaton, L. Heyd, T. Thorne, L. Shturman, P. Hoffmeister, K. Story, V. Zak, D. Dowling, J. H. Traverse, R. E. Olson, J. Flanagan, D. Sodano, T. Murayama, A. Kawamoto, K. F. Kusano, J. Wollins, F. Welt, P. Shah, P. Soukas, T. Asahara, and T. D. Henry, "Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial," Circulation, vol. 115, no. 25, pp. 3165–3172, Jun. 2007.

[17] E. J. Marrotte, D.-D. Chen, J. S. Hakim, and A. F. Chen, "Manganese superoxide dismutase expression in endothelial progenitor cells accelerates wound healing in diabetic mice," J. Clin. Invest., vol. 120, no. 12, pp. 4207–4219, Dec. 2010.

[18] O. M. Tepper, R. D. Galiano, J. M. Capla, C. Kalka, P. J. Gagne, G. R. Jacobowitz, J. P. Levine, and G. C. Gurtner, "Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures," Circulation, vol. 106, no. 22, pp. 2781–2786, Nov. 2002.

[19] N. Werner, S. Wassmann, P. Ahlers, S. Kosiol, and G. Nickenig, "Circulating CD31+/annexin V+ apoptotic microparticles correlate with coronary endothelial function in patients with coronary artery disease," Arterioscler. Thromb. Vasc. Biol., vol. 26, no. 1, pp. 112–116, Jan. 2006.

[20] **S. Sen**, J. Merchan, J. Dean, M. Ii, M. Gavin, M. Silver, T. Tkebuchava, Y. Yoon, J. E. J. Rasko, and R. Aikawa, "Autologous transplantation of endothelial progenitor cells genetically modified by adeno-associated viral vector delivering insulin-like growth factor-1 gene after myocardial infarction," Hum. Gene Ther., vol. 21, no. 10, pp. 1327–1334, Oct. 2010.

[21] Domingues CC*, **Kundu N***, Dore F J and **Sen S**. Genetic Modification of Stem Cells in Diabetes and Obesity. Agricultural and Biological Sciences. Genetic Engineering - An Insight into the Strategies and Applications, book edited by Farrukh Jamal., 2016 .

[22] I. Jialal, S. Devaraj, U. Singh, and B. A. Huet, "Decreased number and impaired functionality of endothelial progenitor cells in subjects with metabolic syndrome: implications for increased cardiovascular risk," Atherosclerosis, vol. 211, no. 1, pp. 297–302, Jul. 2010.

[23] K. E. Jie, M. A. Zaikova, M. W. T. Bergevoet, P. E. Westerweel, M. Rastmanesh, P. J. Blankestijn, W. H. Boer, B. Braam, and M. C. Verhaar, "Progenitor cells and vascular function

are impaired in patients with chronic kidney disease," Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc., vol. 25, no. 6, pp. 1875–1882, Jun. 2010.

[24] Nabanita Kundu, Cleyton Domingues, Cyril Chou, Neeki Ahmadi, Sara Houston, Joseph Jerry, Sabyasachi Sen: Use of p53-silenced Endothelial Progenitor Cells (EPCs) to treat ischemia in diabetic peripheral vascular disease. J Am Heart Assoc. 2017 Apr 1;6(4). pii: e005146. doi: 10.1161/JAHA.116.005146.

[25] Fadini GP, Boscaro E, Albiero M, et al: The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1alpha. Diabetes Care 2010; 33:1607-1609).

[26] De Falco E, Porcelli D, Torella AR, et al: SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. Blood 2004; 104:3472-3482.

[27] Malin SK, Gerber R, Chipkin SR, Braun B. Independent and combined effects of exercise training and metformin on insulin sensitivity in individuals with prediabetes. Diabetes Care. 2012 Jan;35(1):131-6. Epub 2011 Oct 31.

[28] Cabou C, Campistron G, Marsollier N, Leloup C, Cruciani-Guglielmacci C, Pénicaud L, Drucker DJ, Magnan C, Burcelin R. Brain glucagon-like peptide-1 regulates arterial blood flow, heart rate, and insulin sensitivity. Diabetes. 2008 Oct;57(10):2577-87.

[29] Anamil M. Khiyami, Fiona J. Dore, Aytan Mammadova, Richard Amdur, Sabyasachi Sen., The Correlation of Arterial Stiffness with Biophysical Parameters and Blood Biochemistry, Metab Syndr Relat Disord. 2017 May;15(4):178-182. doi: 10.1089/met.2016.0136. Epub 2017 Feb 22.

[30] R. P. Wadwa, E. M. Urbina, A. M. Anderson, R. F. Hamman, L. M. Dolan, B. L. Rodriguez, S. R. Daniels, D. Dabelea, and SEARCH Study Group, "Measures of arterial stiffness in youth with type 1 and type 2 diabetes: the SEARCH for diabetes in youth study," Diabetes Care, vol. 33, no. 4, pp. 881–886, Apr. 2010.

[31] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-419.

1. [32] **Sen S**, Cleyton Dominguez, Carol Rouphael, Cyril Chou, Chul Kim, Nagendra Yadava: Genetic modification of human mesenchymal stem cells helps to reduce adiposity and improve glucose tolerance in an obese diabetic mouse model: Stem Cell Res Ther. 2015 Dec 9;6:242.35.

.

[33] Sumner AE, Sen S, Ricks M, Frempong B, Sebring N, Kushner H: Determining the waist circumference in African Americans which best predicts insulin resistance. Obesity (Nature Publications). 2008; 16(4):841-845.

[34] Wadwa RP, Urbina EM, Anderson AM, Hamman RF, Dolan LM, Rodriguez BL, Daniels SR, Dabelea D; SEARCH Study Group. Measures of arterial stiffness in youth with type 1 and type 2 diabetes: the SEARCH for diabetes in youth study. Diabetes Care. 2010 Apr;33(4):881-6. Epub 2010 Jan 12.

[35] M. Sandri, V. Adams, S. Gielen, A. Linke, K. Lenk, N. Kränkel, D. Lenz, S. Erbs, D. Scheinert, F. W. Mohr, G. Schuler, and R. Hambrecht, "Effects of exercise and ischemia on mobilization and functional activation of blood-derived progenitor cells in patients with ischemic syndromes: results of 3 randomized studies," Circulation, vol. 111, no. 25, pp. 3391–3399, Jun. 2005.

[36] L. Frison and S. J. Pocock, "Repeated measures in clinical trials: analysis using mean summary statistics and its implications for design," Stat. Med., vol. 11, no. 13, pp. 1685–1704, Sep. 1992.

[37] G. Fitzmaurice, N. Laird, and J. Ware, "Applied Longitudinal Analysis," West Sussex, England: John Wiley & Sons, 2004, pp. 401–424.

[38] J. Singer and J. Willett, Applied Longitudinal Data Analysis. New York: Oxford University Press, 2003.

[39] Zhou H, Yuen PS, Pisitkun T, Gonzales PA, Yasuda H, Dear JW, Gross P, Knepper MA, Star RA: Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney international 2006, 69(8):1471-1476.

[40] Fernandez-Llama P, Khositseth S, Gonzales PA, Star RA, Pisitkun T, Knepper MA: Tamm-Horsfall protein and urinary exosome isolation. Kidney international 2010, 77(8):736-742.

41. The synergistic effects of saxagliptin and metformin on CD34+ endothelial progenitor cells in early type 2 diabetes patients: a randomized clinical trial. Fiona J. Dore†, Cleyton C. Domingues†, Neeki Ahmadi, Nabanita Kundu, Yana Kropotova, Sara Houston, Carol Rouphael, Aytan Mammadova, Linda Witkin, Anamil Khiyami, Richard L. Amdur and **Sabyasachi Sen**. *Cardiovascular Diabetology* 2018, **17**:65.

42. Skeith MD, Healey LA, Cutler RE. Effect of phloridzin on uric acid excretion in man. Amer J Physiol. 1970;219:1080–1082

42. Quarles LD. Skeletal secretion of FGF-23 regulates phosphate and vitamin D metabolism. Nat Rev Endocrinol. 2012;8:276–286