PROTOCOL TITLE:

Include the full protocol title.

Response: **An Investigation into the Effects of Dapagliflozin on Ketogenesis In Type 1 Diabetes**

PRINCIPAL INVESTIGATOR:

Response:

Principal Investigator: **Paresh Dandona, MBBS, PhD**
Co- Investigators: **Husam Ghanim, PhD**
Antoine Makdissi, MD
Ajay Chaudhuri, MD
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VERSION:

Include the version date or number.

Response: 09/03/2020, V4

Complete Research Protocol (HRP-503)

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Template Instructions

Sections that do not apply:

- *In several sections, the addition of checkboxes for **Not Applicable** have been added to the template as responses.*
    - *If an N/A checkbox is present, select the appropriate justification from the list.*
    - *If an N/A checkbox is not present, or if none of the existing checkboxes apply to your study, you must write in your own justification.*
- *In addition:*
    - *For research where the only study procedures are records/chart review: Sections 19, 20, 22, 23, 24, 25, 31, and 32 do not apply.*
    - *For exempt research: Sections 31 and 32 do not apply.*

Studies with multiple participant groups:

- *If this study involves multiple participant groups (e.g. parents and children), provide information in applicable sections for each participant group. Clearly label responses when they differ. For example:*

  Response:

  Intervention Group:

  Control Group:

Formatting:

- *Do not remove template instructions or section headings when they do not apply to your study.*

If you are pasting information from other documents using the "Merge Formatting" Paste option will maintain the formatting of the response boxes.

Amendments:

- *When making modifications or revisions to this and other documents, use the **Track Changes** function in Microsoft Word.*
- *Update the version date or number **on Page 3.***

PROTOCOL TITLE:

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GRANT APPLICABILITY:

Indicate whether this protocol is funded by a grant (e.g. NIH, foundation grant). For a grant with multiple aims, indicate which aims are covered by this research proposal.

NOTE: This question does not apply to studies funded by a sponsor contract.

Include a copy of the grant proposal with your submission.

Response: Astra Zeneca

RESEARCH REPOSITORY:

Indicate where the research files will be kept, including when the study has been closed. The repository should include, at minimum, copies of IRB correspondence (approval, determination letters) as well as signed consent documents. This documentation should be maintained for 3 years after the study has been closed.

Response: Diabetes Endocrinology Research Center of WNY

Address:1000 Youngs Road, Suite 105, Williamsville NY 14221

Department: Diabetes Endocrinology

1.0 Objectives

1.1 Describe the purpose, specific aims, or objectives of this research.

Response:

Hypothesis 1: Addition of dapagliflozin results in increased levels of ketone bodies in basal and insulinopenic conditions in Type 1 diabetes (TID) compared to addition of exenatide/Exenatide extended release.

Aim 1.1: To compare levels of ketone bodies (beta-hydroxybutyrate and acetoacetate) in plasma and urine in basal state and in insulinopenic state following single doses treatments before and after 12 weeks treatment with dapagliflozin, exenatide/Exenatide extended release, a combination of both drugs or placebo in T1D.

Aim 1.2: To evaluate levels of FFA, glycerol, diglycerides and hormone sensitive lipase (HSL) in plasma in basal state and in insulinopenic state following single doses treatments before and after 12 weeks treatment with dapagliflozin, exenatide/Exenatide extended release, a combination of both drugs or placebo in T1D.

Aim 1.3: To measure and compare electrolytes, serum bicarbonate, cortisol and catecholamines in basal state and in insulinopenic state following single doses treatments before and after 12 weeks treatment with dapagliflozin, exenatide/Exenatide extended release, a combination of both drugs or placebo in T1D.

Hypothesis 2: Addition of dapagliflozin induces hyperglucagonemia in basal and insulinopenic conditions in TID compared to addition of exenatide/Exenatide extended release.

Aim 2.1: To compare glucagon, glucose and GLP-1 levels in basal state and in insulinopenic state following single doses treatments before and after 12 weeks treatment with dapagliflozin, exenatide/Exenatide extended release, combination of both drugs or placebo in T1D.

Aim 2.1: To correlate changes in glucagon and FFA concentrations, ketone bodies levels with total daily insulin dose following 12 weeks of treatment.

Hypothesis 3 (exploratory): Dapagliflozin treatment induces lipolysis in adipose tissue in basal and insulinopenic conditions in TID compared to addition of exenatide/Exenatide extended release.

Aim 2.1: To evaluate FFA, glycerol and HSL levels in adipose tissue extracts in basal state and in insulinopenic state following single doses treatments before and after 12 weeks treatment with dapagliflozin, exenatide/Exenatide extended release, combination of both drugs or placebo in T1D.
Aim 2.2: To investigate the expression of SGLT-2 in adipose tissue and correlate it with FFA and ketone body generation in basal state and in insulinopenic state following single doses (acute) treatments before and after 12 weeks (long term) treatment with dapagliflozin, exenatide (acutely)/Exenatide extended release (long term), a combination of both drugs or placebo in T1D.

1.2 State the hypotheses to be tested, if applicable.

NOTE: A hypothesis is a specific, testable prediction about what you expect to happen in your study that corresponds with your above listed objectives.

Response:

Hypothesis 1: Addition of dapagliflozin results in increased levels of ketone bodies in basal and insulinopenic conditions in Type 1 diabetes (TID) compared to addition of exenatide/Exenatide extended release.
Hypothesis 2: Addition of dapagliflozin induces hyperglucagonemia in basal and insulinopenic conditions in TID compared to addition of exenatide/Exenatide extended release.
Hypothesis 3 (exploratory): Dapagliflozin treatment induces lipolysis in adipose tissue in basal and insulinopenic conditions in TID compared to addition of exenatide/Exenatide extended release.

2.0 Scientific Endpoints

2.1 Describe the scientific endpoint(s), the main result or occurrence under study.

*NOTE: Scientific endpoints are outcomes defined before the study begins to determine whether the objectives of the study have been met and to draw conclusions from the data. Include primary and secondary endpoints. Some example endpoints are: reduction of symptoms, improvement in quality of life, or survival. Your response should **not** be a date.*

Response:

The study investigations include evaluation of the acute effects of a single dose of dapagliflozin (10mg), exenatide (5µg), a combination of exenatide and dapagliflozin or placebo under insulinopenic condition and the long term effect under basal conditions before and after 12 weeks treatment with dapagliflozin, Exenatide extended release, a combination of Exenatide extended release and dapagliflozin or placebo on ketogenesis, glucagon and lipolysis.
In the acute effects study, qualified patients will come fasting to the research center. Review of study procedures and vitals will be performed. Insulin infusion (pump) will be

stopped and basal blood, urine and adipose tissue biopsy samples will be obtained. Placebos, exenatide (5µg) or dapagliflozin (10mg) (with appropriate placebos) or combination of exenatide and dapagliflozin will be administered, according to a randomized fashion, and blood samples will be collected every 30 min for up to 8 hours after starting the treatment. Urine will be collected every hour up to 8 hours and a second adipose tissue biopsy will be collected at 6 hours after the start of the treatment. Additional blood samples will be collected at 1, 2, 4 and 6 and 8 hour marks for MNC isolation. Plasma and mononuclear cell (MNC) fractions will be prepared from the blood samples. The patient will then come back at the 24 hour mark for a fasting blood and urine collection. The long term study will then commence. Exenatide extended release or dapagliflozin (along with appropriate placebos) or a combination of both drugs will be started and continued for 12 weeks according to original randomization of day 0. In the long term study, dapagliflozin (or the appropriate placebo) will be started at 5mg dose and will be titrated to 10mg/day after 5 days. Fasting blood and 24hr urine samples will be collected at 1, 4 and 8 weeks. At 12 weeks, the 2nd acute effects testing study will be repeated as described above.
Measurements of beta-hydroxybutyrate, acetoacetic acid, glucose, FFA and glucagon, will be measured from all blood samples collected. HSL, GLP-1, glycerol, electrolytes, serum bicarbonate, cortisol and catecholamines will be measured at 0, 60, 120, 240, 360, 480 min and at 24hr following single dose studies at 0 and 12 weeks visits and at baseline on the 1, 4 and 8 weeks visits. Ketone bodies will be measured in all urine samples. All MNC and adipose tissue samples will be tested for HSL and SGLT2 expression.

3.0 Background

3.1 Provide the scientific or scholarly background, rationale, and significance of the research based on the existing literature and how it will contribute to existing knowledge. Describe any gaps in current knowledge. Include relevant preliminary findings or prior research by the investigator.

Response:

Insulin therapy in patients with type 1 diabetes was started soon after the discovery of insulin. Majority of such patients are still not at their glycemic targets. Clearly, therefore, it is necessary to look for means of further improvement. Novel approaches with GLP-1 receptor agonists (GLP-1RA) like liraglutide and with sodium-glucose transport protein-2 (SGLT2) inhibitors, have recently provided additional strategies for the treatment of this condition when used in combination with insulin (1-5). Initial studies with either liraglutide or dapagliflozin,(5-7) an SGLT2 inhibitor, have been successful but have still not allowed patients to be at their target level of glycemic control. We have, therefore, pioneered the use of 'triple therapy', combining insulin, GLP-1RAs and SGLT-2 inhibitors.

While the search for new and novel strategies for glycemic control in type 1 diabetes is of great significance, it is also important to evaluate these new therapies for their associated risks and effects on known complications of type 1 diabetes such as Diabetic Ketoacidosis (DKA). DKA is an important complication and cause of mortality and morbidity in type 1 patients. The decreased ratio of insulin to glucagon in insulin deficient subjects promotes ketogenesis (8). In patients with type 1 diabetes, the suppressive effect of hyperglycemia

and the paracrine inhibitory effect of insulin and GABA from the β cell on α cell are absent. Thus, plasma glucagon concentrations are elevated and in combination with insulin deficiency, lead to lipolysis, increased plasma FFA concentrations and an increased fatty acid supply to the liver. Thus, both fatty acid oxidation and ketogenesis are enhanced (9).

Data from recent studies, including our own (2), show that GLP-1RA can lower blood glucose concentrations in patients with type 1 diabetes through their protean actions, independently of the beta cell. These actions include suppression of appetite and carbohydrate intake, slowing of gastric emptying and the suppression of glucagon. Our recent work has shown that liraglutide not only markedly suppresses the post prandial excursion of glucagon, it also suppresses fasting plasma FFA concentrations (Fig 1). In this context, it is important that the deletion of the glucagon receptor protects both from hyperglycemia and ketosis following a high dose of streptozotocin in mice (10). Therefore, it is possible that GLP-1 mimetics may protect from DKA in presence of sufficient insulin.

SGLT-2 inhibitors are of special importance since their mode of action is independent of insulinogenesis. They are selective antagonists to sodium-glucose transporter in the proximal convoluted tubules in the kidneys. They act by inhibiting glucose re-absorption in the renal tubules and thus inducing the excretion of 60-100 grams per day of glucose in urine (7). They have been studied extensively for use in type 2 diabetes however; there is still paucity of data regarding any benefits in type 1 diabetes. Recent studies have shown that they have similar effects of enhanced 24 hour urine glucose excretion in type 1 diabetes, just like in patients with type 2 diabetes with improved glycemic indices. Indeed, Mudaliar et al (3) demonstrated that use of remogliflozin in patients with type 1 diabetes significantly reduced glucose concentrations relative to a placebo after a standard glucose load and glucose improvements were sustained for up to 10 hours. Perkins et al (6) studied use of 8 weeks of empagliflozin in type 1 diabetes and demonstrated significant reduction in HbA1c, fasting blood glucose, the rate of hypoglycemia and the daily insulin dose. Mean urinary excretion of glucose increased and significant weight loss was observed. Cherney et al(1) also demonstrated significantly increased glucose excretion and fall in HbA1c despite lower insulin doses with use of empagliflozin 25mg daily for 8 weeks in patients with type 1 diabetes.

Preliminary studies involving treatment with SGLT-2 inhibitors in patients with type 1 diabetes is yielding data showing increased ketogenesis and in some instances resulted in DKA. In our retrospective study investigating data from triple therapy patients on liraglutide and dapagliflozin, preliminary data in 20 patients show increased ketogenesis and two cases of DKA despite impressive reductions in glycemia. The most remarkable feature of these clinical episodes was that the patient's glucose levels were normal but the ketoacidosis was florid. She was treated with intravenous insulin and fluids following which DKA reversed rapidly.

These two intriguing DKA episodes lead us to ask several mechanistic questions. Why should the addition of an SGLT2 inhibitor lead to DKA while glycemia improves further? There are several possibilities. Firstly, there is the possibility that the aggressive reduction in the insulin dose due to restoration of normoglycemia by liraglutide and dapagliflozin

may have led to systemic insulinopenia in spite of normal glucose concentrations (which is being maintained by the glycosuria induced by SGLT2 inhibition), thus resulting in uncontrolled ketogenesis and euglycemic DKA. The second possibility is that SGLT2 inhibitors are known to induce hyperglucagonemia which on the one hand, increases gluconeogenesis (11, 12) and on the other, also promotes ketogenesis. The hyperglycemic effect of gluconeogenesis is more than neutralized by the glucosuric effect of SGLT2 inhibitors but the potential ketogenic effect probably adds on to that induced by insulinopenia. It should also be mentioned that SGLT2 inhibitors increase appetite and carbohydrate intake in patients with type 1 diabetes, as was observed in this case. This increase was observed in this patient in spite of the fact that this patient was on liraglutide which is known to suppress appetite, induce weight loss and to suppress postprandial glucagon in patients with type 1 diabetes. Whether liraglutide is able to neutralize the glucagon inducing effect of an SGLT2 inhibitor is not known.

Therefore, we embarked on this study to investigate in detail the mechanisms involved in ketogenesis, lipolysis and glucagon changes in patients with type 1 diabetes treated with dapagliflozin or Bydureon (exenatide extended release, a long term acting GLP-1 RA) for 12 weeks and in an acute condition of insulinopenia. This study will be the first randomized placebo controlled prospective study investigating the effect of an SGLT-2 inhibitor and Exenatide extended release on ketogenesis.

Preliminary data:

Effect of liraglutide on glucose, glucagon, Ketogenesis and FFA I type 1 diabetes

Sixty-three patients with type 1 diabetes were randomized to placebo (17) or liraglutide 0.6 mg (18), liraglutide 1.2 mg (13) and liraglutide 1.8 mg (15) treatment for 12 weeks. There was a statistically significant decrease in average blood glucose concentrations in patients randomized to 1.2 and 1.8 mg groups as compared with placebo. HbA1c fell by 0.78% in 1.2 mg group (7.84±0.17% to 7.06±0.15% ($p<0.0001$) and by 0.42% in 1.8 mg group (7.41±0.15 to 6.99±0.15 ($p=0.001$). Basal and bolus insulin doses fell in both 1.2 and 1.8 mg groups. Fasting plasma FFA fell significantly in the 1.8mg group from 0.55±0.07 to 0.45±0.05mM, ($p<0.05$, Figure 1A). There was no significant change in fasting levels of glucagon following any treatment compared to placebo group while at the end of 12 weeks, the area under curve (AUC) for the HFHC meal induced increase in glucagon was lower by 47±12% and 72±12% in the 1.2mg and 1.8mg groups, respectively ($p<0.05$ for both as compared to 0 week response, Figure 1B). Overall the suppressive effect of liraglutide on postprandial glucagon was dose dependent. There was no significant change in fasting or postprandial concentrations of ketone bodies (acetoacetate acid and β-hydroxybutyrate) following liraglutide treatment.

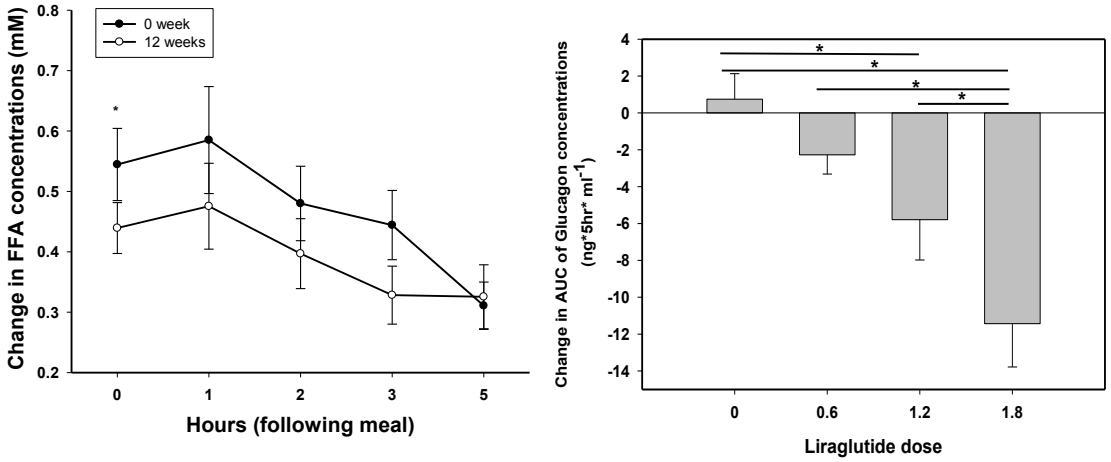

Figure 1: Change in (A) FFA concentrations following meal challenge before and following 12weeks of liraglutide 1.8mg treatment and (B) change in AUC of glucagon change following meal challenge before and following 12 weeks of different doses of liraglutide in patients with type 1 diabetes. *=P<0.05 between groups as indicated.

Dapagliflozin as Additional Treatment to Insulin and Liraglutide in Patients with Type 1 Diabetes Mellitus: Preliminary Observations

We have now investigated whether the addition of dapagliflozin, to insulin and liraglutide would improve glycemia further. We conducted a retrospective analysis of 10 patients on continuous glucose monitoring system (CGMS) treated with this combination. They were under treatment with insulin and had received liraglutide for 11±2 months (baseline HbA1c: 8.01±0.22 %; mean age: 56±4 years; mean age of diabetes diagnosis: 29±5 years; mean BMI: 29±1 Kg/m^2; mean body weight: 86.4±4.5 kg; mean BP: 125/75±3 mm Hg). In all patients, dapagliflozin was started at a dose of 5 mg daily and was increased to 10 mg daily 7±1 days later. At the end of 12±1 weeks of dapagliflozin therapy, mean HbA1c fell by 0.66±0.22% (p=0.0004); mean glucose fell by 28±2mg/dl from a baseline of 172±9 mg/dl (p=0.016,); the daily carbohydrate intake increased from 166±3g to 196±4g (p= 0.04); mean body weight and BMI fell from 87±5 Kg to 85±5 Kg and 29±1 to 28±1Kg/m^2, respectively (p=0.02). Total insulin dose remained unchanged at 0.7±0.1 u/Kg daily. There was no additional hypoglycemia (<70 mg/dl). One patient developed diabetic ketoacidosis (DKA) in spite of normal blood glucose concentrations within 48 hours of increasing the dose of dapagliflozin to 10 mg. The dose of insulin in this patient had declined from 0.45 to 0.39 u/kg (total dose: 32.9 to 28.5 units). Carbohydrate intake had increased from 50 to 95g daily. Along with improved glycemic control there was a significant increase in FFA (from 0.34±0.04 to 0.59±0.11mM, p<0.05), β-hydroxybutyrate (from 0.11±0.02 to 0.39±0.09mM, p<0.05) and acetoacetate (from 0.32±0.09 to 0.53±0.11mM, p<0.05) at the end of follow-up period compared insulin and liraglutide alone. Dapagliflozin intake for 1 2weeks also increased glucagon concentrations by 35±13% (from 91±12 to 114±19pg/ml, p<0.05, Figure 3)

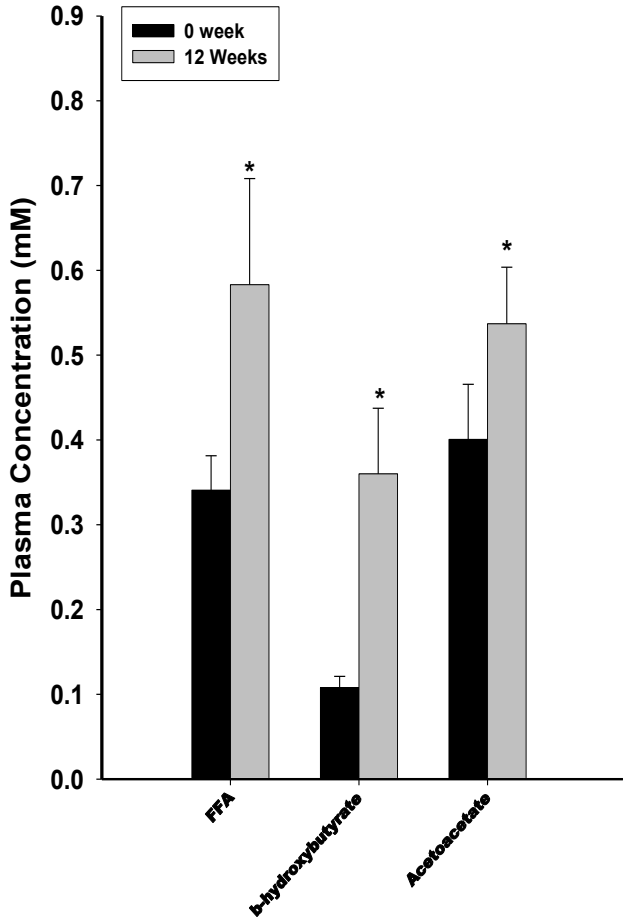

Figure 2. Change in FFA, Ketone bodies following 12 weeks of treatment with dapagliflozin in patients with type 1 diabetes already on liraglutide in addition to insulin. *=

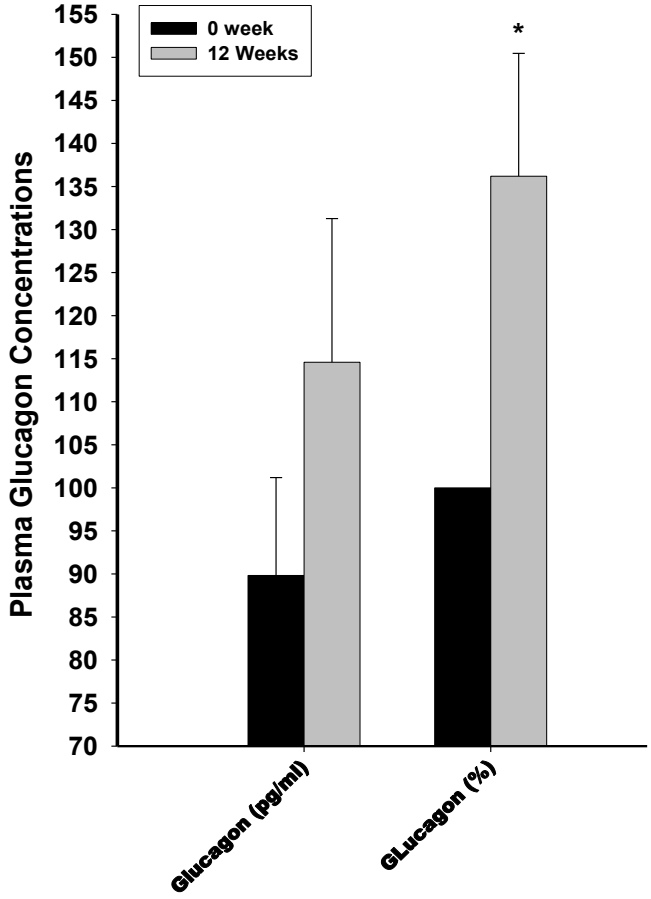

Figure 3. Change in glucagon concentrations pg/dl and % change) following 12 weeks of treatment with dapagliflozin in patients with type 1 diabetes already on liraglutide in addition to

3.2 Include complete citations or references.

Response:

1. *Cherney, D.Z., Perkins, B.A., Soleymanlou, N., Maione, M., Lai, V., Lee, A., Fagan, N.M., Woerle, H.J., Johansen, O.E., Broedl, U.C., et al. 2014. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation 129:587-597.*

2. *Kuhadiya, N.D., Dhindsa, S.S., Mehta, A., Makdissi, A., Sandhu, S., Ghanim, H., Batra, M., Chaudhuri, A., Hejna, J., Green, K., et al. 2014. Liraglutide As Additional Treatment to Insulin in Patients with Type 1*

Diabetes Mellitus: A Randomized Clinical Trial. ICE/ENDO Annual Meeting, Chicago OR-26-4.

3. *Mudaliar, S., Armstrong, D.A., Mavian, A.A., O'Connor-Semmes, R., Mydlow, P.K., Ye, J., Hussey, E.K., Nunez, D.J., Henry, R.R., and Dobbins, R.L. 2012. Remogliflozin etabonate, a selective inhibitor of the sodium-glucose transporter 2, improves serum glucose profiles in type 1 diabetes. Diabetes Care 35:2198-2200.*

4. *Varanasi, A., Bellini, N., Rawal, D., Vora, M., Makdissi, A., Dhindsa, S., Chaudhuri, A., and Dandona, P. 2011. Liraglutide as additional treatment for type 1 diabetes. Eur J Endocrinol 165:77-84.*

5. *Kuhadiya, N.D., Malik, R., Bellini, N.J., Patterson, J.L., Traina, A., Makdissi, A., and Dandona, P. 2013. Liraglutide as additional treatment to insulin in obese patients with type 1 diabetes mellitus. Endocr Pract 19:963-967.*

6. *Perkins, B.A., Cherney, D.Z., Partridge, H., Soleymanlou, N., Tschirhart, H., Zinman, B., Fagan, N.M., Kaspers, S., Woerle, H.J., Broedl, U.C., et al. 2014. Sodium-glucose cotransporter 2 inhibition and glycemic control in type 1 diabetes: results of an 8-week open-label proof-of-concept trial. Diabetes Care 37:1480-1483.*

7. *Abdul-Ghani, M.A., Norton, L., and Defronzo, R.A. 2011. Role of sodium-glucose cotransporter 2 (SGLT 2) inhibitors in the treatment of type 2 diabetes. Endocr Rev 32:515-531.*

8. *Beylot, M. 1996. Regulation of in vivo ketogenesis: role of free fatty acids and control by epinephrine, thyroid hormones, insulin and glucagon. Diabetes Metab 22:299-304.*

9. *Eledrisi, M.S., Alshanti, M.S., Shah, M.F., Brolosy, B., and Jaha, N. 2006. Overview of the diagnosis and management of diabetic ketoacidosis. Am J Med Sci 331:243-251.*

10. *Conarello, S.L., Jiang, G., Mu, J., Li, Z., Woods, J., Zycband, E., Ronan, J., Liu, F., Roy, R.S., Zhu, L., et al. 2007. Glucagon receptor knockout mice are resistant to diet-induced obesity and streptozotocin-mediated beta cell loss and hyperglycaemia. Diabetologia 50:142-150.*

11. *Ferrannini, E., Muscelli, E., Frascerra, S., Baldi, S., Mari, A., Heise, T., Broedl, U.C., and Woerle, H.J. 2014. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest 124:499-508.*

12. *Merovci, A., Solis-Herrera, C., Daniele, G., Eldor, R., Fiorentino, T.V., Tripathy, D., Xiong, J., Perez, Z., Norton, L., Abdul-Ghani, M.A., et al. 2014. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest 124:509-514.*

4.0 Study Design

4.1 Describe and explain the study design (e.g. case-control, cross-sectional, ethnographic, experimental, interventional, longitudinal, observational).

Response: The first 60 patients (cohort 1) will be involved in a single center, prospective, double blind, randomized, placebo –controlled in a parallel design study. The last 20 patients (cohort 2) will be enrolled into a single centered, prospective, randomized, open labeled study. The study will be conducted at the Diabetes – Endocrinology Center of Western New York under the direction of Dr. Paresh Dandona, M.D, Dr. Ajay Chaudhuri, MD, Antoine Makdissi, MD and Dr. Husam Ghanim, PhD.

In the first cohort, sixty (60) patients with T1D will be enrolled and randomized into 4 groups (15 each) to receive placebo, or dapagliflozin, or exenatide (5µg acutely)/Exenatide extended release (2mg weekly subcutaneous injection, long term), or exenatide (5µg acutely)/Exenatide extended release (2mg weekly subcutaneous injection, long term) and dapagliflozin treatments acutely and for 12 weeks.
The treatment arms of this study are:

1. Placebo Arm: dapagliflozin placebo (oral tablet) and exenatide (5µg acutely)/Exenatide extended release (long term) placebo (subcutaneous injection)
2. Dapagliflozin Arm: dapagliflozin 10mg (oral tablet) and exenatide (5µg acutely)/Exenatide extended release (long term) placebo (subcutaneous injection)
3. Exenatide/Exenatide extended release Arm: subcutaneous injection of Exenatide (5µg acutely)/Exenatide extended release 2 mg weekly injection (long term) and dapagliflozin placebo
4. Exenatide/Exenatide extended release & dapagliflozin Arm: Exenatide (5µg acutely)/Exenatide extended release 2 mg weekly injection (long term) and dapagliflozin 10mg

In the second cohort, twenty (20) patients will be randomized into three groups.

1. Open-label Dapagliflozin
2. Open-label Bydureon/Byetta
3. Open label Bydureon/Byetta and open label Dapagliflozin

5.0 Local Number of Subjects

5.1 Indicate the total number of subjects that will be enrolled or records that will be reviewed locally.

Response: 80

5.2 If applicable, indicate how many subjects you expect to screen to reach your target sample (i.e. your screen failure rate).

Response: All screened and qualified patients will be enrolled and randomized

5.3 Justify the feasibility of recruiting the proposed number of eligible subjects within the anticipated recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?

Response: The Diabetes and Endocrinology Center of WNY is the largest Diabetes center in the WNY area, seeing between 60 to 100 type 1 diabetic patient every month. Therefore, majority of recruited patients are our clinic patients. We do recruit a few patients through advertisement and researchmatch.org. These sources will suffice to recruit the needed number to subjects.

6.0 Inclusion and Exclusion Criteria

*6.1 Describe the criteria that define who will be **included** in your final study sample.*

NOTE: This may be done in bullet point fashion.

Response: **Inclusion Criteria**

- Type 1 Diabetes for at least 1 year on stable use of continuous subcutaneous insulin infusion (CSII) or multiple (four or more) injections of insulin per day for last 3 months.
- HbA1c of 7-10% (inclusive)
- Ages 18-65 years (inclusive of ages 18 and 65)
- BMI $\geq$ 22 kg/m^2.
- Well versed in CHO counting.

*6.2 Describe the criteria that define who will be **excluded** from your final study sample.*

NOTE: This may be done in bullet point fashion.

Response: **Exclusion Criteria**

- Inability to give informed consent
- Inability or refusal to comply with protocol
- Use of GLP-1 Receptor Agonists in the last 3 months or DPP-IV and SGLT-2 inhibitors therapy in the last 1 month.
- Risk for pancreatitis (e.g., history of gallstones, alcohol abuse, and hypertriglyceridemia)
- History of pancreatitis and or chronic pancreatitis
- Coronary event or procedure (myocardial infarction, unstable angina, coronary artery bypass, surgery or coronary angioplasty) or stroke in the previous 3 months.
- Congestive Heart Failure class III or IV or tachyarrhythmia.

- Hepatic disease: Severe hepatic insufficiency and/or significant abnormal liver function defined as:
    1. Aspartate aminotransferase (AST) >3x upper limit of normal (ULN) and/or alanine aminotransferase (ALT) >3x ULN
    2. Total bilirubin >2.0 mg/dL (34.2 μmol/L)
    3. Positive serologic evidence of current infectious liver disease including Hepatitis B viral antibody IGM, Hepatitis B surface antigen and Hepatitis C virus antibody
    4. Liver function tests more than 3 times the upper limit of normal
- Renal impairment (e.g., serum creatinine levels ≥1.4 mg/dL for women, or eGFR <60 mL/min/1.73 m2) or history of unstable or rapidly progressing renal disease or end stage renal disease.
- History of unexplained microscopic or gross hematuria, or microscopic hematuria at visit 1, confirmed by a follow-up sample at next scheduled visit.
- HIV positive
- History of gastroparesis
- History of medullary thyroid carcinoma or MEN 2 syndrome
- History of recurring UTI
- Uncontrolled thyroid disease (documented normal TSH), Cushing's syndrome, congenital adrenal hyperplasia or hyperprolactinemia.
- Prior history of a malignant disease requiring chemotherapy or patients with a prior history of bladder cancer regardless of treatment
- Alcoholism or drug addiction.
- Hypertriglyceridemia (>400 mg/dl).
- Any other life-threatening, non-cardiac disease
- Uncontrolled hypertension (BP > 160/95 mm of Hg)
- Patients with hypotension or at risk for volume depletion due to co-existing conditions or concomitant medications, such as loop diuretics or recently donated >500ml of blood should have careful monitoring of their volume status
- Pregnant or breastfeeding patients or patient not willing to use two barrier method contraception during study period (unless sterilized or have an IUD)
- Use of hormonal medications, anti-obesity drugs or weight loss medications (prescription or OTC) and medications known to exacerbate glucose tolerance (such as isotretinoin, GnRH agonists, glucocorticoids, anabolic steroids, C-19 progestins) stopped for at least 8 weeks. Use of anti-androgens that act peripherally to reduce hirsutism such as 5-alpha reductase inhibitors (finesteride, spironolactone, flutamide) stopped for at least 4 weeks
- Presence of hypersensitivity to dapagliflozin or other SGLT2 inhibitors (e.g. anaphylaxis, angioedema, exfoliative skin conditions
- Known hypersensitivity or contraindications to use GLP1 receptor agonists (exenatide, liraglutide)
- Known hypersensitivity to heparin/ IV catheter equipment.
- Eating disorders (anorexia, bulimia) or gastrointestinal disorders
- Having a history of bariatric surgery

- Debilitating psychiatric disorder such as psychosis or neurological condition that might confound outcome variables
- Use of an investigational agent or therapeutic regimen within 30 days of study
- Participation in any other concurrent clinical trial

6.3 Indicate specifically whether you will include any of the following special populations in your study using the checkboxes below.

NOTE: Members of special populations may not be targeted for enrollment in your study unless you indicate this in your inclusion criteria.

Response: NA

☐ Adults unable to consent

☐ Individuals who are not yet adults (infants, children, teenagers)

☐ Pregnant women

☐ Prisoners

6.4 Indicate whether you will include non-English speaking individuals in your study. ***Provide justification if you will exclude non-English speaking individuals.***

In order to meet one of the primary ethical principles of equitable selection of subjects, non-English speaking individuals may ***not*** *be routinely excluded from research as a matter of convenience.*

In cases where the research is of therapeutic intent or is designed to investigate areas that would necessarily require certain populations who may not speak English, the researcher is required to make efforts to recruit and include non-English speaking individuals. However, there are studies in which it would be reasonable to limit subjects to those who speak English. Some examples include pilot studies, small unfunded studies with validated instruments not available in other languages, studies with numerous questionnaires, and some non-therapeutic studies which offer no direct benefit.

Response: We have no non-English speaking patients in this population. We have patients that English is a second language, but they are able to read, write and understand it. This population is less than 10% of the total population.

7.0 Vulnerable Populations

If the research involves special populations that are considered vulnerable, ***describe the safeguards included to protect their rights and welfare.***

NOTE: You should refer to the appropriate checklists, referenced below, to ensure you have provided adequate detail regarding safeguards and protections. You do not, however, need to provide these checklists to the IRB.

*7.1 For research that involves **pregnant women,** safeguards include:*
NOTE CHECKLIST: Pregnant Women (HRP-412)

Response: We will not be using subjects from vulnerable populations

☒ **N/A**: This research does not involve pregnant women.

*7.2 For research that involves **neonates of uncertain viability or non-viable neonates,** safeguards include:*
NOTE CHECKLISTS: Non-Viable Neonates (HRP-413), or Neonates of Uncertain Viability (HRP-414)

Response:

☒ **N/A:** This research does not involve non-viable neonates or neonates of uncertain viability.

*7.3 For research that involves **prisoners**, safeguards include:*
NOTE CHECKLIST: Prisoners (HRP-415)

Response:

☒ **N/A:** This research does not involve prisoners.

*7.4 For research that involves **persons who have not attained the legal age for consent to treatments or procedures involved in the research ("children")**, safeguards include:*
NOTE CHECKLIST: Children (HRP-416)

Response:

☒ **N/A:** This research does not involve persons who have not attained the legal age for consent to treatments or procedures ("children").

*7.5 For research that involves **cognitively impaired adults**, safeguards include:*
NOTE CHECKLIST: Cognitively Impaired Adults (HRP-417)

Response:

☒ **N/A:** This research does not involve cognitively impaired adults.

*7.6 Consider if other specifically targeted populations such as students, employees of a specific firm, or educationally or economically disadvantaged persons are vulnerable. **Provide information regarding their safeguards and protections, including safeguards to eliminate coercion or undue influence.***

Response: No specific populations or vulnerable groups will be targeted.All subjects enrolled in this study will be of legal adult consenting age with the ability to speak, read and interrupt the English language. Patients will have the ability to speak with the research team regarding any questions or concern they have before signing the consent. Patients are made aware that this study is voluntary and they are able to stop participating at any time they feel uncomfortable. Patients are not be pressured into participating and their clinic standard of care will remain the same if they participate or choose not to participate.

8.0 Eligibility Screening

*8.1 Describe **screening procedures** for determining subjects' eligibility. Screening refers to determining if prospective participants meet inclusion and exclusion criteria.*

Include all relevant screening documents with your submission (e.g. screening protocol, script, questionnaire).

Response:

Prospective participants will be asked to read and understand the consent and any questions they may have regarding the protocol will be answered. If the subject wants to participate in the study, they will be asked to sign the informed consent form. The subject's medical history and current medications will be obtained as well as their blood pressure and vitals. A physical examination will also be done. Blood samples will be taken in order to evaluate HbA1c, CBC, CMP and liver and kidney functions and pregnancy status. The patient's insulin pump will be uploaded for pump patients, and food/insulin logs will be collected for MDI patients. Patients meeting all the inclusion and exclusion criteria based on all screening tests will be enrolled in the study.

☐ **N/A:** There is no screening as part of this protocol.

9.0 Recruitment Methods

☐ **N/A:** This is a records review only, and subjects will not be recruited. NOTE: If you select this option, please make sure that all records review procedures and inclusion/exclusion screening are adequately described in other sections.

9.1 Describe when, where, and how potential subjects will be recruited.

NOTE: Recruitment refers to how you are identifying potential participants and introducing them to the study. Include specific methods you will use (e.g. searching charts for specific ICD code numbers, Research Participant Groups, posted advertisements, etc.).

Response: Participants will be identified by prescreening clinical charts, patient doctor interaction at the time of their visits, flyers advertisements and researchmatch.org. Diabetes Endocrinology Center of WNY Locations include:

1. 1020 Youngs Road, Williamsville NY 14221

2. 705 Maple Road, Williamsville NY 14221

3. 462 Grider Street, Buffalo NY 14215

4. 1000 Youngs Road, Suite 105, Williamsville NY 14221

Patients will also be recruited at UBMD Pediatrics, Division of Endocrinology/Diabetes. 1001 Main Street 5th floor Buffalo, NY 14203

The study clinical team will evaluate their clinic patients for possible participation in this study according to the inclusion and exclusion criteria at the Diabetes and Endocrinology Center of WNY. Patients that may qualify for the study at all sites are referred to the research team at 1000 Youngs Rd for further eligibility evolution. Patients meeting the inclusion and exclusion criteria based on preliminary phone evaluation will be invited to participate in the study.

- Facebook and Craigslist will be used for recruitment.
- The approved study advertisement will be posted on the main advertising board at Erie County Medical Center.
- A generic flyer will also be utilized for recruiting within the Kaleida Health system.

9.2 Describe how you will protect the privacy interests of prospective subjects during the recruitment process.

NOTE: Privacy refers to an individual's right to control access to him or herself.

Response: Patient charts will be screened according to the study inclusion and exclusion criteria by our trained clinical staff and physicians. If the patient qualifies and is of consenting age, the physicians will speak to them about their interests in participating in research. If the patient agrees, their information will be given to the research coordinator to be contacted for further evaluation. All personal information will be kept confidential and locked in the coordinator office.

9.3 Identify any materials that will be used to recruit subjects.

NOTE: Examples include scripts for telephone calls, in person announcements / presentations, email invitations.

For advertisements, include the final copy of printed advertisements with your submission. When advertisements are taped for broadcast, attach the final audio/video tape. NOTE: You may submit the wording of the advertisement prior to taping to ensure there will be no IRB-required revisions, provided the IRB also reviews and approves the final version.

Response: In addition to screening clinical charts, participations will be identified through; flyer advertisement, researchmatch.org, craigslist.com, and facebook.

10.0 Procedures Involved

*10.1 Provide a description of **all research procedures or activities** being performed and when they are performed once a subject is screened and determined to be eligible. Provide as much detail as possible.*

NOTE: This should serve as a blueprint for your study and include enough detail so that another investigator could pick up your protocol and replicate the research. For studies that have multiple or complex visits or procedures, consider the addition of a schedule of events table in in your response.

Response:

The study investigations include evaluation of the acute effects of a single dose of dapagliflozin (10mg), exenatide (5µg), a combination of exenatide and dapagliflozin or placebo under insulinopenic condition and the long term effect under basal conditions before and after 12 weeks treatment with dapagliflozin, Exenatide extended release, a combination of Exenatide extended release and dapagliflozin or placebo on ketogenesis, glucagon and lipolysis. This applies to the first 60 patients in cohort 1. For cohort 2,we will evaluate the acute effects of a single dose of dapagliflozin (10mg), exenatide (5µg), or a combination of exenatide and dapagliflozin under insulinopenic condition and the long term effect under basal conditions before and after 12 weeks treatment with dapagliflozin, Exenatide extended release, or a combination of Exenatide extended release and dapagliflozin on Ketogenesis.

In the acute effects study, qualified patients will come fasting to the research center. Review of study procedures and vitals will be performed. Insulin infusion (pump) will be stopped and basal blood, urine and adipose tissue biopsy samples will be obtained. Placebos, exenatide (5µg) or dapagliflozin (10mg) (with appropriate placebos) or combination of exenatide and dapagliflozin will be administered, according to a randomized fashion and cohort assigned, and blood samples will be collected every 30 min for up to 8 hours after starting the treatment. Urine will be collected every hour up to 8 hours and a second adipose tissue biopsy will be collected at 6 hours after the start of the treatment. Additional blood samples will be collected at 1, 2, 4 and 6 and 8 hour marks for MNC isolation. Plasma and mononuclear cell (MNC) fractions will be prepared from the blood samples. The patient will then come back at the 24 hour mark for a fasting blood and urine collection. The long term study will then commence. Depending on the cohort, Exenatide extended release or dapagliflozin (along with appropriate placebos) or a combination of both drugs will be started and continued for 12 weeks according to original randomization of day 0. In the long term study, dapagliflozin (or the appropriate placebo) will be started at 5mg dose and will be titrated to 10mg/day after 5 days. Fasting blood and 24hr urine samples will be collected at 1, 4 and 8 weeks. At 12 weeks, the 2nd acute effects testing study will be repeated as described above.

Drug Blinding:

All drugs used and their matching placebos will be provided by AstraZeneca in an unlabeled form. Drug or placebo assignment will be mailed separately. A designated coordinator/investigator (or University Pharmacy) who is not participating in the study will receive the drug/placebo assignment cods and will be responsible for relabeling the drugs and placebos containers/vials according to Good Manufacturing Practice and in compliance with FDA regulations and local guidelines. Relabeled drug/ placebos will be dispensed by study investigators according to pre-generated randomization log generated using Microsoft –Excel randomization feature. Records of drug and placebo assignment will be kept with the independent investigator/coordinator (or UB Pharmacy) in a secure place (locked office or enclosure) or on password protected computer and folder (if digital) For each patients and in at every dispensing visit, 2 drugs and/or placebos combo will be dispensed to maintain blinding of both patient and study team. For example, patients in the dapagliflozin only arm will receive dapagliflozin active drug tablet and exenatide (acute study) and exenatide extended release (long term) matching placebos.

Drugs will be stored in a locked cabinet or locked temperature controlled refrigerator at 4C at the research facility of the Diabetes and Endocrinology Center of WNY. IND application for use of all drugs (dapagliflozin, Exenatide extended release and exenatide) in type 1 diabetes will be submitted to FDA and no study procedure will start before all local and federal approvals are obtained.

Visit 1 (day -7):
Qualifying patients will be scheduled for first this visit within 2 weeks of screening visit. Their insulin pump will be downloaded or log of MDI/carbohydrate intake will be collected and appropriate insulin adjustments will be made by the investigator to target fasting blood glucose concentrations between 70-180 mg/dl if possible at all times without having additional hypoglycemia (less than 70 mg/dl). Urine collection container will be provided with instruction to collect urine starting 24 hours before coming for their next visit.

Visit 2 (day 0):
Qualifying subjects will be asked to come for the visit in a fasting state (10-12hr fasting). They will be fasting for the entire visit. 24hr urine will be collected, vitals and fingerstick blood glucose and ketones (β-hydroxybutyrate) using the Precision Extra glucose and ketones meter (Abbott Laboratories) and urine Ketostix (Bayer) will be measured**.** For MDI patients, baseline blood, spot urine, and adipose tissue will be collected. The remainder of this visits' procedures apply only to pump patients. If, ketones measure positive in blood or in urine (≥0.4 mM in blood or >+1 in urine) or fasting blood glucose <70 or >250 mg/dl, the acute testing will not take place and patient will be rescheduled**.** An IV port will be placed in order to obtain blood samples throughout the duration of the visit. Insulin infusion will be stopped, and baseline blood, spot urine, and adipose tissue will be collected. These samples will constitute the baseline for all study measurements. One of the treatment arms will be administered to the patient depending on their cohort (placebo OR exenatide 5µg subcutaneous (same site will be used in 2nd acute study at end of study) injection OR dapagliflozin 10mg (two 5mg) oral tablets OR combination of exenatide 5µg AND

dapagliflozin 10mg (two 5mg) with appropriate placebos in a randomized fashion). Subjects will be fasting throughout the first 6 hr of the visit. Blood (10ml) will be collected every 30 min and spot urine every 1hr for up to 8 hours. Additional blood samples (20ml) will be collected at 1, 2, 4 and 6 and 8 hours for *MNC* isolation. A second adipose tissue biopsy will be collected at 360 min after starting the treatment. Basal insulin Infusion will be restarted at the end of the 360min sample collections, food will be provided, patient will be instructed to deliver a bolus insulin dose through bolus wizard calculator of the insulin pump after entering the grams of carbohydrates and blood glucose (Bolus wizard matches the bolus insulin dose based on individual patient's insulin to carbohydrate ratio and correction factor for high blood glucose). Patients will be monitored for at least 2hr before discharging from center (8hr time point). The subject's blood glucose and ketones (β-hydroxybutyrate) levels will be monitored every 30 minutes and ketones will be also measured in urine using Ketostix every hour to ensure safety. If the BG concentrations exceed 400 mg/dl or blood β-hydroxybutyrate exceeds 2.5mmol/l (or +3 (80mg/dl) or greater in urine) anytime during the 360 minutes following single dose/doses with or without clinical signs and symptoms of DKA like nausea and vomiting then STAT lab sample for CMP will be collected along with research blood sample. Spot urine and adipose tissue biopsy will be collected only if deemed feasible by the investigator present based on the clinical condition of the participant. Basal insulin will be restarted; bolus insulin as per the bolus wizard calculator will be administered and a meal provided. Patient will be discharged only after all laboratory parameters return to pretesting levels with no clinical symptoms of DKA.

This will now mark the end of the acute testing phase and the beginning of the 12 weeks treatment phase. If the HbA1c is ≥7.5%; then no reduction will be made in dose of insulin while if the HbA1c ranges from 7-7.5% then the basal and pre-prandial insulin doses will be reduced by 5%. All patients will be instructed on how to give the single subcutaneous injection of Exenatide extended release (2mg weekly) or Exenatide extended release placebo (for those in cohort 1), or open label drugs (for those in cohort 2), which will be administered at home the next day. Patients will also be provided with 12 oral tablets of either 5mg placebo or 5mg dapagliflozin (depending on their group assignment and cohort) and instructed to consume 1 tablet in the morning on each of the first 4 days and 2 tablets (10 mg) on days 5, 6 and 7 (and for the rest of the 12 weeks thereafter) and to return to the center on day 7. Urine collection container will be provided and patients instructed to collect 24hr urine before their next visit. The patients will be trained to recognize the symptoms of DKA and to self-test ketones daily and if they develop any symptoms consistent with DKA, including nausea, vomiting, fatigue, confusion, or shortness of breath. Information sheet about above information will be provided to participants. Patients will be instructed to measure ketone bodies in urine samples daily with Ketostix (Bayer) and record it in diary provided. If ketone bodies in urine measure +2 or greater (>4mM in urine), patients are required to notify the research center. The investigators will collect all necessary information and advise the patient of any steps to mitigate the risk of DKA or to visit the closest emergency department and to inform emergency staff of their participation in a research study.

;

Visit 3 (Week 1): Vitals will be measured and fasting blood and 24hr urine samples will be collected. Carbohydrate intake/insulin injection logs, food diaries, or CGM/pump data will be reviewed and documented. Patients will be provided with 3 weeks supply of Exenatide extended release or Exenatide extended release placebo and 10mg dapagliflozin or placebo tablets depending on their cohort. Urine collection container will be provided and patients instructed to collect 24hr urine before their next visit. Insulin doses will be titrated to target blood glucose between 70-180 mg/dl if possible at all times without having additional hypoglycemia (less than 70 mg/dl). Ketostix will be dispensed and patients will be instructed to measure ketone bodies in a spot urine sample daily and reported as described in visit 2.

Visit 4 (Week 4): Patient physical and vitals will be measured, fasting blood and 24hr urine samples will be collected. Carbohydrate intake/insulin injection logs, food diaries, or CGM/pump data will be reviewed and documented. Patients will be provided with 4 weeks supply of Exenatide extended release or Exenatide extended release placebo and 10mg dapagliflozin or placebo tablets depending on their cohort. Urine collection container will be provided and patients instructed to collect 24hr urine before their next visit. Insulin doses will be titrated to target blood glucose between 70-180 mg/dl if possible at all times without having additional hypoglycemia (less than 70 mg/dl). Ketostix will be dispensed and patients instructed to measure ketone bodies in a spot urine sample daily and reported as described in visit 3.

Visit 5 (Week 8): Vitals will be measured and fasting blood and 24hr urine samples will be collected. Carbohydrate intake/insulin injection logs, food diaries, or CGM/pump data will be reviewed and documented. Patients will be provided with 4 weeks supply of Exenatide extended release or Exenatide extended release placebo and 10mg dapagliflozin or placebo tablets depending on their cohort. Urine collection container will be provided and patients instructed to collect 24hr urine before their next visit. Insulin doses will be titrated to target blood glucose between 70-180 mg/dl if possible at all times without having additional hypoglycemia (less than 70 mg/dl). Ketostix will be dispensed and patients instructed to measure ketone bodies in a spot urine sample daily and reported as described in visit 3.

Visit 6 (Week 12): Subjects will be asked to come for the visit in a fasting state (10-12hr fasting). The will be fasting for the entire visit. Once the patient arrives to the research center, a complete physical examination will be done, 24hr urine will be collected, vitals and fingerstick blood glucose and ketones (β-hydroxybutyrate) using the Precision Extra glucose and ketones meter (Abbott Laboratories) will be measured. For MDI patients, baseline blood, spot urine, and adipose tissue will be collected. The remainder of this visits' procedures apply only to pump patients. An IV port will be placed in order to obtain blood samples throughout the duration of the visit. Insulin infusion will be stopped. Baseline blood, spot urine, and adipose tissue will be collected immediately after stopping insulin. One of the treatment arms will be administered to the patient according to their original group assignment as in Visit 2. All samples and monitoring procedures will be done as in Visit 2. Insulin doses will be titrated to target blood glucose between 70-180 mg/dl if

possible at all times without having additional hypoglycemia (less than 70 mg/dl) before discharge from study.

Phone calls will be made by our team at weeks 2, 6 and 10 to ensure safety. Patients will also be instructed to call if they develop unexpected symptoms.

Procedures:

1. **Ketone bodies measurements:** Serum and plasma samples will be collected from whole blood within 30 min and stored at -80C till analysis. Spot and 24 hr urine samples will be centrifuged and clear supernatant stored at -80C till analysis. Plasma and urine samples will be analyzed for ketone bodies using commercially available kits for β-hydroxybutyrate (Cayman Chemicals, Ann Arbor, MI) and for acetoacetate (Biovision, Malpitas, CA). Patients will be instructed to measure ketone bodies in urine samples daily with Ketostix (Bayer).
2. **MNC isolation:** Blood samples (30-40ml) is collected in Na-EDTA and carefully layered on Lympholyte medium (Cedarlane Laboratories, Hornby, ON) according to manufacturer's instructions. Samples are centrifuged and two bands separate out at the top of the RBC pellet. The MNC band was harvested and washed twice with Hank's balanced salt solution (HBSS). This method provides yields greater than 95% MNC preparation.
3. **Fat aspiration procedure:** Subcutaneous fat tissue aspiration will be performed on abdomen at a 10 cm distance from umbilicus. The subjects will not have taken aspirin or NSAIDS in the last 72 hours. If that is the case, then the procedure will not be done. The skin will first be prepared with povidone-iodine (Betadine) and alcohol. A sterile drape will be placed around the appropriate area. 3 cc of 1% lidocaine will then be administered subcutaneously. After adequate anesthesia has been achieved, approximately 20-50cc of 0.5% lidocaine will be injected in the adipose tissue. Dose of lidocaine will not exceed 4.5mg/kg body weight. Aspiration of fat tissue will then be performed with a 3-holed cannula (Tulip Instrumentation, length: 15cm, diameter: 2.1mm) fixed to a 10mL syringe. More than one attempt at aspiration can be done at the same site during the procedure to get adequate sample. After getting adequate fat tissue (500mg-3g), the puncture site will be pressed for at least ten minutes before the patient rises up from supine position (to minimize bruising). The study subjects will then be discharged home. The adipose tissue will be centrifuged to remove blood and fluid contaminants. The upper adipose tissue will be collected into a separate sterile tube, washed twice with cold sterile Phosphate Buffered Saline (PBS) and centrifuged to remove the PBS. The adipose tissue sample will be weighed and approximately 500 mg transferred to a separate tube for analysis. Total RNA, nuclear extracts and total cell lysates will be prepared from the adipose tissue.
4. **Quantification of HSL and SGLT2 Expression:** The mRNA expression is measured in MNC by RT-PCR: Total RNA was isolated from MNC and adipose tissue using commercially available RNAqueous®-4PCR Kit (Ambion, Austin, TX). One μg of total RNA is reverse transcribed using Advantage RT-for-PCR Kit

(Clontech, CA). Real Time RT-PCR was performed using Stratagene Mx3000P QPCR System (La Jolla, CA), Sybergreen master mix (Qiagen, CA) and gene specific primers (Life Technologies, MD).The specificity and the size of the PCR products are tested by adding a melt curve at the end of the amplifications and by running it on a 2% agarose gel. All values were normalized to the expression of a group of housekeeping genes including actin, ubiquitin C and cyclophilin A.

5. **Western blotting:** MNC total cell lysates are prepared by suspending the cells in 150 µL of lysis buffer (10mM Tris-HCl, 150mM NaCl, 0.2% Triton X and proteases and phosphatases inhibitors). After 30 min incubation on ice, samples are centrifuged at 12000 Xg for 10 min, supernatants are collected and total protein concentrations are determined. Sixty µg of total cell lysate are boiled in 2X SDS buffer (100 mM Tris-HCl, 4% SDS, 20% glycerol, 0.06 % bromophenol blue and 200 mM DTT), proteins separated by SDS-PAGE and then transferred to PVDF membrane. Polyclonal or monoclonal antibodies against SGLT2 and HSL, (Abcam, Cambridge, MA) and actin (Santa Cruz Biotechnology, CA) will be used and the membranes are developed using super signal, chemiluminescence reagent (Pierce Chemical, IL). Densitometry is performed using molecular analyst software (Biorad, CA) and all values are corrected for loading with actin.
6. **Plasma glucose, insulin, glucagon, glycerol, HSL and FFAs measurements:** Glucose levels are measured in plasma by YSI 2300 STAT Plus glucose analyzer (Yellow Springs, Ohio). FFAs and glycerol levels are measured by a colorimetric assay (Roche, Richmond, VA) and (Cayman Chemicals, Inc) respectively. Active GLP-1 and glucagon will be measured using ELISA kit (Millipore, MA) from samples collected in DPP-IV inhibitor (Millipore, MA) and protease inhibitor cocktail (Sigma) to prevent GLP-1 and other peptides degradation. HSL assay in plasma and adipose tissue will be done using ELISA (Biomatik, Wilmington, DE). Cortisol, electrolytes, and catecholamines will measured by standard assays by Quest Diagnostic Labs.

10.2 Describe what data will be collected.

NOTE: For studies with multiple data collection points or long-term follow up, consider the addition of a schedule or table in your response.

Response:.

Measurements of beta-hydroxybutyrate, acetoacetic acid, glucose, FFA and glucagon, will be measured from all blood samples collected. HSL, GLP-1, glycerol, electrolytes, serum bicarbonate, cortisol and catecholamines will be measured at 0, 60, 120, 240, 360, 480 min and at 24hr following single dose studies at 0 and 12 weeks visits and at baseline on the 1, 4 and 8 weeks visits. Ketone bodies will be measured in all urine samples. All MNC and adipose tissue samples will be tested for HSL and SGLT2 expression, as in the table below.

Visit #	Screening	*1*	*2*	*3*	*4*	*5*	*6*
Day #		*-7*	0	8 week 1	28 week 4	56 week 8	84 week12
Study Procedures	Screening	*Insulin titration*	Single dose test	2 days after up titration			End long term/ Single dose test
History	X						
Physical	X				X		*X*
CBC	X						X
CMP/lipid profile	X				X		X
Vitals and BP	X	X	X	X	X	X	X
Pregnancy test	X				X	X	X
HbA1c	X						X
Plasma ketone bodies and metabolic end points			X	X	X	X	X
Urine ketone bodies and oxidative stress end points			X	X	X	X	X
Insulin injection log/Glucose/CGM/pump and food diary review/Insulin dose titrations		*X Review of glucose/ Insulin doses*	X	X	X	X	X
Adipose tissue biopsy			X				X

10.3 List any instruments or measurement tools used to collect data (e.g. questionnaire, interview guide, validated instrument, data collection form).

Include copies of these documents with your submission.

Response: Source documents will be used to collect patient information. The CGM or insulin pump will be used to collect glucose and insulin data, Glucose and ketone meter will be used to measure glucose and ketone in blood. Ketostix for measuring ketones in urine . Food diary will be use to collect food intake.

10.4 Describe any source records that will be used to collect data about subjects (e.g. school records, electronic medical records).

Response: electronic medical records and research files.

*10.5 Indicate whether or not **individual** subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings will be shared with subjects or others (e.g., the subject's primary care physician) and if so, describe how these will be shared.*

Response: Individual participant lab results will be disclosed to the participant upon their request. If the participant requests documentation be shared with another physician, physician office or hospital the participant must come to the research center to collect said documentation or the documentation can be mailed to their given home address.

*10.6 Indicate whether or not **study** results will be shared with subjects or others, and if so, describe how these will be shared.*

Response: Not Applicable. Study results will not be shared with the subjects. However, unidentifiable study results could be published in the form of a manuscript or abstract and will be reported to Astra Zeneca and to clinicaltrials.gov

11.0 Study Timelines

11.1 Describe the anticipated duration needed to enroll all study subjects.

Response: Accrual is expected to be at the rate of 1 subject weekly, thus we should complete enrollment in approximately 18-20 months. We expect to complete all study activities in 30 months.

11.2 Describe the duration of an individual subject's participation in the study. Include length of study visits, and overall study follow-up time.

Response: 12 Weeks

11.3 Describe the estimated duration for the investigators to complete this study (i.e. all data is collected and all analyses have been completed).

Response: 30 Months

12.0 Setting

12.1 Describe all facilities/sites where you will be conducting research procedures. Include a description of the security and privacy of the facilities (e.g. locked facility, limited access, privacy barriers). Facility,

department, and type of room are relevant. Do not abbreviate facility names.

NOTE: Examples of acceptable response may be: "A classroom setting in the Department of Psychology equipped with a computer with relevant survey administration software," "The angiogram suite at Buffalo General Medical Center, a fully accredited tertiary care institution within New York State with badge access," or, "Community Center meeting hall."

Response: Research will be conducted at the Diabetes Endocrinology Research Center of WNY, located at *1000 Youngs Road, Suite 105, Williamsville NY 14221* and at the CTRC located in 875 Ellicott St. Buffalo NY14203.The Diabetes Research Center has facilities and exam rooms available for insulin pump download, CGM device download, meal and infusion studies and presence of study coordinator and registered nurse for data collection and blood work at all times. One of the investigators will be available at all times to address patients' related issues. CTRC location is a fully equipped laboratory with equipment including ultra-low freezers for sample storage, centrifuges, microscopes for sample preparation, infusion pumps, ELISA, PCR and immunoblotting instrumentation. Patients will also be consented at UBMD Pediatrics. 1001 Main Street 5th floor Buffalo NY 14203. Once patients review the consent, and all of their questions are answered, they will be asked to sign the consent. They will then be asked to come to the Diabetes Research Center to be screened and partake in the study. UBMD Pediatrics will only be used as a site for recruiting and consenting; no study procedures will take place here.

12.2 For research conducted outside of UB and its affiliates, describe:

- *Site-specific regulations or customs affecting the research*
- *Local scientific and ethical review structure*

NOTE: This question is referring to UB affiliated research taking place outside UB, i.e. research conducted in the community, school-based research, international research, etc. It is not referring to multi-site research. UB affiliated institutions include Kaleida Health, ECMC, and Roswell Park Cancer Institute.

Response:

☒ **N/A:** This study is not conducted outside of UB or its affiliates.

13.0 Community-Based Participatory Research

13.1 Describe involvement of the community in the design and conduct of the research.

NOTE: Community-Based Participatory Research (CBPR) is a collaborative approach to research that equitably involves all partners in the research process and recognizes the unique strengths that each brings. CBPR begins with a research topic of importance to the community, has the aim of combining

knowledge with action and achieving social change to improve health outcomes and eliminate health disparities.

Response:

☒ **N/A:** This study does not utilize CBPR.

13.2 Describe the composition and involvement of a community advisory board.

Response:

☒ **N/A:** This study does not have a community advisory board.

14.0 Resources and Qualifications

*14.1 Describe the qualifications (e.g., education, training, experience, expertise, or certifications) of the Principal Investigator **and** staff to perform the research. When applicable describe their knowledge of the local study sites, culture, and society. Provide enough information to convince the IRB that you have qualified staff for the proposed research.*

NOTE: If you specify a person by name, a change to that person will require prior approval by the IRB. If you specify a person by role (e.g., coordinator, research assistant, co-investigator, or pharmacist), a change to that person will not usually require prior approval by the IRB, provided that the person meets the qualifications described to fulfill their roles.

Response: All study personnel are educated, trained, and licensed as required for their delegated role in this study. All study personnel have also received the required university training and will be trained by the PI before the study starts

Describe other resources available to conduct the research.

14.2 Describe the time and effort that the Principal Investigator and research staff will devote to conducting and completing the research.

NOTE: Examples include the percentage of Full Time Equivalents (FTE), hours per week. The question will elicit whether there are appropriate resources to conduct the research.

Response: The principal investigator supervises the research project and weekly research meetings are conducted to discuss the recruitment rate, resolve and discuss issues related to the conduct, safety, analysis of the study and related publications. PI is expected to spend 5% of his academic time on this research. The co-investigators and study coordinator provide coverage to the research related activity for 365 days a year.

14.3 Describe the availability of medical or psychological resources that subjects might need as a result of anticipated consequences of the human research, if applicable.

NOTE: One example includes: on-call availability of a counselor or psychologist for a study that screens subjects for depression.

Response: Available medical literature will be provided as deemed appropriate or requested by patient through UB libraries, Pubmed, Google scholar as all the investigators have access to medical literature through listed resources above

The patient will also have access to physician (Investigators and Co-Investigators) who will be available to address any adverse effects or other questions during the course of the study who will be available to address any adverse effects or other questions during the course of the study

14.4 Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions.

Response: Education through training meetings, conferences and discussions

15.0 Other Approvals

15.1 Describe any approvals that will be obtained prior to commencing the research (e.g., school, external site, funding agency, laboratory, radiation safety, or biosafety).

Response: NA

☒ **N/A:** This study does not require any other approvals.

16.0 Provisions to Protect the Privacy Interests of Subjects

16.1 Describe how you will protect subjects' privacy interests during the course of this research.

NOTE: Privacy refers to an individual's right to control access to him or herself. Privacy applies to the person. Confidentiality refers to how data collected about individuals for the research will be protected by the researcher from release. Confidentiality applies to the data.

Examples of appropriate responses include: "participant only meets with a study coordinator in a classroom setting where no one can overhear", or "the participant is reminded that they are free to refuse to answer any questions that they do not feel comfortable answering."

Response: Our clinical providers involved in the study will identify potential patients for recruitment from the Diabetes-Endocrinology Center of WNY Clinics according to the inclusions and exclusion criteria and through advertisements. Patient who qualify will be

asked in private during their one on one consultation time with the physician if they wish to participate in the research study. If the patient agrees, the research coordinator will contact them for a telephone screening privately. The patients who call for potential participation in the study due to advertisement flyers will be screened over the phone with the research coordinator, using our telephone screening form.

When the patient is being seen at our clinics for the first time they sign the "Consent to use and disclosure of protected health information" form which clearly states that their protected health information (PHI) can be used for review in preparation for possible research.

If the patient passes the telephone screening, they will be asked to make an appointment to review and sign the consent. Patient will do this in a private, screen off area of the research department and will be allowed to discuss the consent in detail with the research coordinator and or study doctor. Patient will be no notified that it is completely voluntary to participate in the research study and can withdraw at any time.

We will not be accessing any medical information of the patients for whom the services are not provided by our clinic providers.

16.2 Indicate how the research team is permitted to access any sources of information about the subjects.

NOTE: Examples of appropriate responses include: school permission for review of records, consent of the subject, HIPAA waiver. This question ***does apply*** *to records reviews.*

Response: Consent of the subject and partial HIPAA waiver.

17.0 Data Management and Analysis

17.1 Describe the data analysis plan, including any statistical procedures. This section applies to both quantitative and qualitative analysis.

Response: **DATA ANALYSIS**

This is a prospective study to evaluate ketogenic stress before and after 12 week treatment with dapagliflozin and exenatide/Bydureon. The similarities in baseline values between the study groups will be compared using appropriate parametric tests. Transformations of the data on order to meet statistical assumptions may be considered. All statistical analysis will be carried out using SPSS software (SPSS Inc, Chicago, Illinois) based on intention to treat principle. Data will be presented as mean± standard error. *Data will not be imputed to replace missing data and only observed data will used to run the mixed model for repeated measurement (MMRM) analysis.* The primary endpoint of the study is to detect a significant difference in the percent change in beta-hydroxybutyrate and acetoacetic acid (total ketone bodies) in the plasma samples following 12 weeks of dapagliflozin treatment compared to placebo. *Fasting samples collected at weeks 0, 1, 4, 8 and 12 will be used for this comparison with values at week 0 considered as baseline. The statistical analysis will be done using MMRM* with assigned α value of 0.05. There is no previous data on effects of dapagliflozin alone on ketone bodies generation in T1D but our preliminary data on 12 weeks treatment with

dapagliflozin in patients with T1D using insulin and liraglutide shows more than a 2 fold increase in total ketone bodies.

The secondary end points include the *comparison of the acute changes in $AUC_{0hr-6hr}$ of ketone bodies (plasma and urine), FFA and glucagon between dapagliflozin, exenatide, combination of both and placebo before and after 12 weeks of treatment period using Paired t-test for within group comparisons and student t-test for between groups comparisons.* Additional secondary end points include comparison of basal plasma glucagon, FFA and urinary ketone bodies changes after 12 weeks of dapagliflozin and Bydureon or combination of both treatments compared to baseline using paired t-test and to placebo using 2-way RMANOVA. Changes in insulin dose, glucose levels and carbohydrate intake will be compared after 12 weeks of dapagliflozin and Bydureon treatments to baseline using paired t-test and to placebo with 2-way RMANOVA. Changes in lipolysis mediator (HSL) in plasma and expression of HSL and SGLT-2 in adipose tissue and MNC will be compared to baseline using paired t-test and to placebo with t-test.

17.2 If applicable, provide a power analysis.

NOTE: This may not apply to certain types of studies, including chart/records reviews, survey studies, or observational studies. This question is asked to elicit whether the investigator has an adequate sample size to achieve the study objectives and justify a conclusion.

Response: Conservatively estimating a difference in the change from baseline in ketone bodies generation between the dapagliflozin and placebo of 50%, with standard deviation of no more than 50%, a sample size of 20 patients per group (assuming a drop-out rate of 15%) should provide adequate power ($\beta = 0.2$) to detect a significant difference ($\alpha = 0.05$). A total of 80 patients will be recruited for the study.

17.3 Describe any procedures that will be used for quality control of collected data.

Response: Data will not be imputed to replace missing data and only observed data will used to run the mixed model for repeated measurement (MMRM) analysis

18.0 Confidentiality

A. Confidentiality of Study Data

*Describe the local procedures for maintenance of confidentiality of **study data and any records that will be reviewed for data collection**.*

*18.1 A. Where and how will all data and records be stored? Include information about: password protection, encryption, physical controls, authorization of access, and separation of identifiers and data, as applicable. Include physical (e.g. paper) **and** electronic files.*

Response: All patients' data records will be stored on password protected computers and or in locked cabinets within the research department. The research unit is locked after 5pm daily and all day on weekends. Identifiable patient information along with randomization information for each patient will be stored in locked cabinets in a locked archive room. This will only be accessible by study coordinator and the PI. Electronic data will be stored on password protected computers as coded data based on randomization number eg R-12 without any patients identifiable information attached. These electronic files will only be accessible by authorized study personnel.

18.2 A. How long will the data be stored?

Response: Data and specimens storage has no expiration date and will be stored for a minimum of 7 years. The researchers may continue to rely on this for future use in research study. Identifiable patient information will be destroyed after 3 years of study completion.

18.3 A. Who will have access to the data?

Response: Those physicians, nurses, and laboratory staff that are on all documentation for the study will have access to the data and specimens

18.4 A. Who is responsible for receipt or transmission of the data?

Response: Those physicians, nurses, and laboratory staff that are on all documentation for the study will have access to the data and specimens and can handle transfer of data and samples

18.5 A. How will the data be transported?

Response: All data are stored at one location and is not transported unless it is being archived. At that point files will be transferred to Iron Mountain for storage and archiving.

B. Confidentiality of Study Specimens

*Describe the local procedures for maintenance of confidentiality of **study specimens**.*

☐ **N/A:** No specimens will be collected or analyzed in this research. *(Skip to Section 19.0)*

18.6 B. Where and how will all specimens be stored? Include information about: physical controls, authorization of access, and labeling of specimens, as applicable.

Response: The specimens will be stored in the CRC laboratory located at 1000 Youngs Road, Suite 105, Williamsville NY 14221 and at the CTRC located in 875 Ellicott St. Buffalo NY14203. Samples will be stored in a locked -80^{o} C freezers. Ultimately, all specimens will be transported to CTRC location for banking. Specimens will be labeled as a coded sample, using the patient's randomization number (e.g. R-12), visit time and number and sample type. Specimens will not be stored with any patient identifiable information which is kept in locked cabinets in the CRC at Youngs Rd.

18.7 B. How long will the specimens be stored?

Response: Data and specimens storage has no expiration date and will be stored for a minimum of 7 years. The researchers may continue to rely on this for future use in research study

18.8 B. Who will have access to the specimens?

Response: Those physicians, nurses, and laboratory staff that are on all documentation for the study will have access to the data and specimens

18.9 B. Who is responsible for receipt or transmission of the specimens?

Response: : Those physicians, nurses, and laboratory staff that are on all documentation for the study will have access to the data and specimens and can handle transfer of data and samples

18.10 B. How will the specimens be transported?

Response: All data are stored at one location and is not transported unless it is being archived. At that point files will be transferred to Iron Mountain for storage

and archiving. Samples that are transported will be done so using dry ice in a properly labeled Styrofoam container by the laboratory technician

19.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

☐ **N/A:** This study is not enrolling subjects, or is limited to records review procedures only. This section does not apply.

NOTE: Minimal risk studies may be required to monitor subject safety if the research procedures include procedures that present unique risks to subjects that require monitoring. Some examples include: exercising to exertion, or instruments that elicit suicidality or substance abuse behavior. In such cases, N/A is not an acceptable response.

19.1 Describe the plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe.

Response: Dr. Robert Borowski, an independent clinician has been designated to provide review of accumulating safety date. Along with the principal investigator Paresh Dandona, MD, PhD and co-investigators Husam Ghanim, PhD and Antoine Makdissi, MD they will review the data every 3 months to assess the safety and potential benefits to the participant. Furthermore, they will also assess other risks including the physical, psychological, social, legal and economic harm to these patients. The investigators listed above will carefully watch for any invasion of privacy and breach of confidentiality. The principal investigator will be sharing the results of safety analysis of the study with the sponsor (AZ) and to the IRB. The study groups will remain blinded. If there are any safety concerns, Dr. Ghanim (PhD), who is not directly involved in patients care or insulin titrations, is designated to unblind the study groups based on the discretion of the principal investigator and the recommendation by the independent monitor. Dr Ghanim role will be to tabulate and present unblinded data to the investigators and Dr. Borowski for review. Based on review of the data presented, assessment of potential harm to the patients will be carried out to determine the best course of action. The IRB and sponsor will be informed of this potential harm along with the recommended course of action. Research subjects will be withdrawn from the study if risks outweigh the benefits (see subject withdrawal section). The IRB will be kept well-informed at all times

19.2 Describe what data are reviewed, including safety data, untoward events, and efficacy data.

Response: Patients CGM, dietary intake, BP, weight are monitored every visit to ensure safety. CGM graphs will be evaluated every visit to reduce risk of hypoglycemia or hyperglycemia. STAT CMP will be done when glucose or

ketones exceeds 400mg/dl and 2.5mM, respectively or any signs of DKA is observed during the acute testing phase and will be reviewed the same day. Safety assessments will consist of monitoring and recording all TEAEs, SAEs, AEs leading to discontinuation/withdrawal from study, laboratory evaluation for hematology, blood chemistry, and urine values; pregnancy testing; measurement of vital signs; and performance of physical examinations.

19.3 Describe any safety endpoints.

Response: Safety data (vitals, CBC, CMP, urinary markers) will be collected at screening, during and at end of the study. *STAT CMP will be done when glucose or ketones exceeds 400mg/dl and 2.5mM, respectively or any signs of DKA is observed during the acute testing phase.*

19.4 Describe how the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with participants).

Response: The safety information will be collected at the time of the participants visit, and or during telephone calls with the participant.

19.5 Describe the frequency of safety data collection.

Response: The data collection will be done at all study visits which will be at intervals of either one or two weeks depending on the number of study visit. The patients, however, will be asked to report any adverse event or safety related information via phone as soon as it occurs and it will be reviewed the same day.

19.6 Describe who will review the safety data.

Response: The independent clinician monitor along with the principal investigator Paresh Dandona, MD, PhD and co-investigators, Dr Ajay Chaudhuri, MD, Antione *Makdissi, MD* and Husam Ghanim, PhD will review the data at the completion of all visits by each subject and every 3 months to assess the safety and any potential risks to the participants. Furthermore they will also assess other risks including the physical, psychological, social, legal and economic harm to these patients. The investigators listed above will carefully watch for any invasion of privacy and breach of confidentiality. The principal investigator will be sharing the results of safety analysis of the study with the sponsor (AZ) and with the IRB. The study groups will remain blinded. If there are any safety concerns then co-investigator Husam Ghanim, PhD who is not directly involved with the study participants will unblind the study groups on the discretion of principal investigator and present the analyzed data to the clinician monitor and investigators.

19.7 Describe the frequency or periodicity of review of cumulative safety data.

Response: Safety data will be reviewed every 3 months. Study endpoint data will be reviewed once after half of the recruited patients have completed the study and then at the end of the study.

19.8 Describe the statistical tests for analyzing the safety data to determine whether harm is occurring.

Response: The statistical analysis will be carried out using the student t-test or Wilcoxson's test for paired data

19.9 Describe any conditions that trigger an immediate suspension of the research.

Response:

1. Any episode of DKA occurring in >15% (placebo-adjusted) of participants.
2. Pancreatitis occurring in > 5 % (placebo-adjusted) of participants
3. New information becoming available concerning the safety of study drugs
4. Termination of funding.

20.0 Withdrawal of Subjects

☐ **N/A:** This study is not enrolling subjects. This section does not apply.

*20.1 Describe **anticipated** circumstances under which subjects may be withdrawn from the research without their consent.*

.

a. Patient specific
    i. Pregnancy
    ii. Developing conditions included in the exclusion criteria
    iii. Subjects decision to withdraw from the study
    iv. Change in method of insulin delivery between insulin pumps to MDI or vice versa during the study. Also, patients who upgrade to the new "artificial pancreas" pump (Medtronic's MiniMed 670G hybrid) during the study will be withdrawn.
    v. Side effects specific to study drugs
        1. 2 episodes of UTI.
        2. 2 episodes of mycotic genital infection.
        3. Significant persistent change in eGFR > 15 ml/min
        4. Significant nausea (defined as interfering with normal daily activities). *Those with mild or moderate nausea will continue for at least one more week. If they continue to experience significant nausea (defined as interfering with normal activities), they will discontinue the study.*

vi. DKA

Two episode of DKA (with no identifiable precipitating factors) as defined in the ADA (position statement on hyperglycemic crisis in diabetes (Diabetes Care, Volume 27, Supplement 1, January 2004)

vii. Pancreatitis

1. One episode of pancreatitis.

20.2 Describe any procedures for orderly termination.

NOTE: Examples may include return of study drug, exit interview with clinician. Include whether additional follow up is recommended for safety reasons for physical or emotional health.

Response: The principal investigator of the study can remove a participant from the research study without their approval if for any reason he/she feels is appropriate, including: severe side effect, injury or medical condition which may place you at risk of further complications if you continue to participate, failure to take the medication as instructed, failure to keep your scheduled appointments, cancellation of the study by the sponsor, or other administrative reasons.

If any of the subjects become pregnant during the period of study, they will need to withdraw from the study.

20.3 Describe procedures that will be followed when subjects withdraw from the research, including retention of already collected data, and partial withdrawal from procedures with continued data collection, as applicable.

Response: If a subject withdraws from the research, the data collected to that point will be used toward the research finding. If applicable the subject will have to bring back any unused research drug and or device. If necessary, they will be asked to complete an end of study visit for their safety.

21.0 Risks to Subjects

21.1 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related to their participation in the research. Consider physical, psychological, social, legal, and economic risks. Include a description of the probability, magnitude, duration, and reversibility of the risks.

NOTE: Breach of confidentiality is always a risk for identifiable subject data.

Response: **Risks Associated with Administration of Intravenous (IV) line, adipose tissue and blood collection:**
All subjects will be informed of the complication of administration of intravenous (IV) line, which includes mild bruising at the site of IV line, which should resolve in few days. They will also be informed about the possibility of infiltration of the IV line at the time of

performing blood draws in which case another IV line at different site will be secured and this may lead to bruising at more than one sites. Fat tissue aspiration will probably lead to a bruise at the site of aspiration. The site will therefore maybe painful for 1-2 weeks. Subjects are advised to call us if they have a lot of pain or swelling at the site after the procedure. Rarely some people have side effects such as low blood pressure or heart rate and allergic reaction to lidocaine including swelling of the throat. Serious risks associated with IV puncture or adipose tissue biopsy may also include infections and thrombosis. If any of these serious side effects is observed, the patients will be asked to call us immediately or seek immediate medical help.

Risks Associated with Administration of study drugs:

All subjects will be referred to Bydureon (extended release exenatide) and dapagliflozin labeling safety information, and will be provided a copy of the package insert of the medication. Discussion of the potential side effects and the different warning and precautions will take place at the time of signing the informed consent and prior to treatment. **Patients will be informed they must not discontinue their insulin during the study.**

Potential side effects of GLP-1 agonist and SGLT-2 inhibitors include hypotension, nausea, polydipsia, polyuria, dehydration, reduced GFR. Patients will be provided guidance on potential side effects and how to properly manage and report it. The potential benefits including improved glycemia, improved BP, potential renal protection, improved quality of life (QOL) will most probably overcome the potential side effects.

Bydureon caused rats to develop tumors of the thyroid gland. Some of these tumors were cancer. It is not known if Bydureon will cause thyroid tumors or a type of thyroid cancer called medullary thyroid cancer in people. This will be discussed in details with all participating subjects. Exenatide is contraindicated in patients with a personal or family history of MTC and in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2).

Injection-site reactions: Serious injection-site reactions, with or without bumps (nodules), have happened in some people who use BYDUREON. Some of these injection-site reactions have required surgical intervention. Report any injection site reactions including nodules to study investigators.

All the known adverse effects with SGLT-2 inhibitors will be discussed with patients before the start of the study as follows:-

- Intravascular volume contraction and symptomatic hypotension can occur after initiating SGLT-2 inhibitors.
- Impairment in Renal Function - SGLT-2 inhibitors increases serum creatinine and decreases eGFR. Elderly patients and patients with impaired renal function may be more susceptible to these changes.
- Genital mycotic Infections - SGLT-2 inhibitors increases the risk of genital mycotic infections. Patients with prior history were more likely to develop genital mycotic infections.

- Bladder Cancer - Across 22 clinical studies, newly diagnosed cases of bladder cancer were reported in 10/6045 patients (0.17%) treated with Dapagliflozin and 1/3512 patient (0.03%) treated with placebo. After excluding patients in whom exposure to study drug was less than one year at the time of diagnosis of bladder cancer, there were 4 cases with dapagliflozin and no cases with placebo. There were too few cases to determine whether the emergence of these events is related to Dapagliflozin.
- The dapagliflozin tablets and matching placebo contain lactose, which may cause discomfort in lactose-intolerant individuals.

There is a risk of DKA, as shown by some studies and by our preliminary data, when SGLT2 inhibitors were used in type 1 diabetes. In our own preliminary studies on triple therapy, we have observed euglycemic ketoacidosis. This has led us to analyze the occurrence of DKA. We found that the patients, who developed DKA, had had their insulin doses reduced to less than or equal to 0.5 units/Kg body weight. Thus, this phenomenon is in part due to insulinopenia and in part due to effects of SGLT2 inhibitors at the hepatic level, which are anti-insulin in nature, like increased gluconeogenesis. On the basis of our data, we shall also not allow the dose of insulin to fall below 0.5 units/Kg body weight. Furthermore, we shall use only the lower dose of dapagliflozin (5mg) in patients who exhibit significant ketonuria with the lower dose.

Severe allergic reaction (anaphylaxis) to study drugs or placebo ingredients or other interventions may accrue in rare cases. Epinephrine is the primary treatment for anaphylaxis with no absolute contraindication to its use. Our site and staff are trained and equipped to assist patients with such reactions. Severe conditions that are not satisfactory managed in site will be transferred to the hospital emergency department for further assessment and help.

21.2 Describe procedures performed to lessen the probability or magnitude of risks, including procedures being performed to monitor subjects for safety.

Response:
Safety data (vitals, CBC, CMP, urinary markers) will be collected at screening, during and at end of the study. STAT CMP will be done when glucose or ketones exceeds 400mg/dl and 2.5mM, respectively or any signs of DKA is observed during the acute testing phase. If, ketones measure positive in blood or in urine (≥0.4 mM in blood or >+1 in urine) or fasting blood glucose <70 or >250 mg/dl, the acute testing will not take place and patient will be rescheduled. Injection site reactions, mycotic infections and complications of IV and biopsy collection will also be monitored, volume control and hypoglycemia will be monitored and collected at every visit. Physical examination will be performed at 2, 4, 8 and 12 weeks visits and safety assessments performed. Ketone bodies will measured daily by patient in urine. LDL-C levels will be monitored at screening visit, 4 and 12 weeks. Pregnancy will be tested at screening, 4 and 12 weeks. Insulin dose titration will done at every visit to avoid hypoglycemia. Phone calls will be made for safety and compliance follow-up at least 3 time during the study. The patients, however, will be instructed to

report any adverse event or safety related information via phone as soon as it occurs. All safety data will be reviewed by the PI or sub-investigators within 1-2 days of collection.

If the BG concentrations exceed 400 mg/dl or blood β-hydroxybutyrate exceeds 2.5mmol/l (or +3 (80mg/dl) or greater in urine) anytime during the 360 minutes following single dose/doses with no clinical signs and symptoms of DKA like nausea and vomiting then blood sample, spot urine and adipose tissue biopsy will be collected immediately, basal insulin will be restarted; bolus insulin as per the bolus wizard calculator will be administered and a meal provided. Patients will be instructed to come back at the 24-hour mark.

Patients will be instructed to measure ketone bodies in urine samples daily with Ketostix (Bayer) and record it in diary provided. If ketone bodies in urine measure +2 or greater (>4mM in urine), patients are required to notify the research center.

The lower dose of 5µg exenatide will be used for the acute effects testing part of the study. This will help to reduce the risk of GI side effects which commonly happen when first starting exenatide and is increased with the higher dose. Such side effects including nausea, vomiting, diarrhea, feeling jittery, dizziness, headache, acid stomach, constipation, and weakness may lead to, in addition to increase risk and discomfort to patients, increased drop-out rates and erroneous results. This is in part is based on our experience conducting these acute single dose studies.

*21.3 If applicable, indicate **which procedures** may have risks to the subjects that are currently unforeseeable.*

Response:

Any adverse effects of dapagliflozin or Exenatide not currently known or those unique to their use in Type 1 diabetes may be some of the unforeseeable risks.

21.4 If applicable, indicate which research procedures may have risks to an embryo or fetus should the subject be or become pregnant.

Response: Not applicable, we will not be enrolling pregnant participants or who may become pregnant or with in child baring years without signing the consent stating they will use at least two forms of birth control. If a participant becomes pregnant they will be withdrawn from the study immediately.

21.5 If applicable, describe risks to others who are not subjects.

Response: Not applicable, there is no risk to others not participating in this study.

22.0 Potential Benefits to Subjects

22.1 Describe the potential benefits that individual subjects may experience by taking part in the research. Include the probability, magnitude, and duration of the potential benefits. Indicate if there is no direct benefit.

*NOTE: Compensation **cannot** be stated as a benefit.*

Response: There are no potential benefits individual participants will experience.

23.0 Compensation for Research-Related Injury

☐ **N/A:** The research procedures for this study do not present risk of research related injury (e.g. survey studies, records review studies). This section does not apply.

*23.1 **If the research procedures carry a risk of research related injury,** describe the available compensation to subjects in the event that such injury should occur.*

Response: Routinely, Buffalo General Hospital, Erie County Medical Center, and/or the University at Buffalo, State University of New York, its agents, or its employees do not compensate for or provide free medical care for human subjects/participants in the event that any injury results from participation in a human research project. In the unlikely event that they become ill or injured as a direct result of participating in this study, they may receive medical care, that will be covered by study.

23.2 Provide a copy of contract language, if any, relevant to compensation for research related injury.

*NOTE: If the contract is not yet approved at the time of this submission, submit the current version here. If the contract is later approved with **different language regarding research related injury**, you must modify your response here and submit an amendment to the IRB for review and approval.*

Response: *AstraZeneca shall reimburse the Institution for the direct, reasonable and necessary medical expenses incurred by the Institution for the treatment of any personal injury that is a direct result of adverse drug experiences to subjects resulting directly from any manufacturing defects in the Study Drug to which the Subjects would not have been exposed but for their participation in the Study if (i) the Institutional parties have complied with this Agreement, the Protocol and any written instructions of AstraZeneca concerning the Study Drug and (ii) all the requirements of informed consent have been complied with. AstraZeneca will not provide compensation for lost wages or for any other damages, expenses or losses, or for medical expenses that have been covered by a Subject's medical or other insurance.*

24.0 Economic Burden to Subjects

24.1 Describe any costs that subjects may be responsible for because of participation in the research.

NOTE: Some examples include transportation or parking.

Response: All research expenses will be covered. Participants will not be subjected to any out of pocket cost.

☐ **N/A:** This study is not enrolling subjects, or is limited to records review procedures only. This section does not apply.

25.0 Compensation for Participation

25.1 Describe the amount and timing of any compensation to subjects, including monetary, course credit, or gift card compensation.

Response:

Visit 1 Insulin adjustment $25,

*Visit 2 Single dose $175, Including 2 fat BX, ($50 per bx) and 6 blood draws.**

Visit 3 Blood Draw $50.00,

Visit 4 Blood draw $50.00,

Visit 5 Blood work $50.00,

*Visit 6 Single dose $175, Including 2 fat BX, ($50 per bx) and 6 blood draws.**

*Total compensation for all completed visits: $525.00**

**Those patients that are not on inulin pumps and do not partake in the acute testing will only be compensated for a baseline blood draw and one fat biopsy at Visit 2 and 6 for a total of $100 per visit. Total compensation for these patients that complete all visits will be $375.*

All study participants will receive a check in the mail for their time and effort.

☐ **N/A:** This study is not enrolling subjects, or is limited to records review procedures only. This section does not apply.

☐ **N/A:** There is no compensation for participation. This section does not apply.

26.0 Consent Process

26.1 Indicate whether you will be obtaining consent.

NOTE: This does not refer to consent documentation, but rather whether you will be obtaining permission from subjects to participate in a research study. Consent documentation is addressed in Section 27.0.

☒ ***Yes*** *(If yes, Provide responses to each question in this Section)*

☐ ***No*** *(If no, Skip to Section 27.0)*

26.2 Describe where the consent process will take place. Include steps to maximize subjects' privacy.

Response: All participants will come to 1000 Youngs Rd Williamsville, NY 14221 or 1001 Main Street 5th floor Buffalo, NY 14203 to be consented. The rest of the procedures will take place at 1000 Youngs Rd. Participants will be placed in a private, screened off area and or room where they can review the consent. Participant questions and or concerns will be address with a member of the study team or research doctor if applicable. The research coordinator will discuss in length the participants requests for privacy of their PHI.

26.3 Describe how you will ensure that subjects are provided with a sufficient period of time to consider taking part in the research study.

NOTE: It is always a requirement that a prospective subject is given sufficient time to have their questions answered and consider their participation. See "SOP: Informed Consent Process for Research (HRP-090)" Sections 5.5 and 5.6.

Response: participants will be made aware that participating in research is completely voluntary, and they may withdraw at any time with no consequence to their routine clinic care. If the patients requires time to decide and or discuss partaking in a research study, the subject will be given said time.

26.4 Describe any process to ensure ongoing consent, defined as a subject's willingness to continue participation for the duration of the research study.

Response: The research coordinator and study team are available to answer any question or concerns with the patient during the duration of the research trial. At each study visit, the patient is asked a series of questions to ensure they are on task with the study visits and feel comfortable. Upon departing from their study visit, the patients are told of their next visit and given detail instruction for their next visit. If study is revised or amendment or new information becomes available about drug safety that may affect patients participation, the patient may be re-consented to ensure patient ongoing consent

26.5 Indicate whether you will be following "SOP: Informed Consent Process for Research (HRP-090)." If not, or if there are any exceptions or additional details to what is covered in the SOP, describe:

- *The role of the individuals listed in the application who are involved in the consent process*
- *The time that will be devoted to the consent discussion*
- *Steps that will be taken to minimize the possibility of coercion or undue influence*
- *Steps that will be taken to ensure the subjects' understanding*

Response:

☒ We have reviewed and will be following "SOP: Informed Consent Process for Research (HRP-090)."

Non-English Speaking Subjects

☒ **N/A:** This study will not enroll Non-English speaking subjects. *(Skip to Section 26.8)*

26.6 Indicate which language(s) other than English are likely to be spoken/understood by your prospective study population or their legally authorized representatives.

NOTE: The response to this Section should correspond with your response to Section 6.4 of this protocol.

Response:

26.7 If subjects who do not speak English will be enrolled, describe the process to ensure that the oral and written information provided to those subjects will be in that language. Indicate the language that will be used by those obtaining consent.

NOTE: Guidance is provided on "SOP: Informed Consent Process for Research (HRP-090)."

Response:

Cognitively Impaired Adults

☒ **N/A**: This study will not enroll cognitively impaired adults. *(Skip to Section 26.9)*

26.8 Describe the process to determine whether an individual is capable of consent.

Response:

Adults Unable to Consent

☒ **N/A**: This study will not enroll adults unable to consent. (*Skip to Section 26.13)*

When a person is not capable of consent due to cognitive impairment, a legally authorized representative should be used to provide consent (Sections 26.9 and 26.10) ***and, where possible, assent of the individual should also be solicited*** *(Sections 26.11 and 26.12).*

26.9 Describe how you will identify a Legally Authorized Representative (LAR). Indicate that you have reviewed the "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)" for research in New York State.

NOTE: Examples of acceptable response includes: verifying the electronic medical record to determine if an LAR is recorded.

Response:

☐

26.10 ***For research conducted outside of New York State****, provide information that describes which individuals are authorized under applicable law to consent on behalf of a prospective subject to their participation in the research. One method of obtaining this information is to have a legal counsel or authority review your protocol along with the definition of "legally authorized representative" in "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)."*

Response:

26.11 Describe the process for ***assent of the adults****:*

- *Indicate whether assent will be obtained from all, some, or none of the subjects. If some, indicate which adults will be required to assent and which will not.*

Response:

- *If assent will not be obtained from some or all subjects, provide an explanation of why not.*

Response:

26.12 Describe whether ***assent of the adult*** *subjects will be documented and the process to document assent.*

NOTE: The IRB allows the person obtaining assent to document assent on the consent document using the "Template Consent Document (HRP-502)" Signature Block for Assent of Adults who are Legally Unable to Consent.

Response:

Subjects who are not yet Adults (Infants, Children, and Teenagers)

☒ **N/A**: This study will not enroll subjects who are not yet adults. *(Skip to Section 27.0)*

*26.13 Describe the criteria that will be used to determine **whether a prospective subject has not attained the legal age for consent to treatments or procedures involved in the research** under the applicable law of the jurisdiction in which the research will be conducted **(e.g., individuals under the age of 18 years)**. For research conducted in NYS, review "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)" to be aware of which individuals in the state meet the definition of "children."*

NOTE: Examples of acceptable responses include: verification via electronic medical record, driver's license or state-issued ID, screening questionnaire.

Response:

*26.14**For research conducted outside of New York State**, provide information that describes which persons have not attained the legal age for consent to treatments or procedures involved the research, under the applicable law of the jurisdiction in which research will be conducted. One method of obtaining this information is to have a legal counsel or authority review your protocol along the definition of "children" in "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)."*

Response:

26.15 Describe whether parental permission will be obtained from:

Response: N/A

☐ One parent even if the other parent is alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child.

☐ Both parents unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

☐ Parent permission will not be obtained. A waiver of parent permission is being requested.

NOTE: The requirement for parent permission is a protocol-specific determination made by the IRB based on the risk level of the research. For guidance, review the "CHECKLIST: Children (HRP-416)."

*26.16Describe whether permission will be obtained from individuals **other than parents**, and if so, who will be allowed to provide permission. Describe your procedure for determining an individual's authority to consent to the child's general medical care.*

Response:

*26.17 Indicate whether assent will be obtained from all, some, or none of the **children**. If assent will be obtained from some children, indicate which children will be required to assent.*

Response:

26.18 When assent of children is obtained, describe how it will be documented.

Response:

27.0 Waiver or Alteration of Consent Process

Consent will not be obtained, required information will not be disclosed, or the research involves deception.

☒ **N/A:** A waiver or alteration of consent is not being requested.

27.1 If the research involves a waiver or alteration of the consent process, please review the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" to ensure that you have provided sufficient information for the IRB to make the determination that a waiver or alteration can be granted.

NOTE: For records review studies, the first set of criteria on the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" applies.

Response:

27.2 If the research involves a waiver of the consent process for planned emergency research, please review the "CHECKLIST: Waiver of Consent for Emergency Research (HRP-419)" to ensure you have provided sufficient information for the IRB to make these determinations. Provide any additional information necessary here:

Response:

28.0 Process to Document Consent

☐ **N/A:** A Waiver of Consent is being requested.
(Skip to Section 29.0)

28.1 Indicate whether you will be following "SOP: Written Documentation of Consent (HRP-091)." If not or if there are any exceptions, describe whether and how consent of the subject will be obtained including whether or not it will be documented in writing.

NOTE: If your research presents no more than minimal risk of harm to subjects and involves no procedures for which written documentation of consent is normally required outside of the research context, the IRB will generally waive the

requirement to obtain written documentation of consent. This is sometimes referred to as 'verbal consent.' Review "CHECKLIST: Waiver of Written Documentation of Consent (HRP-411)" to ensure that you have provided sufficient information.

If you will document consent in writing, attach a consent document with your submission. You may use "TEMPLATE CONSENT DOCUMENT (HRP-502)". If you will obtain consent, but not document consent in writing, attach the script of the information to be provided orally or in writing (i.e. consent script or Information Sheet).

Response:

☒ We will be following "SOP: Written Documentation of Consent" (HRP-091).

29.0 Multi-Site Research (Multisite/Multicenter Only)

☒ **N/A:** This study is not an investigator-initiated multi-site study. This section does not apply.

*29.1 If this is a multi-site study **where you are the lead investigator**, describe the processes to ensure communication among sites, such as:*

- *All sites have the most current version of the IRB documents, including the protocol, consent document, and HIPAA authorization.*
- *All required approvals have been obtained at each site (including approval by the site's IRB of record).*
- *All modifications have been communicated to sites, and approved (including approval by the site's IRB of record) before the modification is implemented.*
- *All engaged participating sites will safeguard data as required by local information security policies.*
- *All local site investigators conduct the study appropriately.*
- *All non-compliance with the study protocol or applicable requirements will be reported in accordance with local policy.*

Response:

29.2 Describe the method for communicating to engaged participating sites:

- *Problems*
- *Interim results*
- *Study closure*

Response:

29.3 Indicate the total number of subjects that will be enrolled or records that will be reviewed across all sites.

Response:

29.4 If this is a multicenter study for which UB will serve as the IRB of record, and subjects will be recruited by methods not under the control of the local site (e.g., call centers, national advertisements) describe those methods.

Response:

30.0 Banking Data or Specimens for Future Use

☐ **N/A:** This study is not banking data or specimens for future use or research outside the scope of the present protocol. This section does not apply.

*30.1 If data or specimens will be banked (stored) for **future use, that is, use or research outside of the scope of the present protocol**, describe where the data/specimens will be stored, how long they will be stored, how the data/specimens will be accessed, and who will have access to the data/specimens.*

NOTE: Your response here must be consistent with your response at the "What happens if I say yes, I want to be in this research?" Section of the Template Consent Document (HRP-502).

Response: The study electronic data will be stored in a locked closet and password protected computers located in our research locations in Youngs Rd and CTRC. Data is backed up by university on daily bases. Specimens will be stored in -80 freezer at our CTRC research laboratory at 875 Ellicott St. 14203 for at least 7 years. Samples and electronic data will be labeled as a coded sample, using the patient randomization number (e.g. R12) and visit time and date. Electronic data and specimens will not be identifiable.

The research staff (study personnel including coordinator) only will be authorized to access data and or specimens

30.2 List the data to be stored or associated with each specimen.

Response: Patient ID number, study visit information and date of collection will be stored with specimen. Other data stored will include record files of all patients participating in the study, including data collection sheets, lab results, CGM and insulin pump data. No patient identifiers will be stored with specimen.

30.3 Describe the procedures to release banked data or specimens for future uses, including: the process to request a release, approvals required for release, who can obtain data or specimens, and the data to be provided with specimens.

Response: The copy of the individual patient data collected during the study period will be provided to these individual patients who can choose to hand

carry it to their respective physicians and a copy will be faxed to their respective clinical providers upon verbal request from the patient. The data provided will include the insulin pump or CGM data or any of the lab results obtained during the study period. The results of the completed study will be made available to the patients if requested through published manuscript. Banked samples will not be released to any other party or researchers.

31.0 Drugs or Devices

☐ **N/A:** This study does not involve drugs or devices. This section does not apply.

31.1 If the research involves drugs or devices, list and describe all drugs and devices used in the research, the purpose of their use, and their regulatory approval status.

Response:

Drugs used:

Cohort 1:

Description or Identity of investigational product(s)

Investigational product	***Dosage form and strength***	***Manufacturer***
Dapagliflozin 5 mg	*Green, plain, diamond shaped, film coated 5 mg tablet*	*AstraZeneca*
Matching placebo for dapagliflozin 5 mg	*Green, plain, diamond shaped, film coated tablet*	*AstraZeneca*
Exenatide vials 3ml (0.25mg/ml) solution	*Clear liquid in vials*	*AstraZeneca*
Matching placebo vials (3ml) for Exenatide vials	*Clear liquid in vials*	*AstraZeneca*
Exenatide vials 2.0 mg/vial	*2.0 mg/vial Powder for injection*	*AstraZeneca*
Matching placebo for Exenatide vials	*Powder for injection*	*AstraZeneca*

- *Dapagliflozin and its matching placebo tablets will be supplied in bottles.*
- *Exenatide extended release vials 2.0 mg/vial and matching placebo will be provided in one month kits containing:*
  - *One carton containing 4 vials of active or placebo powder*
  - *One carton containing 4 prefilled syringes with diluent, 5 vial adaptors and 8 needles.*
- *Exenatide for acute study with matching placebo will be provide in vials (3ml) of 0.25mg/ml for subcutaneous injection using 0.3ml Ultra-thin BD needles.* ***The lower dose of 5µg exenatide will be used for the acute effects testing part of the study. This will help to reduce the risk of GI side effects which commonly happen when first starting exenatide and is increased with the higher dose. Such side effects including nausea, vomiting, diarrhea, feeling jittery, dizziness, headache,***

acid stomach, constipation, and weakness may lead to, in addition to increase risk and discomfort to patients, increased drop-out rates and erroneous results. This is in part is based on our experience conducting these acute single dose studies.

Cohort 2:

Investigational product	***Dosage form and strength***	***Manufacturer***
Dapagliflozin 5 mg	*Green, plain, diamond shaped, film coated 5 mg tablet*	*AstraZeneca*
Exenatide pen 1.2ml (0.25mg/ml) solution	*Clear liquid in pens*	*AstraZeneca*
Exenatide pens 2.0 mg/pen	*2.0 mg/pen Powder for injection*	*AstraZeneca*

31.2 Describe your plans to store, handle, and administer those drugs or devices so that they will be used only on subjects and be used only by authorized investigators.

Response: Drugs will be stored in a locked cabinet and temperature controlled refrigerator at 4C at the research facility of the Diabetes and Endocrinology Center of WNY.

If the drug is investigational (has an IND) or the device has an IDE or a claim of abbreviated IDE (non-significant risk device), include the following information:

31.3 Identify the holder of the IND/IDE/Abbreviated IDE.

Response: IND application for use of all drugs (dapagliflozin, Bydureon and exenatide) in type 1 diabetes will be submitted to FDA and no study procedure will start before all local and federal approvals are obtained.

31.4 Explain procedures followed to comply with FDA sponsor requirements for the following:

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	***X***	***X***	
21 CFR 54	***X***	***X***	
21 CFR 210	***X***		
21 CFR 211	***X***		
21 CFR 312	***X***		
21 CFR 812		***X***	***X***
21 CFR 820		***X***	

Response: All FDA sponsor requirements have been reviewed and will be followed during the study procedures

32.0 Humanitarian Use Devices

☒ **N/A:** This study does not involve humanitarian use devices. This does not apply.

32.1 For Humanitarian Use Device (HUD) uses provide a description of the device, a summary of how you propose to use the device, including a description of any screening procedures, the HUD procedure, and any patient follow-up visits, tests or procedures.

Response:

32.2 For HUD uses provide a description of how the patient will be informed of the potential risks and benefits of the HUD and any procedures associated with its use.

Response: