A Phase II Trial to Examine the Effect of Subcutaneous Exenatide (Bydureon®) on Glucose Control in Patients with Type I Diabetes

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Synopsis

Title	A Phase II Trial to Examine the Effect of Subcutaneous Exenatide (Bydureon®) on Glucose Control in Patients with Type I Diabetes
Short Title	Bydureon in T1DM
Clinical Phase	Phase II
Sponsor	Kevan Herold, MD
Conducted by	Yale University, University of Chicago, University of Michigan, Barbara Davis Ctr, University of California at San Francisco, University of Miami, SUNY Upstate Medical University, and Joslin Diabetes Center.
Protocol Chair	Kevan Herold, MD
Accrual Objective	120 Subjects (60 Subjects in the Drug Treatment Group and 60 Subjects in the Placebo Group)
Study Design	This is a multi-site, randomized, placebo controlled, double blind trial of Bydureon in patients with T1DM of at least 2 years duration. Participants will be randomized in a 1:1 ratio to receive Bydureon 2mg x 1/week via subcutaneous injection or placebo over a 6 month treatment period. Subjects will be followed for an additional 6 months after completion of Bydureon or placebo treatment.
Study Duration	Total study duration will be approximately 48 Months (4 years): • Enrollment phase will be approximately 24 Months. • Study participation phase will be approximately 12 Months (1 year), which includes a treatment period of approximately 6 Months and a follow-up period of approximately 6 Months.
Primary Endpoint	Comparison of the change from baseline in HbA1c levels in the Bydureon vs. placebo treated groups at 6 months.
Secondary Endpoints	1. Comparison of the change in HbA1c levels, from baseline at 12 months (6 months off of Bydureon treatment. 2. Comparison between Bydureon treated and placebo groups at 6 and 12 months in insulin use : a. Safety, including frequency of severe hypoglycemia b. Insulin secretory responses to a MMTT (corrected for the response at study entry) c. Insulin Use (corrected for use at study entry) d. Glycemic Variability Calculated from 3 day Measurements of Glucose levels with a glucometer e. Markers of β cell stress including levels of circulating proinsulin:insulin and demethylated insulin DNA **f.** Rate of gastric emptying with measurement of acetaminophen absorption**.**
Inclusion Criteria	Patients must meet *all* of the following criteria to be eligible to

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participate in this study:

1. Male or female aged 18–65 years who meets the American Diabetes Association standard T1DM criteria.
2. Diagnosis of T1DM at least 2 years before Visit 0.
3. Insulin Requirement of ≤ 0.90 units/kg/d
4. Absence of ketoacidosis in the past 6 months
5. HbA1c of ≥ 6.5 and ≤ 9.5%
6. Women of child bearing potential must have a negative pregnancy test and be willing to avoid pregnancy during the study period.
7. Signed informed consent.

Exclusion Criteria

Patients must *not* meet any of the following criteria to be eligible to participate in this study:

1. Inability or unwillingness to give informed consent
2. Current or prior use of immunomodulators or systemic steroids in the last 6 months that could potentially affect diabetes or immunologic status.
3. Known hypersensitivity of Exenatide, Liraglutide or any product components
4. Participation in an investigational treatment trial within the last 6 weeks before enrollment
5. 1 or more episodes of severe hypoglycemia (loss of consciousness or requiring the help of others) within the last 6 months
6. Another condition that would, in the view of the investigator, affect the safety of using Bydureon. This might include, among others a history of MEN 2, pancreatitis, a personal or family history of medullary carcinoma of the thyroid.
7. Known severe renal impairment (creatinine clearance <30 mL/min), end-stage renal disease or renal transplantation.
8. Any history of gastroparesis or other severe gastrointestinal disease, pancreatitis, thyroid nodules or malignancy with the exclusion of a history of localized basal cell carcinoma.
9. Uncompensated heart failure, fluid overload, myocardial infarction or liver disease within the last 6 weeks before enrollment.
10. AST, ALT or Alkaline Phosphatase >2 times upper limit of normal or Total bilirubin >1.5 times upper limit of normal.
11. Clinically active serious infections.
12. Positive pregnancy test in menstruating women or lactating female.
13. Concurrent or prior use of Pramlinitide, other Incretin medications, or other anti-diabetes medications other than insulin within the last 4 weeks.
14. Active or poorly controlled psychiatric disorder that would render the patient incapable of following the protocol and/or history of substance abuse disorder.
15. BMI<18.5 at Screening Visit

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1. SCIENTIFIC BACKGROUND SUPPORTING THE STUDY AND STUDY HYPOTHESIS

There are a number of reasons to consider the use of a GLP-1 receptor agonist as an adjunct agent with insulin to control glucose levels in subjects with Type 1 diabetes (T1D). First, GLP-1 receptor agonists can augment glucose stimulated insulin secretion (*1, 2*). This is thought to be a major mechanism of action in patients with Type 2 diabetes (*3, 4*). In T1D, there may be, in addition to the anatomic loss of β cells, a functional component, possibly due to β cell exhaustion from hyperglycemia. β-cells that are dysfunctional may be able to improve their ability to secrete insulin with a GLP-1 receptor agonist. Indeed, in preclinical studies of the combination of anti-CD3 mAb and exendin-4, given to mice with new onset T1D, we found that exendin-4 augmented recovery of glucose levels primarily by increasing insulin content of β cells(*5*). The improvement in β-cell function may depend on the presence of residual insulin production capacity. While older studies have highlighted the progression of T1D to complete insulin deficiency, more recent studies have identified subjects with long standing T1D with residual insulin production. In these subjects (which, in some cohorts has been as many as 67% of subjects), a β-cell tropic agent may improve glucose control and reduce the needs for exogenous insulin (*6*). Likewise, in a previous clinical study, in which subjects with T1D and residual insulin production were enrolled in a trial of Exenatide and/or Rapamycin there was a trend for improved insulin production with treatment with Exenatide but failed to reach statistical significance(*7*). In patients with T2D, Exenatide has been shown to improve glucose stimulated insulin, and we postulate that the same may occur in subjects with T1D with some residual β-cell function.

Second, Exenatide suppresses glucagon secretion which may improve glucose levels. Studies by Rother et al and our own investigations have shown that glucagon secretion is inappropriately increased in subjects with T1D, possibly because of the significantly impaired insulin secretion(*8*). The increased glucagon secretion itself may contribute to impaired glucose tolerance in addition to the insufficient insulin secretion.

Third, there is experimental evidence that GLP-1 receptor agonists can enhance β cell replication and decrease β-cell death (*9, 10*). Human clinical studies have yet to demonstrate this mechanism, but treating subjects who have received islet transplants with Exenatide has improved metabolic function through mechanisms that may involve improved insulin secretion and/or improved β- cell survival or replication.

Finally, Exenatide may delay gastric emptying (11) and therefore the time to absorption of glucose. This effect may facilitate glucose disposal if patients are well insulinized resulting from the use of basal insulin or still make endogenous insulin.

In addition to these actions, other "off target" actions of GLP-1 receptor agonists may also be beneficial. For example, recent studies have indicated that Exenatide may have anti-inflammatory activity. Inflammatory mediators may participate in the functional impairment and even destruction of β-cells in T1D(*12*). Therefore, through this mechanism, an effect on β-cells as well as on insulin sensitivity may be postulated.

In preliminary studies, we have found that administering Exenatide (5μg sq) prior to commencing a mixed meal tolerance test (MMTT) improves the glucose response to the test in patients with T1D with and without residual insulin production. Our initial studies, in patients with T1D and residual insulin production suggest that the improved glucose excursion is not due to increased insulin production since the rates of insulin secretion were not increased (Figure 1). The subjects took their basal insulin (either as Glargine insulin on the prior night or basal insulin by insulin pump), but did not take bolus insulin

at the meal. The insulin secretory, glucose and glucagon responses for each study are shown (Figure 1).

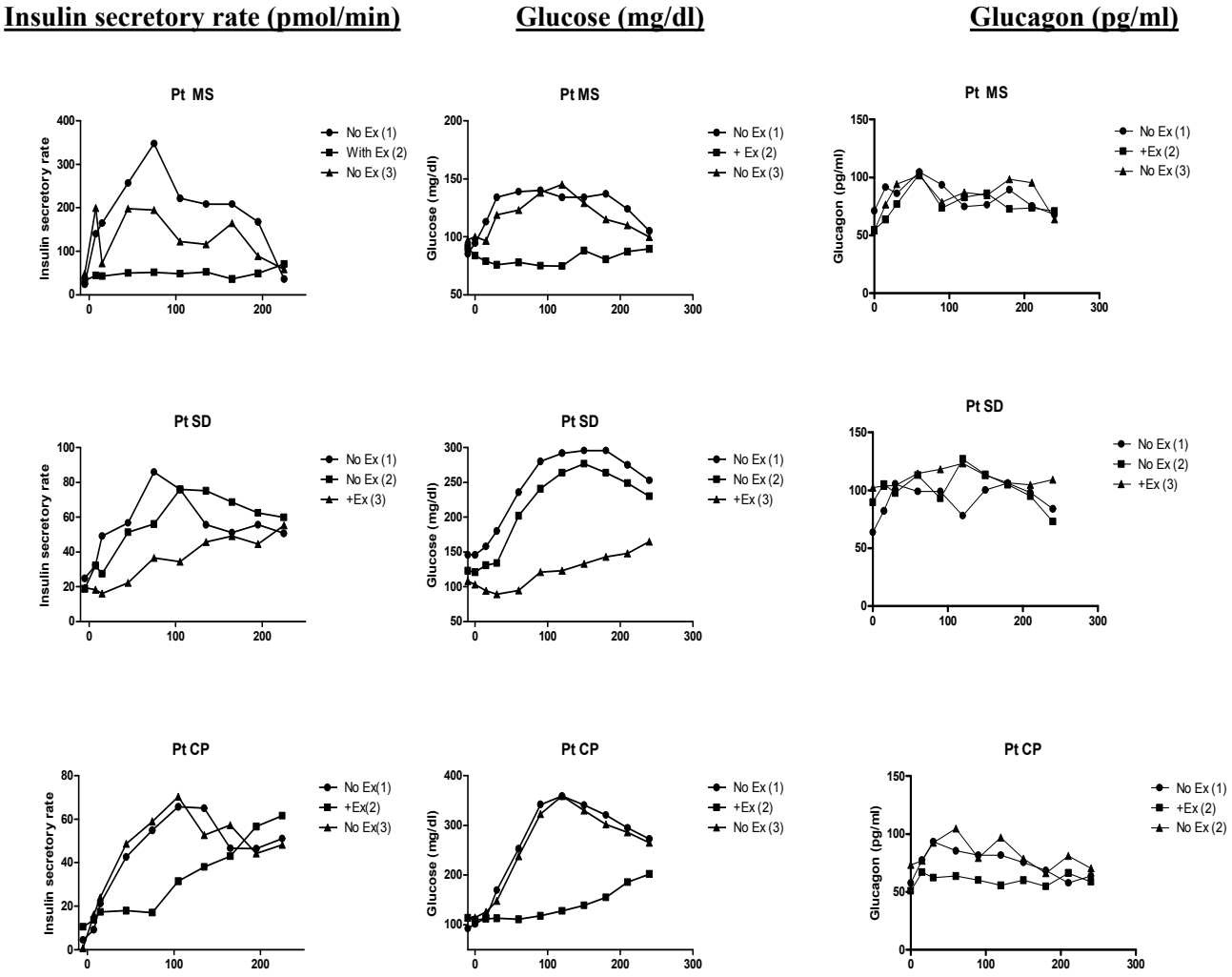

Figure 1: Results of MMTT in 3 patients with T1D and residual insulin production. The subjects ages were 22, 21, and 33 yrs and duration of disease 10, 11, and 11 yrs. They underwent 3 MMTT's. At the start of one of the studies (+Ex) the subjects received 5 μg of Exenatide (Byetta) sq. Data from each subject is shown in a row.

Interestingly, the insulin secretion and glucose responses were reduced when the subjects were given Exenatide. There were variable effects of the Exenatide on the rise in glucagon levels to the meal. These studies are similar to those reported by Rother et al and Raman et al of the acute effects of Exenatide in patients with T1D and confirm the beneficial effect of Exenatide treatment on the meal related rise in glucose levels that occur in the absence of insulin treatment (13; 14). Our studies also highlight the responsiveness of the residual β cells to the physiologic stimulus but also show that this response is not acutely augmented by Exenatide. Nonetheless, based on the glucose responses we have found with acute administration of Exenatide, we predict that Exenatide therapy will result in improved glucose control with reduced requirements for exogenous insulin in patients with T1D.

As part of the study we have also performed an intravenous glucose tolerance test (IVGTT) in one of the subjects with residual insulin production with and without pretreatment with Byetta. The glucose excursion as well as the insulin secretion was similar (Figure 2).

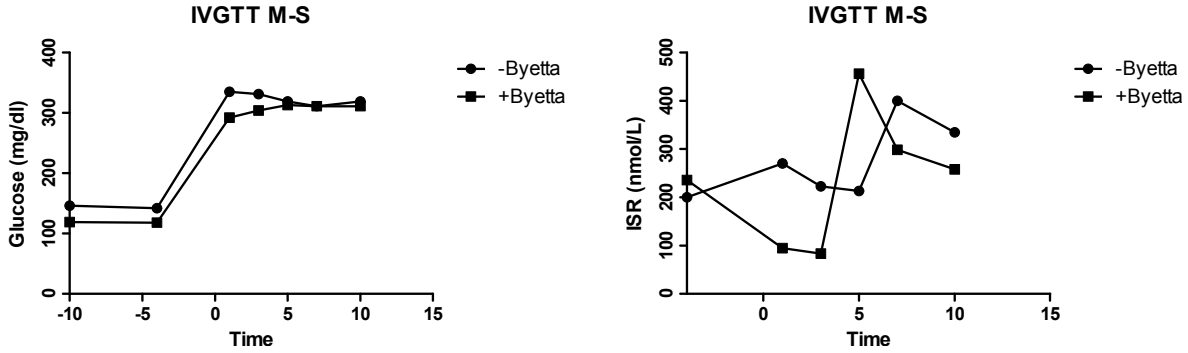

Figure 2: Glucose levels and C-peptide secretion during IVGTT in 1 patient with T1D with and without Byetta. An IVGTT was performed in Subject 1 (in Figure 1) in whom residual insulin production was identified during a MMTT. Byetta (5 µg sq) was administered 15 min before the first blood sampling. The levels of glucose and C-peptide are shown.

These responses are not limited to subjects with residual insulin production. We have characterized the effects of acute administration of Byetta on glucose AUC and glucagon AUC during a MMTT. We included subjects with (n=6) and without (n=7) residual insulin production. We found that overall there was reduced glucose and glucagon AUC (p=0.0003 and p=0.0019 respectively).

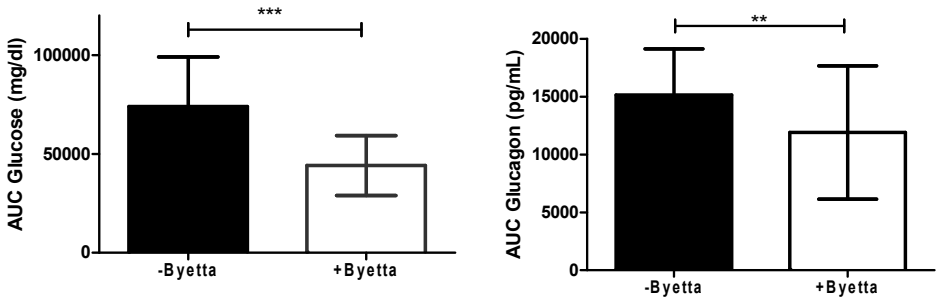

Figure 3: Glucose and glucagon AUC in 14 individuals with > 2yrs duration T1D during a MMTT with and without pretreatment with Byetta (5µg sq prior to the liquid meal. (***p=0.0003 and **p=0.0019 by paired t-test).

Although the effects of Byetta treatment on responses to a mixed meal are profound, the longer term effect of exenatide on glucose control remain to be established. Furthermore the effects of the treatment on insulin secretion are not clear since in our studies, of the 6 subjects with residual insulin production, an increase in insulin production was seen with 2 subjects but a decrease was also seen in 4.

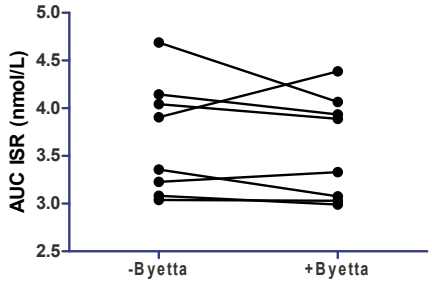

Figure 4: Effects of sq exenatide on insulin secretory response during MMTTs

Therefore, the goal of this proposal is to test whether metabolic control can be improved in subjects with T1D with or without residual insulin production by treatment with Byetta and to test the effects of the drug on insulin responses to a mixed meal.

2. PREVIOUS CLINICAL EXPERIENCE

Previous studies have addressed the actions of GLP-1 agonists in patients with T1D. In a study of Exenatide alone and in combination with Daclizumab on β cell function in long standing T1D for 6 month in an open labeled trial, insulin requirements were reduced but hemoglobin A1c levels and C-peptide responses did not improve. Liraglutide treatment for 4 weeks improved HgbA1c levels (15;16). In the study done by Danish colleagues 2 patients with residual Insulin secretion were able to completely discontinue Insulin treatment after receiving Liraglutide for 4 weeks (16). In a study of the acute effects of Exenatide, glucose excursions were decreased which appeared to be due to delayed gastric emptying (14). However, all of these studies were small and, based on our data on HgbA1c levels from our clinic, underpowered to detect an effect of the drug. Interestingly, two previous studies of Exenatide showed similar effects on C-peptide, glucagon, and glucose responses that we have observed above.

3. CLINICAL STUDIES

3.1 CLINICAL EFFICACY DATA AND SAFETY DATA FOR BYDUREON

A randomized, open-label non-inferiority trial (DURATION-1) in 295 patients with Type 2 diabetes not adequately controlled with diet and exercise or with antidiabetic drugs compared the addition of ER Exenatide 2 mg once weekly with immediate-release (IR) exenatide 5 mcg twice daily for 4 weeks, followed by 10 mcg twice daily. At 30 weeks, hemoglobin A1c (A1c) reductions were 1.9% with ER Exenatide and 1.5% with the IR formulation. Weight loss was about 3.6 kg with either formulation. After the 30-week trial, patients taking IR Exenatide were switched to the ER drug and followed for a total of 2 years; over that period of time, the improvements in fasting glucose, A1c and weight persisted (17).

In a similar open-label 24-week comparative trial in 252 patients with Type 2 diabetes (DURATION-5), ER Exenatide lowered A1c 1.6%, while the IR formulation lowered it 0.9%. Patients taking the ER formulation lost about 2.3 kg, while those taking the IR formulation lost about 1.4 kg. Nausea was less common with the ER formulation (18).

In an unpublished, open-label, 26-week non-inferiority trial in patients with Type 2 diabetes (DURATION-6), about 900 patients with uncontrolled diabetes were randomized to ER Exenatide 2

mg once weekly or Liraglutide 1.8 mg daily. ER Exenatide lowered A1c 1.3% compared to 1.5% lowering with Liraglutide. Gastrointestinal adverse effects occurred more frequently with liraglutide (19).

4. SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR HUMAN PARTICIPANTS

4.1. CONTRAINDICATIONS

- **Medullary Thyroid Carcinoma**

  Bydureon is contraindicated in patients with a personal or family history of medullary thyroid carcinoma (MTC) or in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2).

- **Hypersensitivity**

  Bydureon is contraindicated in patients with a prior serious hypersensitivity reaction to Exenatide or to any of the product components.

4.2. WARNING AND PRECAUTIONS

- **Risk of Thyroid C-cell Tumors:** In both genders of rats, Exenatide extended-release caused a dose-related and treatment-duration dependent increase in the incidence of thyroid C-cell tumors (adenomas and/or carcinomas) at clinically relevant exposures compared to controls. A statistically significant increase in malignant thyroid C-cell carcinomas was observed in female rats receiving Exenatide extended-release at 25-times clinical exposure compared to controls and higher incidences were noted in males above controls in all treated groups at ≥2-times clinical exposure. The potential of Exenatide extended-release to induce C-cell tumors in mice has not been evaluated. Other GLP-1 receptor agonists have also induced thyroid C-cell adenomas and carcinomas in male and female mice and rats at clinically relevant exposures. It is unknown whether Bydureon will cause thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans, as the human relevance of Exenatide extended-release-induced rodent thyroid C-cell tumors could not be determined by clinical or nonclinical studies. Serum calcitonin was not assessed in the clinical trials supporting the approval of Bydureon.

  Serum calcitonin is a biological marker of MTC. Patients with MTC usually have calcitonin values >50 ng/L. Patients with thyroid nodules noted on physical examination or neck imaging should be referred to an endocrinologist for further evaluation. Routine monitoring of serum calcitonin or using thyroid ultrasound is of uncertain value for early detection of MTC in patients treated with Bydureon. Such monitoring m**a**y increase the risk of unnecessary procedures, due to the low specificity of serum calcitonin testing for MTC and a high background incidence of thyroid disease.

- **Acute Pancreatitis:** Based on post marketing data, Exenatide has been associated with acute pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis.
- **Hypoglycemia:** The risk of hypoglycemia is increased when Exenatide is used in combination with a sulfonylurea. Therefore, patients receiving Bydureon and a sulfonylurea may require a lower dose of the sulfonylurea to minimize the risk of hypoglycemia. It is also possible that the

use of Bydureon with other glucose-independent insulin secretagogues (e.g. Meglitinides) could increase the risk of hypoglycemia.

- **Renal Impairment:** Bydureon should not be used in patients with severe renal impairment (creatinine clearance < 30 mL/min) or end-stage renal disease and should be used with caution in patients with renal transplantation. In patients with end-stage renal disease receiving dialysis, single doses of Byetta 5 mcg were not well tolerated due to gastrointestinal side effects. Because Bydureon may induce nausea and vomiting with transient hypovolemia, treatment may worsen renal function. Use Bydureon with caution in patients with moderate renal impairment (creatinine clearance 30 to 50 mL/min). Bydureon has not been studied in patients with end-stage renal disease or severe renal impairment.

  There have been post marketing reports of altered renal function with Exenatide, including increased serum creatinine, renal impairment, worsened chronic renal failure and acute renal failure, sometimes requiring hemodialysis or kidney transplantation. Some of these events occurred in patients receiving one or more pharmacologic agents known to affect renal function or hydration status such as angiotensin converting enzyme inhibitors, nonsteroidal anti-inflammatory drugs, or diuretics. Some events occurred in patients who had been experiencing nausea, vomiting, or diarrhea, with or without dehydration. Reversibility of altered renal function has been observed in many cases with supportive treatment and discontinuation of potentially causative agents, including Exenatide. Exenatide has not been found to be directly nephrotoxic in preclinical or clinical studies.

- **Gastrointestinal Disease:** Exenatide is commonly associated with gastrointestinal adverse reactions, including nausea, vomiting, and diarrhea.

- **Immunogenicity:** Patients may develop antibodies to Exenatide following treatment with Bydureon. Antiexenatide antibodies were measured in all Bydureon -treated patients in the five comparator-controlled 24-30 week studies of Bydureon. In 6% of Bydureon -treated patients, antibody formation was associated with an attenuated glycemic response.

- **Hypersensitivity:** There have been post marketing reports of serious hypersensitivity reactions (e.g. anaphylaxis and angioedema) in patients treated with Exenatide.

- **Macrovascular Outcomes:** There have been no clinical studies establishing conclusive evidence of macrovascular risk reduction with Bydureon or any other antidiabetic drug.

4.3. ADVERSE EFFECTS

Nausea, vomiting, dyspepsia, constipation, diarrhea, headache and injection-site pruritus were the most common adverse effects reported with ER Exenatide in clinical trials. Gastrointestinal effects occurred in about 35% of patients, but decreased over time. Acute renal failure and acute pancreatitis have been reported in patients with diabetes taking Exenatide; whether they were caused by the drug or the disease is not clear.

Injection-site nodules have been reported in about 77% of patients and injection-site reactions in about 17% of patients.

Weight loss is reported with Exenatide. In subjects with T2D who took Exenatide for 4 weeks, the average wt loss was 1.3±0.3 kg. Weight loss was also seen in the DURATION-5 trial of Bydureon. (18; 20).

Impaired recovery from hypoglycemia is a potential adverse effect of GLP-1 receptor agonists. However, the risk of hypoglycemia in patients with T2D treated with combination of Exenatide (Byetta) and exogenous insulin has been low (21). In the DURATION 4 study, a non-inferiority study comparing Bydureon to Metformin, Pioglitazone, and Sitagliptin, no major hypoglycemia was seen (22).

Exenatide may affect absorption of other drugs such as oral contraceptives, antibiotics, digoxin, lovastatin and warfarin. Participants will be informed to take their routine medications at least 1 hour prior to the study drug.

Thyroid C-cell hyperplasia has been reported with use of Exenatide in rats, and the FDA has required a boxed warning about the risk of thyroid C-cell tumors in the package insert.
In pregnant animals, Exenatide at 3 times the human dose had adverse effects on the fetus, including reduced growth, skeletal abnormalities and other teratogenic effects; it is classified as category C (risk cannot be ruled out) for use during pregnancy.

4.4. BENEFITS

Bydureon has been found to be effective for glucose management in patients with T2D. The presumed mechanism of action involves enhanced glucose stimulated insulin release. We propose to test the effects of Bydureon in patients with T1D of at least 2 years duration with or without detectable insulin production.

Our hypothesis is that Bydureon will improve glucose control in insulin treated patients with T1D which would suggest a role for the drug as an adjunct to treatment with insulin. Our preliminary data, as well as a previous pilot study with Liraglutide strongly support this hypothesis. In a 4 weeks study with Liraglutide 2 C-peptide positive patients were able to completely discontinue Insulin treatment without loss of glycemic control at the end of the trial (16). Our view is that if successful, the impact of Bydureon will be greater than either Byetta or Liraglutide because of the long half-life: dosing is once weekly. We anticipate that Bydureon would be used as an adjuvant to insulin therapy in patients with T1D and would not completely replace the need for exogenous insulin. Because patients will already still require up to 4 injections of exogenous insulin daily, we believe it is unlikely that a drug that must be delivered subcutaneously 2 or 3 times daily in addition to insulin would be widely accepted. If we find that Bydureon improves hemoglobin A1c in this pilot trial, it would suggest that a larger, clinical Phase III trial in patients with T1D would be indicated.

In addition to improving HgbA1c overall, the drug may modulate glycemic excursions, which represents the most difficult problem for achieving metabolic control with conventional use of exogenous insulin. The results of the DCCT emphasized the increased frequency of serious hypoglycemia when near normal glucose levels were achieved (23). Insulin pump therapy has reduced the rates of severe hypoglycemia compared to conventional insulin use but they remain high (24). Because of the effects of GLP-1 receptor agonists on inhibiting glucagon release, it is possible that an increased frequency of hypoglycemia may be seen in the Bydureon treated subjects, but our pilot data do not suggest that this complication is likely to occur.

If we find that insulin production improves in the patient population with residual insulin production, it could either reflect increased β cell function or even increased β cell mass. The kinetics of the change in β cell responses will be important as well as the duration of the efficacy. While an effect of GLP-1 receptor agonists on increasing β cell mass or decreasing β cell apoptosis, has been shown in rodents, the data from human trials are not supportive of lasting effects of the drug after it is discontinued, which would be expected if an improvement in β cell mass had been induced(25; 26). In the proposed trial, in the event that we find improved insulin secretion which is maintained beyond the end of drug treatment

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(at 6 month), further evaluation of the potential effects of the drug on stimulating improved β cell mass, similar to that seen in preclinical models, would be indicated.

This trial will be the first in its category to include a larger sample of patients and also have a longer follow up period compared to previous studies in this field. These factors and hopefully accomplishment of the primary and secondary endpoints, we believe will have a great impact on the scientific community and hopefully transform the current treatment of DM type I. These are all points that would support the novelty of this trial.

5. OBJECTIVES

5.1. PRIMARY OBJECTIVE

The goal of the proposed pilot study is to determine whether glucose control can be improved with Bydureon treatment in patients with T1D.

5.2. SECONDARY OBJECTIVES

1. To determine whether there is a lasting effect, 6 months off of treatment with Bydureon, on glucose control.
2. To determine whether Bydureon will affect insulin secretory responses to a mixed meal and gastric emptying.
3. To compare Bydureon treated and placebo groups in:
    a. Safety, including frequency of severe hypoglycemia
    b. Glycemic Variability Calculated from 3 day Measurements of Glucose levels with a glucometer.
    c. Markers of β cell stress including levels of circulating proinsulin: insulin and the levels of circulating demethylated insulin DNA.

6. TRIAL DESIGN

6.1. DESCRIPTION

We propose to conduct a multi-site randomized placebo controlled trial of Bydureon in patients with T1D of at least 2 years duration who may or may not still have detectable levels of C-peptide during a MMTT. Bydureon is a recently approved long acting form of Exenatide. Because of the lack of safety data for Bydureon in children, we propose to conduct the trial in adults (18-65 yrs.).

Patients will receive standard management of their diabetes by their own treating physician. Samples will also be collected for additional mechanistic studies.

To ensure rapid enrollment, we plan to recruit subjects from 9 academic medical centers.

We plan to enroll a total of 120 subjects. To avoid recruitment of individuals with extreme insulin resistance or very labile glucose control, we propose to screen clinic attendees for: i) insulin use

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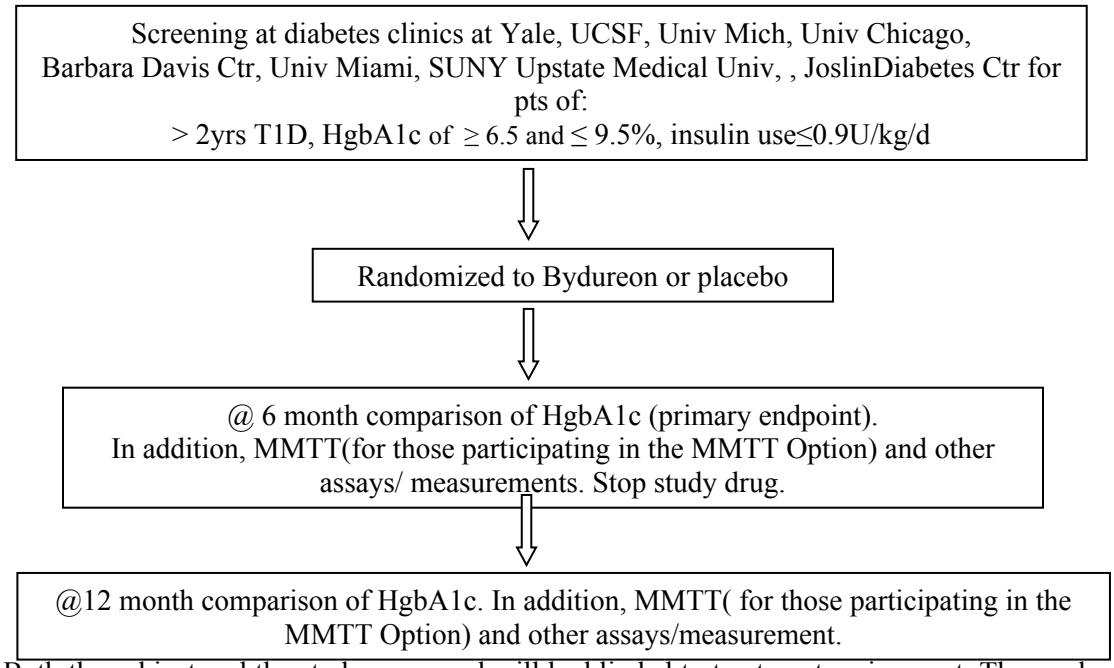
≤0.9U/kg/d; ii) HgbA1c of ≥ 6.5 and ≤ 9.5%; iii) absence of ketoacidosis in the previous 6 mos. Subjects who meet these screening criteria will be invited to participate in the study. The study flow is illustrated in the following block diagram (Figure 5).

Figure 5: Trial Flow Diagram



Both the subject and the study personnel will be blinded to treatment assignment. The randomization will be done, 1; 1, by the coordinating site (at Yale) and study personnel will be told which study kit to use for each subject. AstraZeneca (AZ) will provide study drug and placebo. As a secondary analysis, we propose to determine whether the presence of residual insulin production modifies the drug effect. To do this, we plan to stratify patients for randomization on the basis of detectable C-peptide levels at any time point. We will therefore wait for the results of the C-peptide levels from the enrollment MMTT prior to randomization. The study investigators will not be told in which stratum the patient is being randomized and will be blinded to the C-peptide results of the MMTT until the conclusion of the study.

In order to enhance participation on this trial, newly enrolled subjects will now have the option of entering the trial with plans to complete all scheduled MMTT or entering the trial without completing the MMTT except at the screening visit. All study participants must complete the screening MMTT.

The subjects will learn how the drug is administered at the study site. Participants will also be informed about the common adverse effects of Bydureon and it will be recommended that they stop eating once they feel satiety to decrease the risk of nausea and vomiting during the treatment period.

Study subjects will receive standard medical care from their endocrinologist, but the study team will contact (by telephone or email) the patients 7 days after visit 1 and then on a monthly basis to assess adverse events. At these phone or email contacts and at the study visits, the frequency of symptomatic hypoglycemia will be documented.

6.2. STUDY DURATION AND PACE OF ENROLLMENT

Total study duration will be approximately 48 months (4 years):

- Enrollment phase will be approximately 12 months.
- Study participation phase will be approximately 12 months (1 year), which includes a treatment period of approximately 6 months and a follow-up period of approximately 6 months.

6.3. STUDY ENDPOINTS

6.3.1. PRIMARY ENDPOINT

The primary endpoint is change from baseline in the HbA1c level in the Bydureon vs. placebo treated groups at 6 months.

6.3.2. SECONDARY ENDPOINTS

1. *Assessment of Safety*
   a. Specification of safety parameters:
      i. Frequency of hypoglycemia: We will compare the frequency of hypoglycemic events captured on the participant's glucometer (i.e. glucose < 70 mg/dl) and the frequency of symptomatic or otherwise identified (by glucose measurement) hypoglycemia between visits between the study groups.
      ii. Weight loss: this will be assessed at each study visit.
      iii. Other adverse events: These will be captured on case report forms. Grade III and IV adverse events will be reported to the IRB's of study sites, per local policy.
2. *The type and duration of the follow-up of subjects after adverse events*: All subjects will be followed to month 12 whether or not they have discontinued study drug. In the event that an adverse event is felt to be study drug related, the individual site investigators will be responsible for follow up of subjects as medically indicated.
3. *Effects on insulin secretory responses*:

   C-peptide responses to a mixed meal, adjusted for baseline, will be compared between the two treatment groups at 6 and 12 months. For values that are below the lower limit of detection, a value of the lower limit of detection will be assigned for analysis. We will also compare the effects of drug vs. placebo treatment between groups and within subjects using the AUC from the MMTT at 3 and 6 months (on drug), adjusting for baseline at entry, and 9 and 12 months (off drug), adjusting for the level at 6 mos.
4. *Insulin use* over 3 days prior to each study visit will be compared. The average U/kg/d will be compared, adjusting for baseline.
5. *Glycemic excursion*: We will determine the mean amplitude of glycemic excursion, as described previously; using data from the glucometer.
6. *Additional mechanistic/exploratory measurements*: We will test the effects of Bydureon on gastric emptying by comparing the AUC of the levels of acetaminophen during a MMTT for the patients on which this has been collected. Measurement of GLP-1 and GIP were conducted as part of the MMTT at week 0, 24 and 52 and AUC was calculated and compared for the 2 groups. Participants not taking part in the MMTT's and newly enrolled participants will not have these measurements done.

7. *Measurement of β cell death*: This will be measured in serum samples (0.5 ml) as described (27). In addition, as an exploratory analysis we will attempt to measure insulin and proinsulin levels in serum. The latter may be a reflection of β cell stress and may be stimulated by Exenatide (28).

6.4. RATIONALE FOR SELECTION OF DRUG, ROUTE, DOSE AND REGIMEN

The active drug product to be used in the present study is Exenatide (Bydureon). Neither Byetta nor Bydureon are currently approved by the FDA for treatment of T1DM.

For residual insulin augmentation in type 2 diabetics the recommended dosage of Bydureon is 2mg once every 7 days administered by subcutaneous injection. Bydureon has been selected over Byetta since it is only administered weekly as opposed to twice a day.

We propose a course of 24 weekly injections of Bydureon/placebo.

6.5. STOPPING RULES

The progress of the study will be monitored by the DSMB which will review safety data and make recommendations regarding continuation, termination, or modification of the study. Based on a 12 month enrollment period and an additional study period of 12 months, the DSMB will formally review the safety data at least yearly and after participant number 20 reaches month 2 of the study. The number of subjects who discontinue study treatment will also be included in the reports prepared for the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the protocol chair and site investigators to warrant review, or when an event occurs that contributes to a stopping rule listed below. The study will be monitored in accordance with the monitoring plan.

I. **Patient stopping rules**: Subjects who have three severe hypoglycemic reactions on separate days or who have nausea or vomiting that precludes adherence to diet will discontinue the use of the study drug. A sever hypoglycemic reaction is defined by a blood sugar reading <70 and loss of consciousness or requiring assistance from another person to treat. Subjects who lose more than 5 kg will also discontinue study drug. Subjects who discontinue the drug treatment will not be replaced.

Trial stopping rules: The trial will be discontinued if any 10 subjects meet the patient stopping rules above.

7. ELIGIBILITY

7.1. INCLUSION CRITERIA

Patients must meet *all* of the following criteria to be eligible to participate in this study:

1. Male or female aged 18–65 years who meets the American Diabetes Association standard T1DM criteria.
2. Diagnosis of T1DM at least 2 years from Visit 0.
3. Insulin Requirement of ≤ 0.90 units/kg.

4. Absence of ketoacidosis in the past 6 months.
5. HbA1c of ≥ 6.5 and $\leq 9.5\%$.
6. Women of child bearing potential must have a negative pregnancy test and be willing to avoid pregnancy during the study period.
7. Signed informed consent.

7.2. EXCLUSION CRITERIA

Patients must *not* meet any of the following criteria to be eligible to participate in this study:

1. Inability or unwillingness to give informed consent
2. Current or prior use of immunomodulators or systemic steroids in the last 6 months that could potentially affect diabetes or immunologic status.
3. Known hypersensitivity to Exenatide, Liraglutide or any product component.
4. Participation in an investigational treatment trial within the last 6 weeks before enrollment.
5. 1 or more episodes of hypoglycemia (loss of consciousness or requiring the help of others) within the last 6 months.
6. Another condition that would, in the view of the investigator, affect the safety of using Bydureon. This might include, among others a history of MEN 2, pancreatitis, a personal or family history of medullary carcinoma of the thyroid.
7. Known severe renal impairment (creatinine clearance <30 mL/min), end-stage renal disease or renal transplantation.
8. Any history of gastroparesis or other severe gastrointestinal disease, pancreatitis, thyroid nodules or malignancy with the exclusion of a history of localized basal cell carcinoma.
9. Uncompensated heart failure, fluid overload, myocardial infarction or liver disease within the last 6 weeks before enrollment.
10. AST, ALT or Alkaline Phosphatase >2 times upper limit of normal or Total bilirubin >1.5 times upper limit of normal.
11. Clinically active serious infection.
12. Positive pregnancy test in menstruating women or lactating females.
13. Concurrent or prior use of Pramlinitide, other Incretin medications, or other anti-diabetes medications other than insulin within the last 4 weeks.
14. Active or poorly controlled psychiatric disorder that would render the patient incapable of following the protocol and/or history of substance abuse disorder.
15. BMI<18.5 at Screening Visit

7.3. PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

- **Withdrawal of consent.** Participants who withdraw consent will be asked to complete all the assessments listed for the week 24 visit
- **Failure to return.** Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered lost to follow-up.

- **Investigator judgment.** A severe or serious AE occurs, which, based on the medical judgment of the investigator, prevents completion of participation in the study.

Discontinuation of study drug in an individual patient:

The dosing and administration of investigational medication according to study specification will be discontinued for an individual participant if *any* of the following criteria is met:

- Subjects who have 3 severe hypoglycemic reactions on separate days (requiring assistance from another individual)
- Subjects who have nausea or vomiting that precludes adherence to diet will discontinue the use of the study drug.
- Subjects who lose more than 5 kg in weight from baseline.
- Any grade 3 or higher AE occurs that, based on the medical judgment of the investigator, prevents completion of the course of infusions.
- Evidence of pancreatitis
- Evidence of thyroid carcinoma
- Any unexpected, treatment-related SAE resulting in permanent treatment discontinuation and not related to glycemic events.
- The investigator determines that it is in the participant's best interest to discontinue treatment.
- The participant, or participant's legal representative, requests that treatment be halted.
- The participant becomes pregnant.

Further care will be provided according to the judgment and practice of the investigator.

The participant will be asked to remain in the study and participate in follow-up.

Participants who prematurely terminate from the study will not be replaced.

8. INVESTIGATIONAL MEDICATION

8.1. FORMULATION, PACKAGING, RECONSTITUTION, STORAGE AND ADMINISTRATION

8.1.1. HOW SUPPLIED

The study drug for once every seven days (weekly) subcutaneous administration will be supplied in kits of 4 single-dose vials for use. The kit contains four vials with study drug, four prefilled syringes delivering 0.65 mL diluents, five vial connectors, eight custom needles (23G, 5/16") specific to this delivery system (one is a spare needle for each injection). The subjects will learn how the drug is administered at the study site.

8.1.2. STORAGE AND HANDLING

The study drug is stored in the refrigerator at 36°F to 46°F (2°C to 8°C) and protected from light. The study drug needs to be brought down to room temperature by taking the drug out of the refrigerator for a minimum of 10 hours (overnight) and a maximum of 7 days before administration. For bringing the drug to room temperature, the drug should be removed from the refrigerator and stored in cabinet/drawer protected from light. The study drug powder is suspended in the diluents by mixing thoroughly for several minutes until a solution is formed and there are no residual powdery clumps in the vial. A well-mixed drug solution looks cloudy and should be administered by subcutaneous injection immediately. The study drug is administered as a subcutaneous (SC) injection in the abdomen, thigh or upper arm region.

8.1.3. REQUIRED MEDICATIONS

Insulin preparations as advised by the investigator or the referring physician.

8.1.4. PROHIBITED MEDICATIONS

The use of immunomodulators or systemic steroids in the last 6 months that could potentially affect diabetes or immunologic status is prohibited. Concurrent usage of Pramlinitide, other incretin medications or anti-diabetes medications other than insulin are also prohibited.

If a participant receives, or if the investigator believes that a participant must receive, a prohibited medication, the case must be immediately discussed with the protocol chair. They will determine whether the participant may continue in the trial or should be prematurely terminated.

The use of prohibited medications must be documented on the source document and a protocol deviation must be indicated.

8.1.5. DRUG ACCOUNTABILITY

Federal regulations (21CFR 312.62) will be followed. The date and quantity of drug that was received, the participants to whom drug was dispensed (participant by participant accounting), and an account of any drug accidentally or deliberately destroyed will be recorded. The investigator will ensure that the investigational product supplies are stored as specified. .

Records for receipt, storage, use, and disposition of the study drug will be maintained by the study sites. A drug-dispensing log will be kept current for each participant and will contain the identification of each participant and the date and quantity of drug dispensed. All remaining unused investigational product will be returned to the drug manufacturer or their representative after study termination, or destroyed with the permission of the manufacturer and the sponsor in accordance with applicable law and study site procedures. If investigational product is to be destroyed locally, the investigator will provide documentation in accordance with sponsor's specifications.

8.1.6. ASSESSMENT OF COMPLIANCE WITH STUDY MEDICATION

Bydureon will be self-administered subcutaneously. The subjects will be asked to note the injections of the study drug in their log book (which will be recovered from study subjects at each visit).

9. STUDY PROCEDURES

9.1. DIABETES MANAGEMENT

During the study period, all participants will receive "intensive" management of their diabetes. The sites that are involved all have diabetes clinics that provide intensive diabetes management. The patients will continue to see their primary Endocrinologist during the study period. All participants will be expected to take a sufficient number of daily insulin injections to meet the glycemic targets. In general, the expectation is that all participants will receive at least three injections of insulin daily, including short- and long-acting insulin preparations, or will utilize continuous subcutaneous insulin infusion (CSII insulin pump). All participants will be instructed to reduce their prandial insulin dosages by 50% for the first bolus immediately after receiving study drug. . If the participant's glycemic control worsens they can adjust accordingly.

Glucose levels should be checked at least 6 times daily via the study participant's glucometer for 3 days prior to each visit starting at visit 1. Records of glucose measurements and communication with the participant will be kept as source documentation. Insulin use and hypoglycemia events will be recorded at each visit. Participants will be required to record the daily amount of insulin they have used during the 3-day period immediately preceding each study visit. Log books will be provided to participants at the start of the study and information regarding their insulin usage and glucose levels will be collected at each visit and will serve as source documents. Upon review of these records, the investigator may make adjustments in the insulin regimen, refer a participant to a registered dietician or if necessary take other approaches that will help to maintain or improve a participant's glucose control. Participants will also be contacted by the study coordinator or study nurse 7 days after Visit 1 and then monthly between visits to assess their diabetes. We will also ask the participants to record the date, time and dose of each Bydureon/Placebo injection taken in their log book. This information will be collected at each visit and serve as source documents.

9.2. STUDY VISIT WINDOWS

Refer to SOE, Appendix 1. Study visits must occur as scheduled or within these time frames:

- Visits 2-4: >24hrs but <72hrs after administration of study medication/placebo.
- Visits 5 and 6 ±3 days

9.3. RANDOMIZATION

Individuals complying with all inclusion and exclusion criteria and consenting to study participation will be randomized to one of the two groups (Bydureon vs. placebo) in a 1:1 ratio. We plan to stratify patients for randomization on the basis of detectable C-peptide levels at any time point. We will therefore wait for the results of the C-peptide levels from the enrollment MMTT prior to randomization. The biostatistician will produce the randomization code and scheme through a

computer generated process. Subject identification numbers (SIN) will be randomly pre-assigned to study participants and the corresponding treatment group will be stored and assigned from our web-based system to assure concealment of treatment allocation. The coordinating center at Yale will sequester the randomization schedule until the study is unmasked and will have no involvement in assessment of participants. For each subject, the SIN, treatment and participant's name will be entered into the study log.

9.4. GENERAL ASSESSMENTS

- Medical history: To determine if there are any clinically significant diseases or medical procedures other than the disease under study.
- Physical examination: Includes any body system with clinical signs or reported symptoms of adverse events and weight loss.
- Adverse events: Participants will be assessed for AEs.
- Concomitant medications: All concomitant medications will be reviewed.
- Laboratory Assessments

Central laboratory assessments:

- C-peptide
- Glucose
- HbA_{1C}
- Glucagon
- GAD antibodies
- GLP-1
- GIP

Local laboratory assessments:

- Urine HCG (dip): For female participants of child bearing potential to monitor for pregnancy
- Blood Glucose
- Liver function test
- Lipid Profile
- Amylase

9.5. DISEASE-SPECIFIC ASSESSMENTS

- MMTT: 2-hour and 4-hour
- Insulin use: As U/kg body weight/day.

- Hypoglycemia events
- Glucometer readings: The mean amplitude of glycemic excursion (MAGE) will be calculated from this data.

9.6. MECHANISTIC ASSESSMENTS

- Peripheral Blood Mononuclear Cells (PBMCs)
- Serum for Epigenetic studies
- RAGE-expression on peripheral T-cells

9.7. RETENTION OF SAMPLES

Biological specimens collected in this trial may be used to reevaluate biologic responses as new research tools become available. The samples stored for future use are optional. If a participant consents to have these samples drawn. the specimens will be stored at the laboratory of the individual investigators or Dr. Kevan Herold's laboratory at Yale University. Specimens for mechanistic studies will be obtained throughout the study. Residual specimens may be used by the investigators for studies of immunologic mechanisms involved in T1D with participant consent.

10. ADVERSE EVENTS

10.1. DEFINITION

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

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). For grading, classification and attribution of adverse events see section 10.4.2. Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject.

10.1.1. DISEASE-SPECIFIC ADVERSE EVENTS

For the purposes of this study, hypoglycemia events will be recorded as follows:

- Minor hypoglycemia, defined as a glucose concentration of 55–65 mg/dL. Minor hypoglycemic events are not required to be documented as adverse events in the case report forms.

- Major hypoglycemia, defined as a glucose concentration <55 mg/dL (grades 2–5, NCI-CTCAE manual version 4.0), or clinically: involving seizure or loss of consciousness

(coma), or requiring assistance from another individual in order to recover. Major hypoglycemic events must be reported as adverse events on the case report forms. *All episodes of hypoglycemia that require hospitalization and/or emergency care will be reported as SAEs to the DSMB as described in Section 10.6.*

Hypoglycemia grading according to CTCAE version 4.0

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Hypoglycemia	<LLN-55 mg/dL	<55 -40 mg/dL	<40-30 mg/dL	<30 mg/dL; life threatening consequences; seizures	Death

All reported hypoglycemic episodes accruing during the treatment period or within 4 weeks of treatment end will be defined as possibly related to study treatment (see table 1).

Hyperglycemia will not be collected as an adverse event, unless it results in an inpatient hospitalization.

10.2. Nonserious Adverse Events

A ***nonserious adverse event*** is an AE not classified as serious.

10.3. Serious Adverse Events

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

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g. medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.) Potential drug induced liver injury (DILI) is also considered an important medical event. (See Section 10.10 for the definition of potential DILI.)

Suspected transmission of an infectious agent (e.g. pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs. (See Section 10.6 for reporting pregnancies).

Any component of a study endpoint that is considered related to study therapy (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported) should be reported as SAE (see Section 10.6 for reporting details).

NOTE:

The following hospitalizations are not considered SAEs:

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

10.4. COLLECTING AND RECORDING ADVERSE EVENTS

10.4.1. METHODS OF COLLECTION

Adverse events will be collected from the time of consent and will be followed to resolution or until 30 days after the participant completes participation, whichever comes first.

Adverse events may be collected as follows:

- Observing the participant.
- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event. If this is the case, then the evaluation that produced the value or result should be repeated until the value or result returns to normal or can be explained and the participant's safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be indicated as such on the appropriate laboratory evaluation form(s), and must also be reported as an adverse event on the adverse event form. Abnormal vital sign measurements or laboratory finding deemed not clinically significant by the site investigator must be documented as such and are not required to be listed in the adverse event source documents or adverse event case report form.

10.4.2. GRADING, CLASSIFICATION AND ATTRIBUTION OF ADVERSE EVENTS

10.4.2.1. GRADING CRITERIA

The study site will grade the severity of AEs experienced by study participants according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version 4.0*. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

- Grade 1 = mild adverse event.
- Grade 2 = moderate adverse event.
- Grade 3 = severe and undesirable adverse event.
- Grade 4 = life-threatening or disabling adverse event.
- Grade 5 = death.

For additional information and a printable version of the NCI-CTCAE manual, go to http://ctep.cancer.gov/reporting/ctc.html

All adverse events will be reported and graded whether they are or are not related to treatment.

10.4.2.2. DEFINITION OF ATTRIBUTION

The site investigator will determine the relation, or attribution, of an AE to the investigational product. The site investigator will also record the determination of attribution on the appropriate CRF and/or SAE report form. The relationship of an adverse event to the study treatment will be defined by using the descriptors provided in table 1

Table1. *Attribution of Adverse Events*

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is *clearly* not related.
2	Unlikely	The adverse event is *unlikely* related.
Related Categories		
3	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
4	Probable	The adverse event is *likely* related.
5	Definite	The adverse event is *clearly* related.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE website: http://ctep.cancer.gov/reporting/ctc.html.

10.5. Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information should begin at consent.
Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious (see Section 10.6.). Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate. All identified non-serious AEs must be recorded and described on the non-serious AE page of the CRF (paper or electronic).

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

10.6. Serious Adverse Event Collection and Reporting

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

An SAE report should be completed for any event where doubt exists regarding its seriousness.

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

The investigator is responsible for prompt reporting of serious adverse events. Serious adverse events will be recorded on the adverse event CRF and on the SAE form. SAEs will be reported to the institutional IRB according to the IRB's requirements. They will be reported to the lead site within 24 hours with the following information:

- Name and contact phone number of the investigator
- Subject number
- SAE term
- Date of Event Onset
- Date(s) of administration of the investigational product
- Current status of subject
- Investigator causality assessment
- Reason why the event is serious

The following documents should also be sent to the lead site within 24 hours of the occurrence of the SAE:

- Serious adverse event report form
- Concomitant medication information

- Results of relevant laboratory or diagnostic tests
- Relevant medical record progress notes
- Supplementary CRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, death.

As additional details become available, the SAE CRF should be updated and submitted. Every time the SAE CRF is submitted, it should be signed by the site investigator or sub-investigator.

SAEs, whether or not related to study drug and pregnancies must be reported to AZ (or designee) within 48 hours. The lead site will be responsible for reporting to AZ.

SAEs and pregnancies should be recorded on the SAE MedWatch form;. Reports are to be transmitted via email or confirmed facsimile (fax) transmission to:

SAE Email Address: AEMailboxClinciaTrialTCS@AstraZeneca.com

SAE Facsimile Number: (302) 886 4114

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 48 hours to the AZ (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

The lead site is responsible for reporting these events to the health authorities as applicable.

All investigators must report SAEs to their respective IRBs as mandated by them.

10.7. Laboratory Test Result Abnormalities

The following laboratory test result abnormalities should be captured on the non-serious AE CRF page or SAE Report Form (paper or electronic) as appropriate:

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

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g. anemia versus low hemoglobin value).

10.8. Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including up to

12 weeks after last product administration, the investigational product will be permanently discontinued..

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g. x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated.

They must be reported to the lead site within 24 hours. The lead site must complete and forward a MedWatch form to AZ (or designee) within 48 hours and in accordance with SAE reporting procedures described in Section 10.6. The lead site will be responsible for reporting the event.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information should be reported on MedWatch form.

Any pregnancy that occurs in a female partner of a male study participant should be reported to the lead site. Information on this pregnancy will be collected on the MedWatch form.

10.9. Overdose

All occurrences of overdose must be reported as SAEs (see Section 10.6. for reporting details).

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE (see Section 10.6 for reporting details.).

10.10. Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs (see Section 10.6 for reporting details).

Potential drug induced liver injury is defined as:

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

   AND

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

   AND

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

10.11. Other Safety Considerations

APPROVED BY THE YALE UNIVERSITY IRB 7/26/2018 VALID THROUGH 8/1/2019

Any significant worsening noted during interim or final physical examinations, electrocardiogram, x-ray filming, any other potential safety assessment required or not required by protocol should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

10.12. FOLLOW UP OF ADVERSE EVENTS

AEs will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first. All SAEs will be reported as specified in Section 10.6 whether they are or are not related to disease progression or study participation.

11. STATISTICAL CONSIDERATIONS

11.1. STATISTICAL METHODS

This is a randomized, double blind, placebo controlled, parallel group trial to evaluate the efficacy of Bydureon to improve glucose tolerance, other markers of glycemic control and safety. Data analysis will be conducted in collaboration with the Yale Center for Analytic Sciences. For all analyses, a type I error of 5% (two-sided) will be used to test for statistical significance and will be performed using SAS v9.2 (SAS Institute, Cary, NC) (29) and GraphPad Prism 6.

11.2. BASELINE COMPARABILITY

Because of the size of this study, we expect that the randomization process will produce reasonably comparable groups. However, the adequacy of the randomization will be assessed by comparing the distribution of baseline demographic and clinical characteristics among the intervention groups. Comparability for continuous variables will be examined graphically and by summary statistics (means, medians, quartiles, etc.). Categorical variables will be examined by calculating frequency distributions.

11.3. JUSTIFICATION OF SAMPLES SIZE

The primary endpoint is change from baseline in the HbA1c level in the Bydureon vs. placebo treated groups at 6 months. The subjects will be followed for another 6 months (to 12 months from study entry) to determine whether the effects of Bydureon are persistent after discontinuation of the drug. No interim efficacy analysis is planned. The sample size calculation is based on data collected from our clinic on subjects with long standing T1D. In these individuals (n=10) the standard deviation (SD) of the HbA1c level was 1.25% and the correlation between measurements of HbA1c, performed 6 months apart was 0.88. This is a strong correlation but reflects our actual clinic data and it suggests a standard deviation for the change in HbA1c of 0.61%. A sample size of 54 subjects per group will provide 90% power at the two-sided 0.05 significance level to detect a difference in the change in HbA1c of 0.40%. Even with a more conservative estimate of correlation of 0.75 (0.65), this sample size will provide 81% (82%) power to detect a difference of 0.50% (0.60%). We will enroll a total of 120 participants to accommodate up to a 20% dropout. Of additional note, the given sample size will have 80% power to detect differences 0.08 U/kg/day in insulin dose and 3417 in the area under the curve for ISR from the mixed meal tolerance test, both key secondary outcomes. The final analysis will involve all study subjects who complete the enrollment visit and in whom at least 1 post-randomization assessment of HbA1c is available.

We anticipate that each study site will be able to enroll about 17 subjects.

11.4. Analysis for Primary Outcome

The primary objective of the analysis is to demonstrate that HbA1c will be reduced in subjects receiving Bydureon compared to those receiving placebo at 6 months. A likelihood-based ignorable analysis using a linear mixed model will be used to compare HbA1c between groups [Lachin, 2000 #2444; Molenberghs, 2004 #2273] (30, 31). The primary advantage of the mixed model when compared to commonly used methods such as complete case analysis and single imputation (e.g. last observation carried forward) is its flexibility in handling missing data. This analysis will assume that missing data occurs at random. The inclusion of baseline HbA1c as a covariate as well as the 3, 6 and 12 month data as outcomes in the model will assist in meeting this assumption. Superiority of Bydureon vs. placebo will be concluded if the linear between group contrast is significant (two-sided $p<0.05$) with a lower mean HbA1c in the Bydureon group at 6 months. The mixed model will include fixed effects for treatment (Bydureon vs. placebo), time (3, 6, 12 months), and the interaction of treatment with time. Additional fixed effects will be included for baseline covariates (baseline A1c, detectable/non-detectable baseline C-peptide, site, gender, race, BMI). We will evaluate the 2 and 3 way interactions of the stratification factor (baseline C-peptide) with treatment and time using a multiple degree of freedom likelihood ratio test and omit from further analysis if $p>0.10$. Linear contrasts will be used to estimate intervention group differences and 95% confidence intervals at each time point. Durability of the treatment effect will be determined by comparing groups at 12 months.

11.5. Analysis of Secondary Outcome

There are a number of secondary outcomes including indices and summaries from the insulin, glucagon and C-peptide AUC response to a mixed meal tolerance test, insulin usage, glycemic excursion as captured by a glucometer, and absorption of acetaminophen (gastric emptying). Linear mixed effect models similar to those described above will be used to evaluate continuous outcomes. The frequency of hypoglycemia and other safety parameters will be assessed and compared between groups using a Fisher's exact test.

In a post-hoc subgroup analysis, we will determine whether the presence of residual insulin production affects the clinical response to treatment. Individuals will be classified based on the presence or absence of residual insulin production. Interactions of treatment, residual insulin indicator and time will be added to the models described above. Linear contrasts comparing the treatment difference in those with or without residual insulin production will be evaluated to determine whether the impact of Bydureon is modified by residual insulin.

The subjects will discontinue Bydureon after 6 mos but will be followed for another 6 mos. At 12 mos, the same assessments will be performed.

11.6. Assessment of Safety

1. Specification of safety parameters:
    a. Frequency of hypoglycemia: We will compare the frequency of hypoglycemic events captured on the participant's glucometer (i.e. glucose < 70 mg/dl) and the frequency

of symptomatic or otherwise identified (by glucose measurement) hypoglycemia between visits between the study groups.

b. Weight loss: this will be assessed at each study visit
c. Other adverse events: These will be captured on case report forms. Grade III and IV adverse events will be reported to the IRB's of study sites per local policy.

2. The type and duration of the follow-up of subjects after adverse events: All subjects will be followed to Month 12 whether or not they have discontinued study drug. In the event that an adverse event is felt to be study drug related, the individual site investigators will be responsible for follow up of subjects as medically indicated.

11.7. PLAN FOR MISSING DATA

Several strategies will be imposed to accommodate the likelihood that missing data will occur during this study. Prevention is the most obvious and effective manner to control bias and loss of power from missing data. This protocol will follow the intent to treat principle, requiring follow-up of all subjects randomized regardless of the actual treatment received. Telephone or email visit reminders will be delivered to participants prior to protocol specified collection times. Alternative contacts will be identified on entry into the study to minimize loss-to follow-up. Despite these prevention efforts it is reasonable to assume missing data will occur. Our primary analysis is valid under the assumption that missing data is missing at random (MAR). We will evaluate the plausibility of this assumption by determining the extent of missing data and missing data patterns, describing reasons for dropout and using logistic regression to identify factors associated with dropout. While we do not expect differential rates of dropout between groups or high loss to follow-up, sensitivity analysis using pattern-mixture and selection models under missing not at random (MNAR) assumptions will be performed to examine the robustness of conclusions of the primary analysis to missing data.

11.8. REPORTING DEVIATIONS FROM ORIGINAL STATISTICAL PLAN

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the statistical analysis plan (SAP). Any change in these features requires either a protocol or an SAP amendment, which is subject to review by the study sponsor(s), and the health authorities. These changes will be described in the final study report as appropriate.

12. ACCESS TO SOURCE DATA/DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational sites must permit authorized representatives of the sponsor (s), and health authorities to examine (and to copy when required by applicable law) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (and any personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

13. QUALITY CONTROL AND QUALITY ASSURANCE

The study will run through the Yale Center for Clinical Investigation (YCCI). The statistical core will be responsible for randomization, collection of CRF's, and statistical analysis. The YCCI will also be responsible for the regulatory support. All data will be collected using the OnCORE software for trial management.

14. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

14.1. STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—adopting the principles of the Declaration of Helsinki—and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate ethics review committee or institutional review board (IRB). Any amendments to the protocol or consent materials must also be approved by IRB before they are implemented.

14.2. INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before participating in the study, taking the study drug, and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever important new safety information is available, when required by a protocol amendment, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The attending physician, in the presence of a witness, will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

14.3. PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number. This number, rather than the participant's name, will be used to collect, store, and report participant information.

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Appendix 1. Schedule of Events

Visit	0	1	1a	2	3	4	5	6
Week	**-2**	**1**	**2**	**4**[1]	**12**[1]	**24**[1]	**38**[2]	**52**[2]
GENERAL ASSESSMENTS								
Informed Consent	X							
Medical History	X							
Adverse Events		X		X	X	X	X	X
Concomitant Medications	X	X		X	X	X	X	X
Phone Assessment for Adverse Events and Concomitant Medications			X		Monthly			
Physical Examination	X					X		X
DISEASE-SPECIFIC ASSESMENTS								
4-hour Mixed Meal Tolerance Test (MMTT)	X					X[6]		X[6]
2-hour Mixed Meal Tolerance Test					X[6]		X[6]	
Insulin Use	X	X		X	X	X	X	X
Blood Glucose Readings[3]		X		X	X	X	X	X
Hypoglycemia Events[4]	X	X		X	X	X	X	X
INVESTIGATION THERAPY/PLACEBO								
Study Medication/Placebo Distribution		X		X	X			
Study Medication/Placebo Administration		X	Weekly					
LOCAL LABORATORY ASSESSMENTS								
Urine HCG[5]	X	X		X	X	X	X	X
Lipid Profile	X					X		X
Liver Function Test	X				X	X		X
Amylase						X		X
CENTRAL LABORATORY ASSESSMENTS								
C-peptide (send to NWLRL)	X				X[6]	X[6]	X[6]	X[6]
Glucose (send to NWLRL)	X				X[6]	X[6]	X[6]	X[6]
GADA (send to Barbara Davis Center)	X					X		X
HbA_{1C} (send to NWLRL)	X				X	X	X	X
Glucagon (send to Core Lab at Yale)	X				X[6]	X[6]	X[6]	X[6]
GLP-1 & GIP (send to Core Lab at Yale)	X[6]					X[6]		X[6]
Proinsulin:insulin ratio (only in C-pep+) (send to Core Lab at Yale)	X					X		X
PBMCs (including RAGE-expression) (send to Dr. Herold's Lab)	X					X		X
Serum Archive (including demethylated insulin DNA) (send to Dr. Herold's Lab)	X					X		X

[1] Visit Window: >24hrs but <72hrs after administration of study medication/placebo.

[2] Visit Window: +/- 3 Days.

[3] To be obtained via participant's glucometer for 3 days prior to each visit except for visit 0.

[4] See Section 10.1.1. for definition.

[5] For females of child bearing potential.

[6] Only for subjects who have agreed to complete all the MMTT's

Appendix 2. Procedures for Performing the 2 hour Mixed-Meal Tolerance Test

The mixed-meal tolerance test (MMTT) is performed in the morning before 10:00 a.m., which means that administration must begin within this time. Please see appendix 1 for additional blood draws during the MMTT.

The mixed meal used in this protocol will be Boost® Complete Nutritional Drink High Protein (Nestlé Health Science). The 2 hr MMTT's stand alone.. The 2-hour MMTT should take 130 minutes.

Dietary Guidelines and Pretest Instructions

Carbohydrates (CHO) should not be restricted from the diet before the test. A general guideline is that adult participants should consume at least 15 kcal (3.75 g) CHO/kg/day for 3 days before the test. These are minimum amounts of CHO; most diets will include greater amounts of CHO. There is no need to alter the participant's diet unless he or she has been on a CHO-restricted diet.

In preparation for the visits including the screening visit, each participant should:

- Fast for 10 hours (but not more than 16 hours) before the test. Fasting should start the night before the test, and should continue up until the start of the test. Participants should not eat or drink anything except water. This means no coffee, tea, soda, cigarettes, alcohol, or chewing gum during the fasting period. However, if hypoglycemia develops, the participant may ingest up to 15 grams of carbohydrates up to 4 hours prior to the start of the MMTT.
- Refrain from vigorous exercise during the fasting period.
- Refrain from working the night before the morning of the test.
- Evaluate concomitant medications being taken by subject and make adjustments if necessary.

The above information including dietary guidelines will be communicated to each study subject by e-mail or phone prior to all clinical visits including screening visit.

Glucose and Insulin before the Test

- Short-acting insulin analogues may be administered up to 4 hours before the test.
- Regular insulin may be administered up to 6 hours before the test.
- Intermediate-acting insulin (such as NPHor Lente) may be administered on the evening before the MMTT, but not on the morning of the test.
- Long-acting basal (such as Glargine) insulin or continuous subcutaneous insulin infusion may be administered before, during and after the test as usual. Participants on Glargine may take their usual injection at the appropriate time, and those on continuous subcutaneous insulin infusion may continue with their usual basal settings.

Target Glucose Level at the Start of Test

The target glucose level at the start of the test is between 70 and 200 mg/dL. If the participant's glucose level is not within this range the glucose reading should be evaluated by the local site investigator to determine if the test can begin. Regular insulin or short acting insulin analogues may be used up to 6 and 4 hours before the test, respectively, to achieve the desired glucose level. The investigator and the study participant should discuss the individual situation for insulin administration to attain the goal of meter capillary glucose values within the range of 70–200 mg/dL at the start of the test. For example, as a practical matter, participants may be instructed to check their blood glucose by meter at home 2 hours before the start of the test so that marked hyperglycemia can be treated with a

short-acting insulin analogue. Alternatively, participants who arrive at the research unit with elevated blood glucose can receive additional short-acting insulin analogues at the time of their arrival, if the test itself does not start until at least 4 hours after insulin administration and occurs before 10 a.m.

IV Placement during the Test

- The IV should be in place for the duration of the test and must be flushed after each draw with saline solution.
- The participant should remain sitting or resting in bed quietly throughout the test and until the test is completed. However, he or she may engage in quiet, non-strenuous activities, such as reading, playing cards, or watching TV. The participant may walk to the bathroom between blood draws if necessary.

Testing Instructions

Time Point −10 minutes

- Draw one 3-mL sample into the purple-top tube for C-peptide and one 4-mL gray-top tube for glucose. After each vacutainer is collected the tubes must be inverted gently at least 8 to 10 times. Chill samples in a bucket of crushed ice or in a refrigerator set at 4˚C for no more than 20 minutes. At the laboratory, spin the tubes in a centrifuge (1000–1300 g, ~3000RPM) for 10 minutes. Tubes must be spun within 20 minutes from blood draw. Aliquot plasma into cyrovial (please ensure vial is properly identified with a label that indicates the time point). Freeze the sample at -70°C.
- Draw one 2mL sample into a syringe and transfer into a chilled 3-mL lavender-top tube containing Aprotinin (250 KIU/mL of blood) for the glucagon sample. Invert tube gently 8 to 10 times. Chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 10 minutes. Centrifuge in a refrigerated centrifuge (1250g) for 10 minutes. Aliquot plasma into cyrovial (please ensure vial is properly identified with a label that indicates the time point). Freeze the sample at -70°C.
- Draw one 0.5 mL sample and transfer into a microfuge/centrifuge tube for glucose. Immediately centrifuge (12.5 rpm) for 15 seconds and test via YSI.

Time Point 0 minutes

- C-peptide, glucose, glucagon and YSI samples should be collected just before the participant drinks the Boost; these are the “0- minute” samples. Process via the instructions above.
- Then the MMTT dose should be given with 6 kcal/kg @ 1 kcal/mL to a maximum of 360 mL. The participant should consume the MMTT dose in no more than 5 minutes.

Time Points 15, 30, 60, 90, 120

For each of the time points specified above:

- Collect C-peptide, glucose, glucagon and YSI samples per the instructions above.
- At the conclusion of the test, check blood glucose by glucometer, and administer insulin as per participant’s standard insulin plan.
- A delayed or missed sample, or other deviation from the protocol must be noted on the Comments section of the MMTT specimen transmittal form.

Appendix 3. Procedures for Performing the 4 hour Mixed-Meal Tolerance Test (MMTT)

The MMTT/AAS combination study is performed only when doing a 4 hr test. The 4 hr MMTT/AAS should be started prior to 10am. The 4-hour MMTT should take 250 minutes to perform. Please see appendix 1 for additional blood draws during the MMTT.

The mixed meal used in this protocol will be Boost ® Complete Nutritional Drink High Protein (Nestlé Health Science). The 4-hour MMTT/AAS should take 250 minutes to perform

Dietary Guidelines and Pretest Instructions

Carbohydrates (CHO) should not be restricted from the diet before the test. A general guideline is that adult participants should consume at least 15 kcal (3.75 g) CHO/kg/day for 3 days before the test. These are minimum amounts of CHO; most diets will include greater amounts of CHO. There is no need to alter the participant's diet unless he or she has been on a CHO-restricted diet.

In preparation for the visits including screening visit, each participant should:

- Fast for 10 hours (but not more than 16 hours) before the test. Fasting should start the night before the test, and should continue up until the start of the test. Participants should not eat or drink anything except water. This means no coffee, tea, soda, cigarettes, alcohol, or chewing gum during the fasting period. However, if hypoglycemia develops, the participant may ingest up to 15 grams of carbohydrates up to 4 hours prior to the start of the MMTT.
- Refrain from vigorous exercise during the fasting period.
- Refrain from working the night before the morning of the test.
- Evaluate concomitant medications being taken by subject and make adjustments if necessary.

The above information including dietary guidelines will be communicated to each study subject by e-mail or phone prior to all clinical visits including screening visit.

Glucose and Insulin before the Test

- Short-acting insulin analogues (such as Lispro or l-aspart) may be administered up to 4 hours before the test.
- Regular insulin may be administered up to 6 hours before the test.
- Intermediate-acting insulin (such as NPH or Lente) may be administered on the evening before the MMTT, but not on the morning of the test.
- Long-acting basal (such as Glargine) insulin or continuous subcutaneous insulin infusion may be administered before, during and after the test as usual. Participants on Glargine may take their usual injection at the appropriate time, and those on continuous subcutaneous insulin infusion may continue with their usual basal settings.

Target Glucose Level at the Start of Test

The target glucose level at the start of the test is between 70 and 200 mg/dL. If the participant's glucose level is not within this range the glucose reading should be evaluated by the local site investigator to determine if the test can begin. Regular insulin or short acting insulin analogues may be used up to 6 and 4 hours before the test, respectively, to achieve the desired glucose level. The investigator and the study participant should discuss the individual situation for insulin administration

to attain the goal of meter capillary glucose values within the range of 70–200 mg/dL at the start of the test. For example, as a practical matter, participants may be instructed to check their blood glucose by meter at home 2 hours before the start of the test so that marked hyperglycemia can be treated with a short-acting insulin analogue. Alternatively, participants who arrive at the research unit with elevated blood glucose can receive additional short-acting insulin analogues at the time of their arrival, if the test itself does not start until at least 4 hours after insulin administration and occurs before 10 a.m.

IV Placement during the Test

- The IV should be in place for the duration of the test and must be flushed after each draw with saline solution.
- The participant should remain sitting or resting in bed quietly throughout the test and until the test is completed. However, he or she may engage in quiet, non-strenuous activities, such as reading, playing cards, or watching TV. The participant may walk to the bathroom between blood draws if necessary.

Testing Instructions

Time Point −10 minutes

- Draw one 3-mL sample into the purple-top tube for C-peptide and one 4-mL gary-top tube for glucose. After each vacutainer is collected the tubes must be inverted gently at least 8 to 10 times. Chill samples in a bucket of crushed ice or in a refrigerator set at 4˚C for no more than 20 minutes. At the laboratory, spin the tubes in a centrifuge (1000–1300 g, ~3000RPM) for 10 minutes. Tubes must be spun within 20 minutes from blood draw. Aliquot plasma into cyrovial (please ensure vial is properly identified with a label that indicates the time point). Freeze the sample at -70°C.
- Draw one 2-mL sample into a syringe and transfer into a chilled 3-mL lavender-top tube containing Aprotinin (250 KIU/mL of blood) for the glucagon sample. Invert tube gently 8 to 10 times. Chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 10 minutes. Centrifuge in a refrigerated centrifuge (1250g) for 10 minutes. Aliquot plasma into cyrovial (please ensure vial is properly identified with a label that indicates the time point). Freeze the sample at -70°C.
- Draw one 0.5 mL sample and transfer into a microfuge/centrifuge tube for glucose. Immediately centrifuge (12.5 rpm) for 15 seconds and test via YSI.

Time Point 0 minutes

- C-peptide, glucose, glucagon and YSI samples should be collected just before the participant drinks the Boost and; these are the “0 minute” samples. Process via the instructions above.
- Then the MMTT dose should be given with 6 kcal/kg @ 1 kcal/mL to a maximum of 360 mL. The participant should consume the MMTT dose in no more than 5 minutes.

Time Points 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes
For each of the time points specified above:

- Collect C-peptide, glucose, glucagon, and YSI samples per the instructions above.
- At the conclusion of the test, check blood glucose by glucometer, and administer insulin as per participant’s standard insulin plan.

- A delayed line or missed sample, or other deviation from the protocol must be noted on the Comments section of the MMTT specimen transmittal form.

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