The Bihormonal iLet™ Bionic Pancreas Feasibility Study
(Study 19-002)

Principal Investigator:
Steven J Russell, MD, PhD

Co-Investigators:
Courtney A Balliro, BS, RN, CDE
Rabab Jafri, MD
Jordan Sherwood, MD

Sponsor: Beta Bionics

Address correspondence to Steven J Russell, MD, PhD, MGH Diabetes Research Center, 50 Staniford Street Suite 301, Boston, MA, 02114, email: sjrussell@mgh.harvard.edu, phone: 617-726-1848, fax: 617-643-0697, page: 617-726-2066

Version 2, February 21, 2019

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I. Background and significance

I. a. Background

Maintaining near-normal blood glucose (BG) levels (70–120 mg/dl) is a challenging and critically important task for people with type 1 diabetes (T1D). The Diabetes Control and Complications Trial (DCCT) Research Group definitively demonstrated that tight BG control can reduce long-term complications. The likelihood and severity of nephropathy, retinopathy, neuropathy, macrovascular disease, and skin disorders is reduced in proportion to reductions in glycated hemoglobin (HbA1c), which is closely correlated with long-term average BG levels. Risks for such complications are elevated by three- to five-fold with diabetes. On the other hand, tight BG control through conventional intensive insulin therapy increases the likelihood of episodic hypoglycemia, which carries acute risks, including convulsions, seizures, coma, and death. Conventional therapy also requires a relentless daily effort to count carbohydrates, frequently monitor BG throughout the day and night, and administer a daily insulin regimen.

A more reliable method for achieving consistent BG control consists of an integrated artificial or bionic pancreas (BP) system, consisting of a continuous glucose monitor (CGM), an infusion pump, and a control algorithm that actuates the pump based on CGM glucose data. Such a system can automate and ease the burden of T1D management and vastly improve glycemic control relative to the current standard of care.

Recent years have seen the development of several competing strategies for automated or semi-automated management of glycaemia. One large difference between competing designs is whether they use insulin alone (insulin-only) and rely on the user treating with carbohydrates if the blood glucose falls too low, or insulin and glucagon (bihormonal) and use glucagon to automatically prevent and treat hypoglycemia, with carbohydrate treatment used only if glucagon treatment is not successful.

Glucagon is an endogenous hormone that binds with high affinity to its cognate receptor. Glucagon is quantitatively the most important counter-regulatory hormone in normal glucose control physiology. In healthy individuals without T1D, glucagon levels rise during exercise, and in the late-postprandial period as glucose levels return to the normal range after a small hyperglycemic excursion. The production of glucagon is dysregulated early in the course of T1D and glucagon production in response to threatened hypoglycemia is lost. Therefore, people with T1D are functionally glucagon deficient.

An important challenge for automated glucose control is that the physiologic need for insulin can change rapidly, but insulin is slowly absorbed when delivered subcutaneously. Even “rapid-acting” insulin analogs such as insulin lispro (Humalog) have a mean time-to-peak of ~70 minutes. This means that if the need for insulin decreases rapidly, such as in the case of exercise, there is already insulin-on-board that cannot be withdrawn. In contrast to insulin, glucagon is absorbed quickly, with a time-to-peak of ~15-20 minutes. Therefore, small doses of glucagon can be given to counter the effects of excess insulin that has already been delivered and cannot be withdrawn, and can prevent hypoglycemic events that could not be prevented by suspending insulin delivery alone.

The use of glucagon provides the BP with a powerful tool to automatically prevent and treat hypoglycemia, but it does present two challenges. First, exogenous glucagon must be shown to be safe when administered in micro-doses intermittently on a chronic basis. A second challenge to the use of glucagon is that a form of glucagon that is stable near body temperature for at least several days in a pump must be available. When we first began developing our BP, there was no stable form of glucagon available; however, several companies are now developing stable analogs (Zealand, Eli Lilly) and stable formulations (Xeris, Adocia). The clinical programs for the Zealand analog are sufficiently advanced that we are now using it in the BP in clinical feasibility trials, and we expect it to be qualified for pivotal studies by the end of 2019. A third challenge is that, as with subcutaneously administered insulin, replacement of

glucagon by subcutaneous administration cannot perfectly mimic normal physiology, and peripheral levels must be higher than normal to generate adequate liver exposure for effectiveness. In our last inpatient study of the BP in adults and adolescents during over 2,300 patient-hours of exposure, frequent blood sampling showed that the aggregate mean glucagon levels were in the normal fasted range (<150 pg/ml by the Millipore radioimmunoassay) between 61% and 91% of the time. Based on these results, we expect that the doses of glucagon used by the bihormonal BP will be safe

Given the potential different user preferences, we have developed a BP system that can be used in either a bihormonal, insulin-only, or glucagon-only mode.

I. b. Bihormonal BP System

We have developed an autonomous, self-learning BP that requires only the participant's weight for initialization, and then autonomously adapts, modestly or dramatically, as needed, to cope with the wide range of insulin requirements of adults, adolescents, and pre-adolescents with T1D. Our BP obviates the need for the patient to know, or even appreciate, their insulin requirements, and renders obsolete any need for patients or caregivers to know carbohydrate-to-insulin ratios, basal rates, or insulin correction factors.

Our core technology is our insulin controller, which orchestrates all subcutaneous (SC) insulin dosing. At its centerpiece is a model-predictive control algorithm, which bases insulin doses on the glucose data and insulin absorption kinetics. We were the first to incorporate insulin pharmacokinetics (PK) into our algorithm, by augmenting it with a mathematical formulation for estimating the concentration of insulin in the blood and predicting its future concentration. It is essential to compensate for the slow absorption rate of SC insulin analogs (peak time in blood of 30–90 min, clearance in 4–8 hr), and to enable the algorithm to refrain from stacking and overdosing insulin. Furthermore, our MPC algorithm automatically adjusts its insulin-doing aggressiveness continuously and in real time to different insulin needs between individuals and variable needs within the same individual. Running in parallel with our MPC algorithm is an algorithm that automatically modulates basal insulin delivery over multiple time scales, and another algorithm that automatically adapts insulin doses in response to optional meal announcements. Unlike current insulin pumps, and all of the insulin-only control algorithms of which we are aware, our adaptive basal insulin algorithm obviates the need for the user to set, or even know, his or her “basal-rate profile”. Instead, it is capable of automatically adapting to, and compensating for, changes in an individual's basal insulin need, such as might occur over a period of hours, days, or weeks (e.g. circadian hormonal fluctuations, intercurrent illness, physical activity, or emotional state) or as might occur over a period of months or years due to developmental changes (e.g. hormonal changes that occur during puberty or menopause). Our adaptive meal dose controller obviates the need for the user to set, or even know, his or her “carbohydrate-to-insulin ratios”, as it makes automatic adjustments based on dosing history for similar meal announcements made on previous days, and customizes the dose for each individual and for time of day. Our BP also includes a proportional-derivative algorithm governing SC micro-doses of glucagon to help prevent impending hypoglycemia. Glucagon dosing is based on the glucose level and rate of descent. It could occur preemptively even if glucose is above target range and it includes a feedback term to account for the pending effects of recent glucagon doses. The amount of glucagon dosed also feeds back on the insulin controller, so that large amounts of glucagon dosing decrease the aggressiveness of the insulin controller.

Taken together, these mathematical algorithms provide a universal framework for a glycemic control strategy that requires no quantitative input from, or participation by, the user (besides entering body weight to initialize the system), but which automatically adapts insulin and glucagon dosing to meet the individual needs of each user. Another challenge we have met is enabling our technology to remain

completely autonomous in managing insulin and glucagon delivery even when the CGM is offline. Specifically, when the CGM is offline, our BP invokes the high-resolution "basal rate profile" that it had recently learned and stored when the CGM was online. Based on what the system learned and stored about meal announcements when the CGM was online, it can respond to meal announcements in the same manner when the CGM is offline. Finally, it automatically responds to user-entered BG values when the CGM is offline by issuing a correction dose of insulin or glucagon based on what it learned about the user's insulin and glucagon needs when the CGM was online. Thus, our BP never relies on, or burdens the user with, the determination of dosing decisions, which inevitably vary in quality and reliability among different users. The BP provides a turnkey solution for people with T1D that comprehensively manages glycaemia across a broad range of individual needs and a across a large spectrum of circumstances and challenges to glycemic control.

I. c. Insulin-Only BP System

The BP can also operate in an insulin-only mode. During operation in this mode, all the other features of the BP operate as usual except that glucagon is not given. In addition, the lowest glucose target that can be chosen by the user (towards which the insulin controller drives down the blood glucose levels) is increased from 100 mg/dl in the bihormonal system to 110 mg/dl in the insulin-only system. This works to reducing the aggressiveness of insulin dosing in the insulin-only system relative to its bihormonal counterpart, with the aim of keeping the amount of hypoglycemia low even at the potential cost of raising the mean glucose level achieved by the insulin-only system. The intended use for such a system would be to provide glycemic control for people with type 2 diabetes who require insulin therapy, and early technology adopters with type 1 diabetes.

I. d. Glucagon-Only BP System

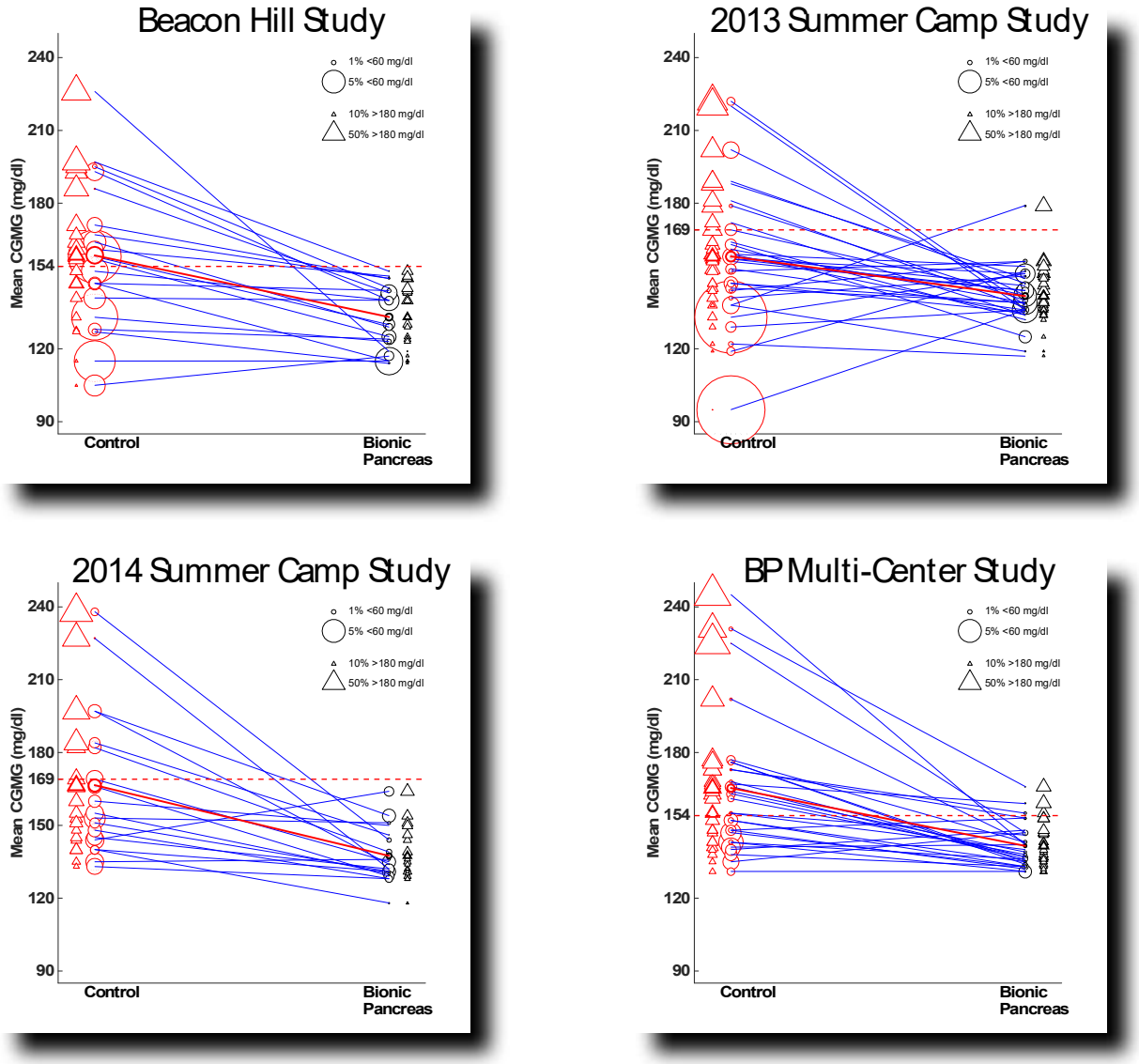
The BP can also operate in a glucagon-only mode. During operation in this mode, all the other features of the BP operate as usual except that insulin is not given. The intended use for such a system would be to treat glycemic disorders associated with chronic hypoglycemia (such as congenital hyperinsulinism, insulinoma syndrome, chronic hypoglycemia in post-bariatric surgery patients, etc.).

I.e. Preliminary Studies

Our BP hardware platform has evolved over the years from a laptop-driven system, which we used in all of our inpatient studies (between 2008-2012), to the first truly mobile wearable iPhone-driven platform, which we have used in all of our outpatient studies thus far (between 2013–2016). Using our iPhone-driven BP system, we have conducted >110 outpatient experiments of 5–11 days in duration in each participant (> 800 patient days or > 2 patient years of data), and across participants ranging in age between 6 and 76 years old and in body mass between 21 and 133 kg. The robust adaptation capabilities of our BP are evident in the fact that the average total daily dose of insulin among these participants varied by over 13-fold (from 11 to 145 units/day).

All of our preclinical studies at BU testing our BP in a diabetic swine model of T1D (between 2005 and 2009), and all of our inpatient clinical trials in the Clinical Research Center at MGH testing our BP in adults and adolescents with T1D (between 2008 and 2012) have set the stage for the outpatient studies that followed. In November 2012 we obtained FDA approval to conduct our first outpatient study testing our bihormonal BP in adults 21 years or older with T1D. The Beacon Hill Study[1] followed a random-order cross-over design in which 20 adults with T1D participated in 5 days on our iPhone-based BP and 5 days of usual care. In the usual care control arm the participants used conventional insulin pump therapy (and their own CGM if they had one), and they wore a CGM with blinded display and muted alarms. In the BP arm, participants kept to a three-square-mile geographic area centered around the Beacon Hill neighborhood in Boston. They ate as they chose at local restaurants, and exercised at will with access to

two gyms. Analysis was pre-specified to focus on Days 2–5, since glycemic control is more representative of BP performance after most of the adaptation by the BP occurs on Day 1. Results are summarized in the plots and table of Figure 1.



Study	Age (years)	Bionic Pancreas (BP)			Control			*p*-value (BP versus Control) for:		
		Mean CGM glucose level (mg/dl)	% of CGM glucose levels <60 mg/dl (%)	70–180 mg/dl (%)	Mean CGM glucose level (mg/dl)	% of CGM goucose values <60 mg/dl (%)	70–180 mg/dl (%)	Mean CGM glucose level	% of CGM glucose values <60 mg/dl	70–180 mg/dl
Beacon Hill (n=20, 5-day experiments)	≥21	133	1.5	80	159	3.7	59	<0.001	0.020	<0.001
2013 Summer Camp (n=32, 5-day experiments)	12–20	142	1.3	76	158	2.2	65	0.004	0.192	<0.001
2014 Summer Camp (n=19, 5-day experiments)	6–11	137	1.2	81	168	2.8	58	0.004	0.001	<0.001
BP Multi-Center (n=39, 11-day experiments)	≥18	141	0.6	78	162	1.9	62	<0.001	<0.001	<0.001

Figure 1. Outpatient results summarizing the distribution of mean CGM glucose levels and hypoglycemia in the bihormonal BP and control arms. Mean CGM glucose levels for each participant under usual care (shown as a red circle on the left) relates to the participant's mean CGM glucose level on the BP (shown as a black circle on the right). For each participant, the circle diameter is proportional to the percentage of CGM glucose values < 60 mg/dl, and the size of the triangle is proportional to the percentage of CGM glucose values > 180 mg/dl. The heavy circles and lines represent the group means. The horizontal red

dashed line refers to the glucose level corresponding to the ADA therapy goal for each age group tested, which corresponds to 154 mg/dl (HbA1c <7%) for adults and 169 mg/dl (HbA1c <7.5%) for children. Results are summarized in the table below the plots, where the co-primary outcomes (mean CGM glucose level and percentage of CGM glucose values < 60 mg/dl) for the BP are highlighted in red for each of the four studies.

In April 2013, we obtained FDA approval to conduct our first outpatient study testing our bihormonal BP in adolescents 12–20 years old with T1D. The 2013 Summer Camp Study[1] followed a random-order cross-over design in which 32 adolescents with T1D participated in 5 days on our BP and 5 days of supervised camp care in the control arm. In the control arm the participants used conventional insulin pump therapy (and their own CGM if they had one), and they wore the BP without pumps and with blinded display and muted alarms for remote monitoring. Participants were monitored remotely according to identical criteria in all arms for proper device functioning and CGM glucose <70 mg/dl lasting more than 15 minutes, which would prompt study staff to call the participant and make sure they were treated. Participants were fully integrated into normal camp activities without restrictions on diet or exercise. The study used the same iPhone-based BP that was used in our Beacon Hill Study. The mean HbA1c of all 32 participants at baseline (pre-study) was 8.2%, which corresponds to a mean BG of 189 mg/dl. Results are summarized in the plots and table of Figure 1.

In April 2014, we obtained FDA approval conduct our first outpatient study testing our bihormonal BP in pre-adolescents 6–11 years old with T1D. The 2014 Summer Camp Study[2], was similar in design to our 2013 Summer Camp Study. Results are summarized in the plots and table of Figure 1.

In April 2014, we obtained FDA approval to conduct our first multi-center study, which was also our first home study, to test our BP in adults 18 years or older with T1D. The Bionic Pancreas Multi-Center Study[3] followed a random-order cross-over design in which 39 adults participated in 11 days on our BP and 11 days of usual care. Participants went to work as usual, and lived and slept at home, all without clinical supervision. There were no restrictions placed on diet or exercise. The study included four medical centers (10 participants per center), which included MGH, the University of Massachusetts Medical School, Stanford University, and the University of North Carolina at Chapel Hill. Results are summarized in the plots and table of Figure 1.

In July 2015, we obtained FDA approval to perform our first study testing the BP at different static glucose targets ("set-points"), including in both the bihormonal and insulin-only configurations. In the MGH Set-point Study[4] 20 adults participated in 7 arms, each lasting 3 days. This study was the first to explore modifying the glucose target towards which the BP attempts to drive the glucose level. In all our previous studies, the target glucose was 100 mg/dl. Since this was the first study to test the BP in a configuration without glucagon, the insulin-only arms initially used significantly elevated glucose targets of 130 mg/dl and 145 mg/dl (not shown). We subsequently obtained approval to test glucose targets of 120 mg/dl and 110 mg/dl in December 2015. Results for the insulin-only and control arms are summarized in Figure 2. The conclusion of this study was that *the insulin-only system was safe, with minimal hypoglycemia,* with the 120 mg/dl glucose target appearing to be a good compromise between mean glucose, amount of hypoglycemia, and insulin utilization.

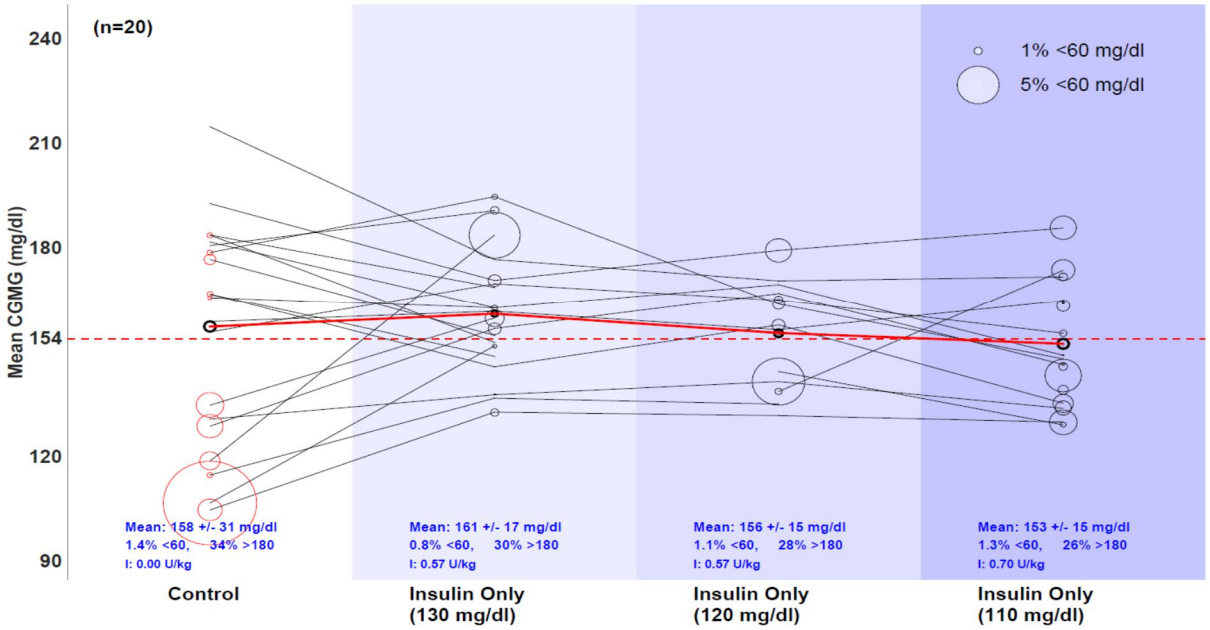


Figure 2 Outpatient results summarizing the distribution of mean CGM glucose levels and hypoglycemia in the insulin-only BP (set-points 130, 120, and 110 mg/dl) and comparator arms. Mean CGM glucose levels for each participant in each arm (shown as a red circles) are connected by black lines. For each participant, the circle diameter is proportional to the percentage of CGM glucose values < 60 mg/dl. The heavy circles and lines represent the group means. The horizontal red dashed line refers to the glucose level corresponding to the ADA therapy goal for each age group tested, which corresponds to 154 mg/dl (HbA1c <7%) for adults.

In July 2015, we obtained FDA approval to perform our first study investigating a feature that allowed the target glucose to be determined automatically by the BP, an additional level of adaptation to the individual participant. In the Stanford Insulin-only Study[5] 16 adults participated in a week of usual care followed by another week on the insulin-only BP. Participants were monitored remotely according to identical criteria in both arms for proper device functioning and CGM glucose <50 mg/dl lasting more than 15 minutes, which would prompt study staff to call the participant and make sure they were treated. The first week was a control arm in which participants managed their own conventional insulin pump therapy (using their own CGM if they had one) and wore the BP without pumps and with blinded display and muted alarms for remote monitoring. In the second week, the BP was initiated with target glucose of 130 mg/dl, which could be lowered to 115 mg/dl if certain criteria were met. All but one participant was kept at a target of 130 mg/dl, and one was lowered to 115 mg/dl, for an overall average target of 129 mg/dl. During this week, the mean CGM glucose achieved was 159 mg/dl. There was only 0.8% time <60 mg/dl in the static set-point week. This was non-significantly lower than the 2.3% observed in the usual care arm. Results are summarized in Figure 3. This provided further reassurance that the insulin-only configuration of the BP is safe and effective.

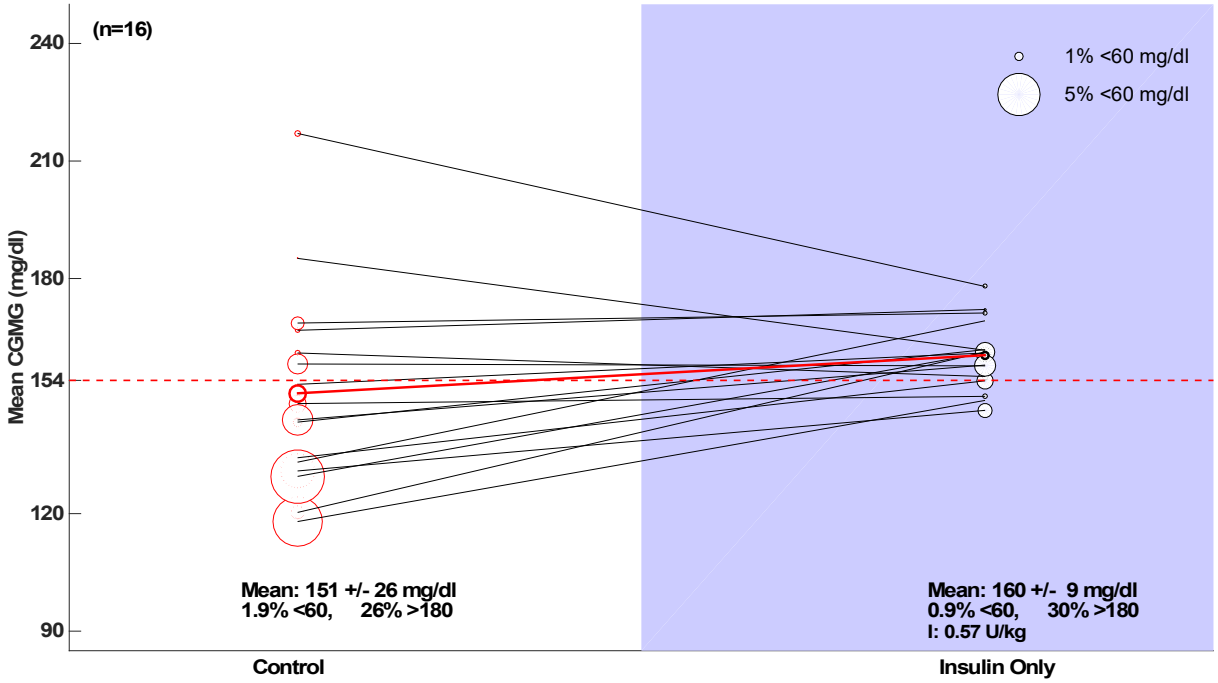


Figure 3 Outpatient results summarizing the distribution of mean CGM glucose levels and hypoglycemia in the insulin-only BP and control arms. Mean CGM glucose levels for each participant under usual care (shown as a red circle on the left) relates to the participant's mean CGM glucose level on the BP (shown as a black circle on the right). For each participant, the circle diameter is proportional to the percentage of CGM glucose values < 60 mg/dl. The heavy circles and lines represent the group means. The horizontal red dashed line refers to the glucose level corresponding to the ADA therapy goal for each age group tested, which corresponds to 154 mg/dl (HbA1c <7%) for adults.

In April 2016, we obtained FDA approval to perform our first study removing remote telemetric monitoring for severe biochemical hypoglycemia from an outpatient study comparing the bihormonal bionic pancreas, the insulin-only bionic pancreas and the subject's own usual care. In the Monitoring Study[6] each arm was repeated with and without remote monitoring to allow for a direct comparison of glycemic control and hypoglycemia. Each BP hormonal configuration used the lowest glucose target previously tested: 100 mg/dl for the bihormonal BP and 110 mg/dl for the insulin-only BP. The results are summarized in figure 4. There was more hypoglycemia without monitoring vs. with monitoring in the two usual care arms (1.95 vs. 1.32%, p=0.02). However, there was no difference in hypoglycemia without monitoring vs. with monitoring in the two bihormonal BP arms (0.99 vs. 1.05%, p=0.82) and two insulin-only BP (1.66 vs. 1.55%, p=0.74) arms. Without monitoring, hypoglycemia was reduced on the bihormonal BP vs. usual care (0.99 vs. 1.95%, p=0.02) and was comparable on the insulin-only BP vs. usual care (1.66 vs. 1.95%, p=0.47). The mean CGMG was significantly lower in all BP vs. usual care arms. There were no mean CGMG differences between the two bihormonal, two insulin-only, and two usual care arms. We concluded that *remote monitoring had no effect on hypoglycemia with the BP, and could be safely omitted from future studies even at the most aggressive set point.* The default glucose set points of the iLet in all future studies will be higher in each configuration (110 mg/dl for bihormonal, and 120 mg/dl for insulin-only), but users will be allowed to lower each set point if they desire without sacrificing their safety.

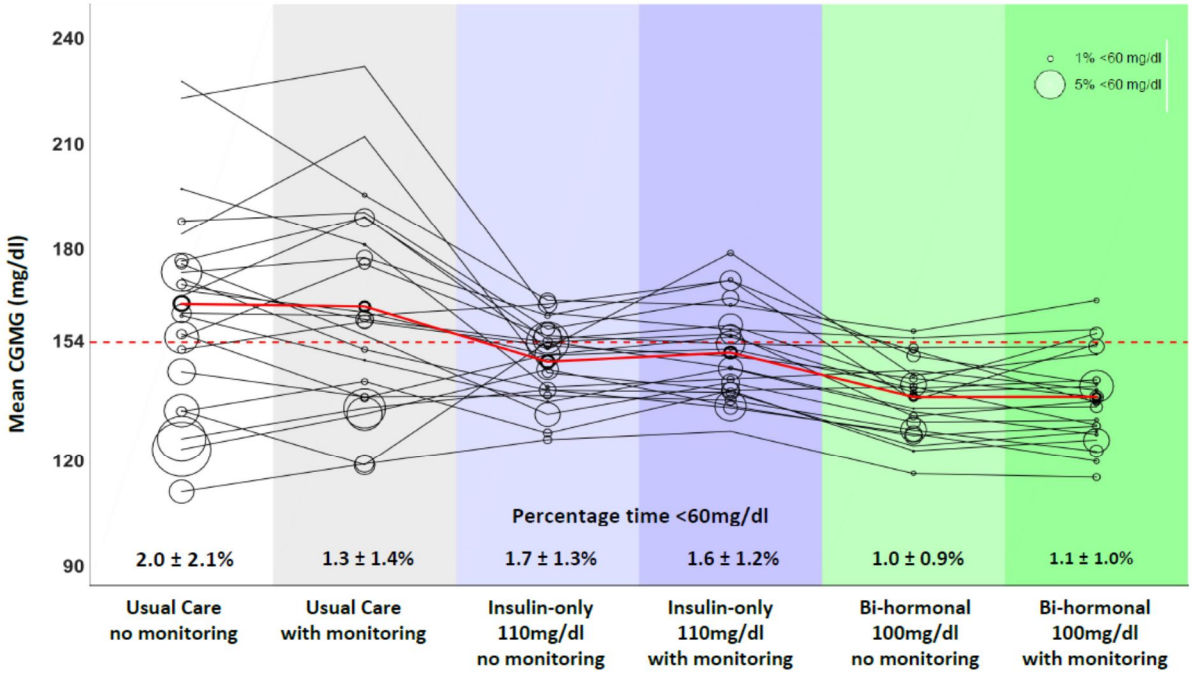


Figure 4. Outpatient results summarizing the distribution of mean CGM glucose levels and hypoglycemia in usual care, on the insulin-only BP and the bihormonal BP, each with and without monitoring. Mean CGM glucose levels for each participant in each arm (shown as a red circles) are connected by black lines. For each participant, the circle diameter is proportional to the percentage of CGM glucose values < 60 mg/dl. The heavy circles and lines represent the group means. The horizontal red dashed line refers to the glucose level corresponding to the ADA therapy goal for each age group tested, which corresponds to 154 mg/dl (HbA1c <7%) for adults.

In June 2016, we obtained FDA approval to perform a feasibility trial designed to compare an investigational glucagon analog, ZP4207 (Dasiglucagon, Zealand Pharma), with freshly reconstituted recombinant human glucagon (Eli Lilly) using the bihormonal BP. In the Dasiglucagon Feasibility Study[7] 20 adults completed two 1-day study visits in random order (bihormonal BP using dasiglucagon and bihormonal BP using Lilly glucagon). The trial was designed to compare dasiglucagon and Lilly glucagon administered via the bihormonal BP under strenuous conditions by having each subject fast prior to each treatment visit, continuing the subject's own insulin pump with up to double the basal insulin administration rate, and including an exercise session, which all together challenged the ability to maintain euglycemia. Under these conditions, the efficacy results demonstrated comparable automated glycemic control with dasiglucagon as compared to Lilly glucagon when used within the bihormonal BP system for up to 8 hours. Both dasiglucagon and Lilly glucagon were well tolerated and able to counteract decreasing and hypoglycemic plasma glucose levels. The mean CGM glucose and glucagon dosing for this trial are summarized in Figure 5. The results of this study allow us to use dasiglucagon in a bihormonal bionic pancreas in longer outpatient phase 2 and 3 trials in place of recombinant human glucagon.

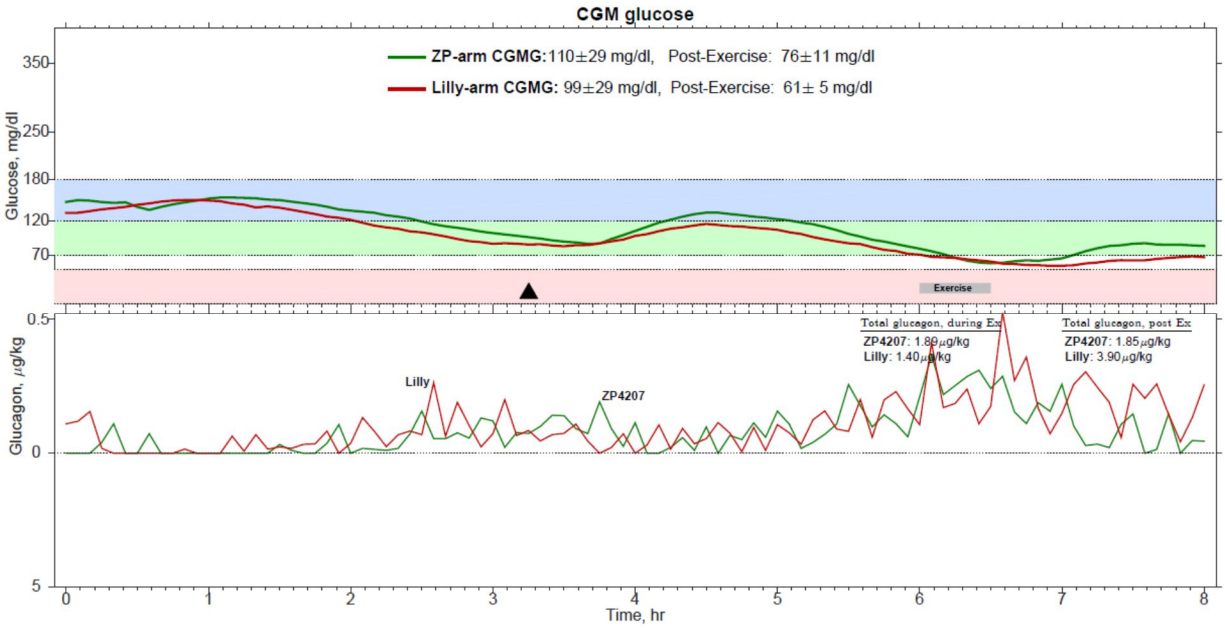


Figure 5. The mean CGM glucose and mean glucagon dosing in the eight-hour study visit for the dasiglucagon arm (ZP-arm) and Lilly glucagon arm.

In May 2018, we obtained FDA approval to perform our first pilot study testing the insulin-only configuration of the iLet in the home-use setting in adults and children. The aims of the Insulin-only Bridging Study were to assess the efficacy, safety, and reliability of the insulin-only configuration of the bionic pancreas in regulating glycaemia in a short-term, outpatient study under real-world conditions. In a preliminary test run we enrolled 7 adult subjects ≥ 18 years old with T1D. The test run was completed in June 2018 and consisted of an uncontrolled, 7-day experimental period that tested the insulin-only configuration of the iLet along with the iLet pigtail adapter, the iLet ready-to-fill insulin cartridge, the Contact Detach infusion set (Unomedical), the Dexcom G5 CGM, and the insulin analog that each subject normally used for their usual care (either Humalog or Novolog). Participants were followed with round-the-clock, remote, telemetric monitoring for hyperglycemia (> 300 mg/dl for ≥ 90 minutes) and hypoglycemia (< 50 mg/dl for ≥ 15 minutes). The primary glucose target was set to 120 mg/dl by default for all subjects. All 7 adult participants had a designated contact, who served as an emergency contact person and were available in the event study staff were unable to reach the participant. The mean CGM glucose over the last five days of the study (days 3–7) for the entire cohort was 145 ± 10 mg/dl and the time < 60 and 54 mg/dl was 1.7 ± 0.6 and 0.8 ± 0.6%, respectively.

The test run was followed by the random-order, cross-over, outpatient Insulin-only Bridging Study[8] comparing the insulin-only mode of the iLet to usual care (UC) for 7 days each. The study enrolled adults with T1D who used either MDI (n=12) or CSII (n=22) for their UC. Participants enrolled at Massachusetts General Hospital (n=17) used the Eversense while those at Stanford (n=17) used the G5 as the input CGM signal for the iLet. There was no statistically significant difference between the iLet and UC in % time <54 mg/dl (0.6 [0.2,1.1] vs. 0.6 [0.1,1.2], p=0.87) or mean CGM glucose (155±12 vs. 162±26 mg/dl, p=0.097). The iLet significantly increased % time within 70-180 mg/dl vs UC (70.1% vs 61.5%, p=0.006). The mean insulin TDD was not significantly different between iLet and UC (44±20 vs. 42±20 u/day). No serious adverse events occurred during either arm. These results suggest that the iLet, using either the Eversense or G5 CGMs, may provide safe and effectively glucose control to users with MDI or CSII as their UC.

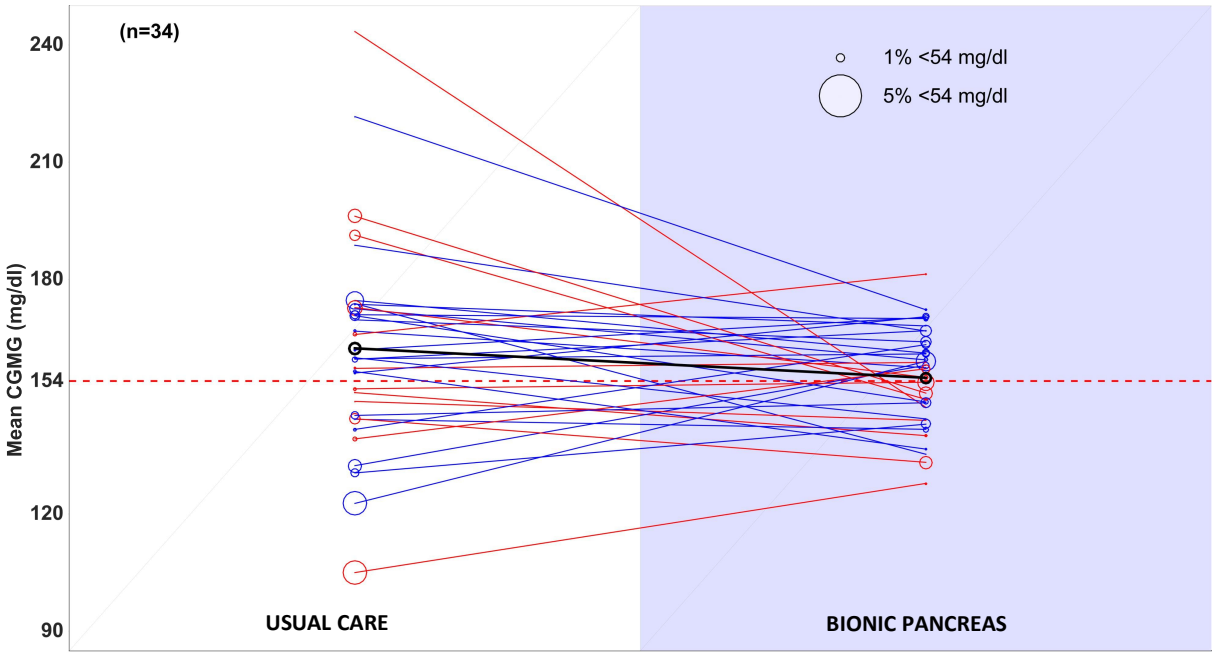


Figure 6. Outpatient results summarizing the distribution of mean CGM glucose levels and hypoglycemia in usual care and on the insulin-only iLet. Mean CGM glucose levels for each participant in each arm (shown as a red or blue circles for participants on MDI or CSII during ususal care, respectively) are connected by lines. For each participant, the circle diameter is proportional to the percentage of CGM glucose values < 54 mg/dl. The heavy circles and lines represent the group means. The horizontal red dashed line refers to the glucose level corresponding to the ADA therapy goal for adults, which corresponds to 154 mg/dl (HbA1c <7%).

I. f. Fully Integrated Insulin-Only, Glucagon-Only, and Bihormonal Configurations of the iLet Bionic Pancreas System

We have designed, built, and tested our first-generation working prototype BP system, which we refer to as the iLet bionic pancreas system, and which consists of a dual-chamber autonomous infusion pump. The iLet has been built according to Class III medical device standards, adheres to a comprehensive and robust quality system, and is fully compliant with ISO 13485 standards and document control practices. The bihormonal configuration of the iLet includes a dual motor and drivetrain assembly, which independently actuates the delivery of insulin and/or glucagon from glass cartridges that are separately loaded into the iLet housing. Each drivetrain utilizes a lead screw, which is driven by a precision micromotor, a gear case assembly, and a motor controller unit, in a manner that is commonly found in many insulin infusion pumps on the market today. Our mathematical control algorithms, the integrated CGM (Dexcom G5 or Senseonics Eversense), and the native user interface (UI) software, are all interconnected through a host controller software module and reside as embedded systems on printed circuit board assemblies contained within the device housing. Our touchscreen-enabled, menu-driven UI and onboard microprocessor provide a comprehensive and standalone platform, which allows the iLet to operate independently of smartphones or other devices and without the need for internet support during routine operation. The iLet has dosing accuracy that is comparable to the most accurate FDA-approved insulin pumps currently on the market.

The iLet is set to either an insulin-only, bihormonal, or glucagon-only configuration by manually selecting the configuration in the user interface. When in the bihormonal configuration, the control algorithm would occasionally and automatically invoke the same insulin-only dosing mode as in the insulin-only configuration during periods when the glucagon cartridge has not been loaded, is empty, or becomes

empty during use, or if there is a pump occlusion detected along the glucagon fluid path. Whenever the control algorithm is in the insulin-only configuration, the minimum glucose target is 110 mg/dl. The minimum glucose target in the bihormonal or glucagon-only configurations, when the glucagon cartridge is available for dosing and the glucagon fluid path is patent, is 100 mg/dl.

In addition to the iLet device itself, the entire iLet bionic pancreas system includes a glass insulin cartridge, a glass glucagon cartridge, pigtail adapters that connect the drug cartridges to infusion sets, and a self-monitored blood-glucose (SMBG) meter. The SMBG meter that we will use is the Contour Next One (Ascensia). This meter is the successor to the Contour Next SMBG meter (Bayer), which was found to be the most accurate meter assessed in all three blood-glucose ranges tested (< 70, from 70 to 179, and ≥ 180 mg/dl) in a comparative accuracy study involving 19 point-of-care glucometers [REF].

II. Objectives and Endpoints

Our objective is to conduct a home-use study testing the Gen 3.2 iLet bionic pancreas system in the insulin-only configuration and the bihormonal configuration with dasiglucagion in 10 adult subjects (≥ 18 years old) with type 1 diabetes in a random-order crossover study under real-world conditions. The study will assess the safety and reliability of both iLet configurations.

Primary objective: To assess whether the iLet operates as designed comparing the insulin-only configuration of the Gen 3.2 iLet bionic pancreas system with the bihormonal configuration of the device using dasiglucagon at a concentration of 4 mg/ml.

Secondary objective: To assess the impact of both configurations of the Gen 3.2 iLet bionic pancreas system on glycemic control, quality of life, and treatment satisfaction among study participants, their caregivers, partners, and/or family members.

Primary endpoint

The primary endpoint will capture the operational characteristics of the Gen 3.2 iLet bionic pancreas system for each of the patient's two treatment periods. A 7-day treatment period will be considered to have successfully met its primary endpoint if each of the following is true during the time that the iLet is powered on over the entire treatment period:

1. The percentage of time that valid CGM glucose readings are captured by the iLet is ≥80%.
2. The percentage of the time that each drug channel (insulin, and if applicable, glucagon) is available is ≥95%.
3. The ratio of cumulative drug doses delivered to cumulative drug doses attempted is between 0.95 and 1.05, inclusive, for insulin and, if applicable, for glucagon.

Key secondary endpoint

- Proportion of time with CGM glucose < 54 mg/dl across days 2-7

Secondary endpoints

- Mean grams of carbohydrate per day ingested to treat hypoglycemic events (reported daily by subjects)
- Mean CGM glucose across days 2-7
- Proportion of time across days 2-7 within the CGM glucose range of 70-180 mg/dl
- Quality of life among study participants, their caregivers, partners and/or family members
- Treatment satisfaction among study participants, their caregivers, partners and/or family members

Safety endpoints

- Frequency of adverse events
- Frequency of severe hypoglycemia
- Frequency of diabetic ketoacidosis

Other endpoints

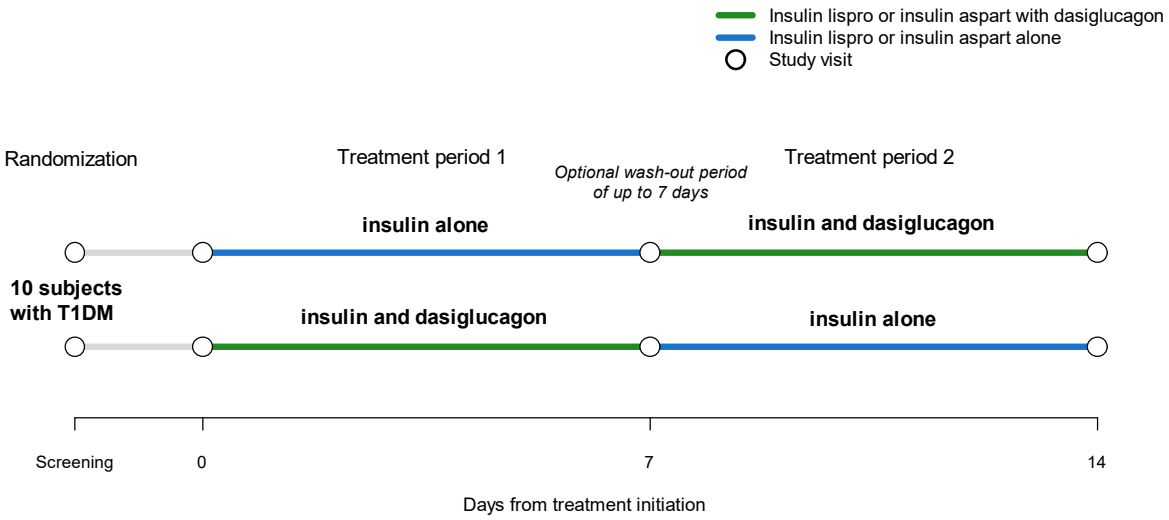
- Proportion of time across days 2-7 within each of the following CGM glucose ranges:
    - < 50 mg/dl
    - < 60 mg/dl
    - < 70 mg/dl
    - 70-120 mg/dl>180 mg/dl
    - >250 mg/dl
- Number of episodes of symptomatic hypoglycemia (reported daily by subjects)
- Percentage of subjects with mean Dexcom CGMG < 154 mg/dl (estimated average glucose corresponding to an A1c of 7%)
- MARD for the Dexcom G5 with the Contour Next One glucose meter as the reference
- Total daily dose of insulin
- Mean daily basal insulin dose
- Mean daily bolus insulin dose
- Total daily dose of glucagon
- Number of user-intiated glucagon doses
- Number of meal announcements
- Glucose variability measured with coefficient of variation (CV) and with mean of daily difference (MODD)
- Mean nausea severity from VAS
- Number of unscheduled insulin and glucagon cartridge/infusion set changes
- Mean glucose target

III. Study Design

III.a Overall Design

The study follows a 2-treatment, 2-period crossover design, 2 and will consist of two 7-day study arms in random order: one bihormonal bionic pancreas arm using insulin lispro or insulin aspart and dasiglucagon, and one insulin-only bionic pancreas arm using insulin lispro or insulin aspart, as seen in Figure 1.

Figure 7. Study design



Questionnaires will be administered to participants and their partners (designated contacts) at the beginning of the study and the end of each arm to gather data on attitudes towards bionic pancreas BG control, confidence, device burden and treatment satisfaction. This information will be used to make the best choices about how the final version of the bionic pancreas should be configured and used.

III.b Scientific Rationale for Study Design

A crossover study design was chosen to reduce the impact of inter-subject variability and to increase the statistical power to detect differences in secondary outcomes despite the relatively small number of subjects.

III.c End of Study Definition

The study will end for subjects when they have completed the second follow-up visit scheduled 25 (± 4) days after the final day 7 visit, or at the time they chose to withdraw from the study or are removed from the study by decision of the principle investigator.

VI. Subject Selection

VI.a. Inclusion Criteria

Subjects

1. Age ≥ 18 years and have had clinical type 1 diabetes for at least one year
2. Diabetes managed using an insulin pump for ≥ 3 months
3. Prescription medication regimen stable for > 1 month (except for medications that will not affect the safety of the participant and are not expected to affect any outcome of the study, in the judgement of the principal investigator)
4. Willing to wear one Dexcom CGM sensor, and up to two steel cannula infusion sets (6 mm Contact Detach) and change infusion sets frequently (if the subject is known not to tolerate steel infusion sets then a plastic set may be used)
5. Have used a CGM for at least one cumulative month over the last 24 months
6. Willing to stay within a 250-mile radius of the designated base throughout the study. Air travel is not permitted.
7. Informed consent obtained before any trial-related activities
8. Have a designated contact (an adult ≥ 18 years of age) willing to serve as an emergency contact for them throughout the study.

Designated Contacts

1. Age ≥ 18 years
2. Have an established relationship with the study participant
3. Willing to answer questionnaires about their experience with the participant's diabetes and the bionic pancreas
4. Willing to answer calls and check on the participant's wellbeing as needed
5. Able to provide informed consent (e.g. no condition that impairs cognition or judement)

VI.b. Exclusion Criteria

1. Unable to provide informed consent (e.g. impaired cognition or judgment)
2. Unable to safely comply with study procedures and reporting requirements (e.g. impairment of vision or dexterity that prevents safe operation of the iLet, impaired memory, unable to speak and read English)
3. Current participation in another clinical trial with administration of investigational drug.
4. Current participation in another diabetes-related clinical trial that, in the judgment of the principal investigator, will compromise the results of this study or the safety of the participant
5. Previous exposure to dasiglucagon (otherwise known as ZP4207)
6. Pregnant (positive urine HCG), breast feeding, plan to become pregnant in the next 12 months, or sexually active without use of contraception
    a. Subjects must use acceptable contraception for the two weeks prior to the study, throughout the study and for the two weeks following the study.
    b. Acceptable contraception methods include:
        - Oral contraceptive pills (OCP)
        - Intrauterine Device (IUD, hormonal or copper)
        - Male condoms
        - Female condoms
        - Diaphragm or cervical cap with spermicide
        - Contraceptive patch (such as OrthoEvra)
        - Contraceptive implant (such as Implanon, Nexplanon)
        - Vaginal ring (such as Nuvaring)
        - Progestin shot (such as Depo-Provera)
        - Male partner with a vasectomy proven to be effective by semen analysis
7. Current alcohol abuse (intake averaging >4 drinks daily in last 30 days) or other substance abuse (use within the last 3 months of controlled substances other than marijuana without a prescription)
8. Unwilling or unable or to avoid use of drugs that may dull the sensorium, reduce sensitivity to symptoms of hypoglycemia, or hinder decision making during the period of participation in the study (use of benzodiazepines or narcotics, even if by prescription, may be excluded according to the judgment of the principal investigator)
9. Renal failure on dialysis
10. History of cystic fibrosis, pancreatitis, or other pancreatic disease, including pancreatic tumor or insulinoma, or history of complete pancreatectomy
11. Coronary artery disease that is not stable with medical management, including unstable angina, angina that prevents moderate exercise (e.g. exercise of intensity up to 6 METS) despite medical management, or within the last 12 months before screening a history of myocardial infarction, percutaneous coronary intervention, enzymatic lysis of a presumed coronary occlusion, or coronary artery bypass grafting
12. Abnormal EKG consistent with increased risk of malignant arrhythmia including, but not limited

to, evidence of active ischemia, proximal LAD critical stenosis (Wellen's sign), or prolonged QT interval (QTc > 500 ms). Other EKG findings, including stable Q waves, are not grounds for exclusion as long as the participant is not excluded according to other criteria. A reassuring evaluation by a cardiologist after an abnormal EKG finding may allow participation.

13. Unwilling or unable to avoid drugs known to cause torasdes de pointe or bradycardia throughout the entire dosing period. Subjects will also be given a list of drug classes to avoid without checking with study staff and will be told to call study staff before any new drug, including over the counter drugs but excluding topicals, so that it may be checked against a comprehensive list of drugs associated with torsades de pointes. Any medications in these classes taken by subjects at baseline will be checked against a comprehensive list of medications associated with torsades de pointes and subjects taking such medications will be excluded. The list of drug classes is intentionally over-inclusive (i.e. the drug classes listed contain many drugs that are NOT associated with torsades de pointes) to facilitate recognition of drugs to be checked against a definitive list.
    a. Antibiotics
    b. Antifungals
    c. Antivirals
    d. Antipsychotics
    e. Antiderpressants
    f. Mood stabilizers
    g. Anticonvulsants
    h. Antiemetics
    i. Diuretics
    j. Antihypertensives
    k. Antihistamines
    l. Anesthetics
    m. Opiods
    n. Cocaine
14. Congestive heart failure with New York Heart Association (NYHA) Functional Classification III or IV
15. History of TIA or stroke in the last 12 months
16. History of liver disease that is expected to interfere with the anti-hypoglycemic action of glucagon (e.g. liver failure or cirrhosis). Other liver disease (e.g. active hepatitis, steatosis, active biliary disease, any tumor of the liver, hemochromatosis, glycogen storage disease) may exclude the subject if it causes significant compromise to liver function or may do so in an unpredictable fashion.
17. Personal history of pheochromocytoma, MEN 2A, MEN 2B, neurofibromatosis, or von Hippel-Lindau disease
    a. Fractionated metanephrines will be tested to rule out pheochromocytoma in patients with symptoms that could be related to a catecholamine secreting tumor, including those with:
        - Episodic or treatment refractory (requiring 4 or more medications to achieve normotension) hypertension
        - Paroxyms of tachycardia, pallor or headache
18. History of adrenal disease or tumor that has not undergone characterization for endocrine function
19. Hypertension with systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥100 mm Hg despite treatment
20. Recent history of diabetic ketoacidosis (DKA) or severe hypoglycemia in the last 6 months. Severe hypoglycemia is defined as an event that required assistance of another person due to altered

consciousness, and required another person to actively administer carbohydrate, glucagon, or other resuscitative actions. This means that the participant was impaired cognitively to the point that he/she was unable to treat himself/herself, was unable to verbalize his/ her needs, was incoherent, disoriented, and/or combative, or experienced seizure or coma.

21. History of more than 1 episode of DKA requiring hospitalization in the last 2 years
22. History of more than 1 episode of severe hypoglycemia in the last year.
23. Untreated or inadequately treated mental illness (indicators would include symptoms such as psychosis, hallucinations, mania, and any psychiatric hospitalization in the last year), or treatment with anti-psychotic medications that are known to affect glucose regulation.
24. Electrically powered implants (e.g. cochlear implants, neurostimulators) that might be susceptible to RF interference
25. Unable or unwilling to completely avoid acetaminophen for duration of study
26. Established history of allergy or severe reaction to adhesive or tape that must be used in the study
27. History of adverse reaction to glucagon (including allergy) besides nausea or vomiting
28. History of severe hypersensitivity to milk proteins or lactose
29. History of eating disorder within the last 2 years, such as anorexia, bulimia, or diabulemia or omission of insulin to manipulate weight
30. Current or planned use of SGLT2 inhibitors (prior use more than 3 months prior to enrollment is acceptable; SGLT2 inhibitors should not be initiated during the trial)
31. If using GLP1, pramlintide, or metformin must be on a stable dose for 3 months prior to enrollment (these agents should not be initiated during the trial)
32. Required use of 2 or more steroid bursts in the 6 months prior to the trial
33. History of intentional, inappropriate administration of insulin leading to severe hypoglycemia requiring treatment
34. Any factors that, in the opinion of the site principal investigator or clinical protocol chair, would interfere with the safe completion of the study, including medical conditions that may require hospitalization during the trial

VI. c. Sources of Subjects

Volunteers who fit the selection criteria will be considered as candidates for this study. We will contact individuals who have previously inquired about participation in our studies and have asked us to have their contact information kept on file. In addition, advertisements for the study may be posted at the MGH Diabetes Center and other places, and may be distributed in the weekly broadcast email of research studies seeking volunteers. A letter may be sent to adult endocrinologists in the Boston metropolitan area as well as selected nearby endocrinologists informing them of the study and asking them to refer to any eligible patients who might be interested. We will post information about the trial along with contact information on our website www.bionicpancreas.org and on www.clinicaltrials.gov.

No individuals will be excluded based on gender or race. An equal gender distribution between males and females is anticipated.

V. Subject Enrollment

V. a. Number of Subjects

It is expected that we will have 10 subjects complete the study with a consistent protocol. We expect that the experiments and the analysis can be accomplished over a period of 6-12 months. Up to 20 subjects will be enrolled. The upper bound is based on the expectation that some volunteers will be excluded after the screening visit and the possibility that some experiments may have to be discontinued before completion (due to, for instance, intercurrent illness or subject withdrawal). Each subject will have up to

two designated contacts enrolled who will serve as an emergency contact for the participant, and complete questionnaires based on their experience with the bionic pancreas. Therefore, total enrollment for the study including these partners will be 60.

V. b. Enrollment and Consent Procedures

Prospective participants will be briefed by a study staff member by phone or by e-mail regarding the study procedure and the inclusion and exclusion criteria. Potential subjects will be sent an informed consent document by mail, fax or e-mail.

Once potential subjects have had time to review the consent document, they will meet with a study provider (MD or NP) that will explain the study, answer any questions, and administer informed consent prior to any data collection or study-specific procedures. If a volunteer is a patient of one of the study MDs or NPs, another staff MD or NP will answer questions and administer consent. A licensed physician investigator will be available to speak with the subjects during the consent process in the event of an NP administering consent. The principal investigator will be responsible for assuring that the informed consent process is properly followed and that each study participant is well informed about the study and the participant's responsibilities.

Once identified by enrolled subjects, designated contacts will be sent a consent form to review. They will then meet with a member of the study team (RN, NP or MD) in person or over the phone that will explain their role, answer any questions and administer informed consent prior to any data collection. A licensed physician investigator will be available to speak with the subjects during the consent process in the event of an RN or NP administering consent. The principal investigator will be responsible for assuring that the informed consent process is properly followed and that each partner is well informed about the study and their responsibilities.

Study staff will answer any questions that the subjects may have during their participation. They will share any new information in a timely manner that may be relevant to the subject's willingness to continue participating in the trial. The subjects may choose to discontinue participation at any time.

VI. Study Procedures

VI. a. Screening Data

Subjects

- Age
- Sex
- Race and ethnicity
- Date of last menstrual period in female pre-menopausal females
- Date of diabetes diagnosis
- Medical, surgical, and social history, allergies and review of systems relevant to inclusion and exclusion criteria
- Medication (prescription and non-prescription) and date of last change in medication regimen
- Duration of insulin pump use
- Type of insulin used in pump
- Average total daily dose of insulin in the last 30 days
- Usage of CGM (type of CGM, days per month worn, usage of data, whether insulin dosing is based on CGM alone, alarm settings)
- Assessment of impaired awareness of hypoglycemia
- Height and weight

- Blood pressure
- Heart rate
- EKG
- Urine HCG (if applicable)
- Hemoglobin A1c
- Fractionated metanephrines (if applicable)

Designated Contacts:

- Age
- Sex
- Race and ethnicity
- Contact information (i.e. e-mail address, mailing address)

VI. b. Drugs

The study involves subcutaneous administration of **insulin lispro** (Humalog, Eli Lilly) or **insulin aspart** (Novolog, Novo Nordisk). Humalog and Novolog are commercially available by prescription and are indicated for patients with type 1 diabetes, but not for use in a bionic pancreas.

The study also involves subcutaneous administration of the glucagon analog, **dasiglucagon**. Dasiglucagon will be made available by Zealand Pharma A/S, Denmark as liquid formulation of 4 mg/mL in 1 mL prefilled cartridges. Dasiglucagon is a peptide analog of human glucagon that is being developed to treat hypoglycemia in patients with insulin-treated diabetes mellitus. Dasiglucagon exhibits improved physical and chemical stability in aqueous media and is suitable for liquid formulation. Dasiglucagon is comprised of 29 amino acids and has 7 amino acid substitutions when compared to native glucagon.

The iLet bionic pancreas system can administer bolus doses of each drug up to every five minutes. A single autonomous bolus of insulin will not exceed 3 units per 5-minute dose [30 µl] (except when it is in response to an isolated BG entry, where the dose will not exceed 12 units [120 µl]) and a single meal-priming dose, which is triggered by the user but automatically determined by the control system, will not exceed 18 units [180 µl]. A single bolus of glucagon will not exceed 80 µg [20 µl]. The iLet can administer as little as 0.10 µl (0.01 units of U-100 insulin) in single bolus doses. Insulin exposure is expected to be comparable to that of participants when not participating in the study. It is expected that the total daily dose of glucagon will be < 1.0 mg daily as in previous studies. The recommended dose of glucagon for adult patients suffering from severe hypoglycemia is 1 mg as a single injection. Mean glucagon levels in our previous inpatient studies have been above the normal fasting range for glucagon only 1% of the time. The mean daily glucagon dose in a previous 7-day outpatient study using the bihormonal BP at a set point of 100 mg/dl was 0.58 mg/day (range 0.20-0.90 mg/day). Our previous data using the bihormonal BP at a set point of 110 mg/dl, which will be used in this study, resulted in a mean daily glucagon dose of 0.41 mg/day (range 0.15-0.78 mg/day). The total dasiglucagon dose delivered over the 8-hour period of our in-clinic study was 0.59 mg (range 0.28-1.33 mg) compared with 0.73 mg (range 0.35-1.33 mg) Lilly Glucagon. Therefore, the glucagon exposure of subjects is expected to be modest.

Subjects will also be provided with an emergency **glucagon** kit, for treatment of severe hypoglycemia as needed.

VI. c. Devices

Infusion Sets: Participants will be provided with leur lock compatible infusion sets for both study arms. Participants will be instructed to replace both their glucagon infusion and their insulin infusion set every

other day throughout the study. If the infusion set fails for any reason during the experiment it will be replaced promptly..

Continuous Glucose Monitors: One transcutaneous glucose sensor for the Dexcom G5 will be inserted in the subcutaneous tissue and will provide input to the controller. The sensor is powered by the battery within the transmitter that clips to the sensor and the whole assembly is held to the skin with an adhesive patch and communicates wirelessly with the iLet. If the Dexcom G5 CGM sensor fails for any reason during the experiment it will be replaced promptly. The G5 sensor will be replaced at least every 7 days.

iPhone: The Dexcom G5 app is installed and will run on a stock iPhone. The Dexcom G5 app captures CGM glucose values via Bluetooth Low Energy from the transmitter worn on the body. The Dexcom G5 CGM app on the iPhone allows for calibration and maintenance of the sensor session.

iLet Bionic Pancreas Control Unit: The Gen 3.2 iLet bionic pancreas system receives the same CGM glucose values from the Dexcom transmitter worn on the body. The iLet has an integrated graphical user interface (GUI) and touchscreen display that displays the current CGM glucose, a graphical history of the CGM glucose, and doses of insulin delivered by the control algorithm. The GUI can also be used to input optional meal announcements, designating the size of the meal as “Large for Me” “Typical for Me”, “Small for Me”, or “Tiny for Me”, and the mealtime as the “Start”, “Middle”, “End”, or “Sleeping” periods of the day. This will trigger a partial meal-priming bolus, the size of which will adapt throughout the course of the trial to meet a target of 75% of the insulin needs for that size and mealtime. Participants will be instructed to announce meals in the same way when using the insulin-only and bihormonal configurations of the iLet.

The factory-set “usual” glucose target level for the bionic pancreas in the bihormonal mode is 110 mg/dl, and in the insulin-only mode is 120 mg/dl. A higher (+10 mg/dl) or lower glucose target (-10 mg/dl) can be set indefinitely as the “usual” target, or as “temporary” for a limited time with automatic expiration, or as “recurring” with automatic renewal and expiration times. When a temporary target is set, or when a recurring target period is on, upon expiration the target will revert to the currently chosen usual glucose target. Although our previous studies showed that the bionic pancreas decreased hypoglycemia and the need for carbohydrate interventions relative to usual care, this will allow participants to raise the glucose target for additional safety, particularly temporarily during periods when hypoglycemia may become problematic, such as when driving or otherwise unable to check or attend to their BG for a period of time, or during periods when hypoglycemia is more likely, such as during exercise. It may also be used to raise the mean BG if the mean is unnecessarily low and the user prefers to further reduce the risk of hypoglycemia.

For the purposes of this trial, the default target will be set at 110 mg/dl for both arms so that any difference in outcomes associated with the use of the bihormonal system vs. the insulin-only system will not be obscured by a difference in the target. Subjects must not change the permanent target without consulting with the study team. A change in the permanent target will be made only if, in the judgement of the principle investigator, this is important for subject safety. For example, the permanent target may be raised in an individual subject if there is excessive hypoglycemia, glucagon utllization, or need for carbohydrate treatment for hypoglycemia at the 110 mg/dl target. This is consistent with the intended clinical use of the iLet, in which a health care provider will consult with the user on the appropriate target. Subject may use the temporary target feature, but will be encouraged to use it only for short periods and for specific situations, such as exercise.

The user will have the option during the bihormonal bionic pancreas arm to trigger the administration of a

glucagon dose, intended to be used prior to device disconnection (e.g. for taking a shower or for swimming). The size of the glucagon dose will be automatically determined by the bionic pancreas based on the subject's body mass and will be between 40 and 80 micrograms. This option will provide a means for subjects to raise their BG if they anticipate they will be at risk for hypoglycemia during a period of disconnection, based on their glucose level and glucose trend at the time.

During periods when the CGM is offline, such as after a sensor is replaced and before the new sensor has been calibrated, the control algorithm will determine and direct the administration of insulin basal rates either based on the participant's weight in the first 24 hours of the experiment, or on the average of adaptively determined basal rates for that time of day once sufficient experience has been accumulated (i.e. 24 hours or more) by the control algorithm. The user will also be able to enter meal announcements in the GUI, in order to trigger automatically calculated meal boluses, in the same way as when the CGM was online. Finally, the user can trigger an automated correction bolus during such periods by entering a BG value in the GUI. The controller will administer insulin or decrease basal insulin as appropriate, in response to entered BG values during such CGM-offline periods, to a large extent as if the BG values were CGM values.

The device also displays visual alarms, sounds audible alarms, and generates vibration alarms for problems with the functioning of the bionic pancreas.

Ascensia Diabetes Care Contour Next One Glucose Meter: The Contour Next One glucometer is FDA approved and commercially available. Blood glucose measurements for Dexcom CGM calibrations and other required BG measurements will be obtained via finger stick with the Contour Next One in all study arms.

Abbott Precision Xtra Ketone Meter: The Precision Xtra ketone meter is FDA approved and commercially available. Blood ketone measurements will be obtained via fingerstick using the Precision Xtra ketone meter in all study arms.

VI. d. Experimental Procedures and Data Collection

VI. d. i. Screening Visit

- All subjects will have a screening visit to confirm eligibility
- The subject will be interviewed and the case report form will be completed by study staff to establish whether the subject is eligible to continue with the screening.
- A urine pregnancy test will be performed in pre-menopausal female volunteers. If the test is positive the volunteer will be informed of the result and the visit will be ended.
- Height, weight, heart rate and blood pressure will be measured. An EKG will be performed.
- If the volunteer is not excluded based on historical criteria, blood pressure, EKG or urine pregnancy test, blood will be drawn for hemoglobin A1c, and fractionated metanephrines if applicable. A study MD or NP will review the case report form to determine subject eligibility. If subjects are not eligible to continue in the study the results of abnormal tests will be reported to the subjects and to a health care provider of their choosing.
- A questionnaire will be administered to assess impaired awareness of hypoglycemia.
- Subjects who have been screened and are eligible can participate without having to be re-screened for a period of 3 months. The study staff should verbally confirm that there have been no health events that would make them ineligible at every study visit, and rescreen subjects for eligibility if it has been greater than three months.
- Once enrolled, subjects will identify up to 2 designated contacts (caregivers/family

members/friends) who are willing to serve as an emergency contact for the participant, requiring them to answer phone calls and check on the participants wellbeing as needed. If possible, they would also be an appropriate person to answer questionnaires about their experiences and attitudes towards the subject's diabetes and the impact of the bionic pancreas.

- Study staff will confirm the designated contacts are able to provide informed consent and are willing and able to serve as an emergency contact, and can recognize and treat hypoglycemia. If possible, they will confirm the contacts also have an appropriate relationship with the study participants to answer these questionnaires. Informed consent will be obtained from these contacts, and study staff will collect basic demographic information (age, sex, race/ethnicity) as well as contact information.
- All designated contacts will be trained in the symptoms of hypoglycemia and response to severe hypoglycemia, including the administration of glucagon. They will also be trained to recognize the symptoms of hyperglycemia, and when to contact study staff and local EMS.
- Designated contacts will complete two validated questionnaires, the Diabetes Distress Scale and the INSPIRE AID, which are both designed to be completed by partners. These questionnaires will be completed at baseline, and at the end of each study arm.
    - They will have the option to complete these forms in person at a study visit, or online using RedCap.

VI. d. ii. Randomization of Visit Order

Once the subject has been enrolled and eligibility has been established, subjects will be randomized to one of two possible visit schedules.

VI. d. iii. General Policies and Protocols for Days 1-7 of Both Study Arms

- Subjects will remain within a geographic boundary established based on 250 miles from the designated base for study personnel at all times and will avoid any air travel.
- Subjects will keep a charged mobile phone on their person (or at their bedside) at all times and will answer calls from the study staff.
- Study subjects will keep a Contour Next One glucometer easily accessible at all times in case a calibration is needed, and they will do all calibrations with this meter. They will keep a glucometer, fast-acting carbohydrates, and a glucagon emergency kit easily accessible at home for hypoglycemia as needed.
- Subjects should use the study provided Contour Next One glucometer for all BG checks throughout the study. They are encouraged to check their BG at least four times a day, before meals and before bedtime. They will also be encouraged to check before exercise and at intervals during exercise, and for any symptoms of hypoglycemia. There are no restrictions on additional checks and subjects should check as often as they wish to confirm the accuracy of the Dexcom CGM and for safety.
- Subjects will wear the study provided 6 mm steel cannula (Contact Detach) infusion sets throughout the study. If the subject is known not to tolerate steel infusion sets or are found not to do well with them during the study, then an alternative infusion set may be used.
- Subjects will not use any recreational drugs or drugs of abuse, other than alcohol. The need to take prescription drugs that dull the sensorium, reduce sensitivity to symptoms of hypoglycemia, or hinder appropriate decision-making may be grounds for exclusion from the trial or discontinuation of participation, a decision that will be made by the principal investigator.
- Subjects may not take acetaminophen throughout the study due to potential interference with CGM sensing. Acetaminophen is known to interfere with the accuracy of the Dexcom CGM.
- Subjects will contact study staff before taking any new drugs. Subjects may not take any drugs known to cause torsades de pointe or bradycardia throughout the study.

- Subjects will not tamper with the bionic pancreas device in any way, including changing any settings.
- Subjects will keep their iPhone charged, which will require charging at least once per day. In addition, subjects will change the iLet batteries every two days and use only Lithium AAA batteries. Alkaline batteries should be avoided unless they are needed temporarily until lithium batteries can be placed. Subjects may change their batteries more frequently as needed.
- The bionic pancreas is not water resistant and therefore must be removed for showering. Subjects are urged to take appropriate precautions when they are disconnected from the bionic pancreas, including frequent BG checks and having carbohydrates readily available.
    - The Dexcom CGM transmitter is water resistant and can be left on for bathing and swimming.
    - Subjects may not remove the bionic pancreas for more than 1 hour at a time (e.g. for bathing) and may not remove it for more than 2 hours total in any 24-hour period.
- Any medical advice needed by the subjects during their participation, which is not directly related to BG control during the experiment, should be obtained by them in the usual manner with their primary care physician or endocrinologist.
    - If a subject develops an illness during the experiment, they can seek medical care as usual. As long as the subject is not hospitalized, the study can be continued. If the subject is unable to eat for a period exceeding one day, they must notify study staff so that the medical staff can assess the safety of continuing in the study.
- Subjects may participate in any activities that they wish, as long as they abide by the policies above.
- There are no restrictions of any kind on diet or exercise, although subjects should attempt to maintain similar dietary habits and exercise habits during each arm of the study. The bionic pancreas must be kept dry during exercise.
- Participants in both study periods will complete a web-based questionnaire daily. Participants will be asked to report certain events occurring during the prior day such as hypoglycemia, other medical conditions, alcohol use, exercise, and use of a personal CGM as a part of usual care. In addition, events during the prior 24 hours including nausea and/or vomiting will be solicited.
- Participants will be encouraged to announce the three major meals of the day to the iLet in both arms. The meal announcement will consist of choosing the timing of the meal relative to one's regular sleep period (first of the day, middle of the day, end of the day, or during what would be regular sleep hours if one occasionally happens to be up) and the size of the meal relative to typical meals for that participant (snack, smaller than typical, typical, larger than typical). Participants will be trained not to announce snacks that occur between major meals.
- The subjects will calibrate the Dexcom CGM twice daily, preferably before breakfast and supper using the Contour Next One.
    - Subjects will be advised to delay calibration if there is a steep rise or fall in the blood glucose (>2 mg/dl/min), if they got a dasiglucagon dose in the last 15 minutes, or if there has been carbohydrate intake in the last 30 minutes. In the immediate aftermath of glucagon dosing or carbohydrate intake, it is possible for the BG to be rising without a change in interstitial fluid glucose. If a calibration is delayed for any of these reasons, it will be performed at the next opportunity.
    - Subjects may perform additional calibrations if the Dexcom CGM is inaccurate relative to a BG measurement as long as they do not calibrate within 30 minutes of food intake. Subjects will be discouraged from performing extra calibrations if the Dexcom CGM is within 15 mg/dl when the BG is ≤ 75 mg/dl and within 20% if the BG is >75 mg/dl at times when the rate of change is low. They will also be trained to understand that the apparent error can be higher than this when the BG is changing rapidly, and that it is typical for the

Dexcom CGM to underestimate BG when the trend is upward and to overestimate BG when the trend is downward as a result of physiologic lag. Errors in these directions should typically not prompt extra calibrations unless they are very large (≥ 50%).

- Subjects may choose to withdraw from the study at any time. If they withdraw from the study, they should contact a provider immediately who will help them transition to their own insulin regimen safely.
- Subjects will be asked to change their insulin infusion set and reservoir at least every other day throughout the study.
- Subjects will be asked to change their glucagon infusion set at least every other day throughout the study. Dasiglucagon is stable in the reservoir for more than seven days, so it may not need to be replaced during the study. Subjects will be provided with an extra reservoir to use as needed.
- Subject will be instructed to change the iLet batteries every two days, or sooner as needed.
- A new Dexcom G5 CGM sensor will be placed every 7 days if no replacement was required before this time. The Dexcom G5 app will generate an alarm when replacement is required.
    - If the CGM sensor fails during the experiment, the system will provide automated basal insulin based on past requirements, and will allow announcement of meals (with 75% of predicted insulin delivered based on past requirements), and entry of fingerstick BG measurements (which will be treated as CGM data and may result in administration of insulin or temporary suspension of basal insulin). The system will alarm and request a BG measurement every two hours when the CGM signal is not available, but the system will remain in closed-loop mode even if CGM data are not available. The CGM sensor will be replaced as soon as possible and normal (online) iLet control will resume when the new sensor is calibrated.
- If there is a technical fault with the iLet, the participant will call the technical support line immediately. If necessary, a staff member will meet the participant to assist with troubleshooting. This meeting may be delayed until morning if the problem occurs overnight - in this case, the participant will use their own pump or use injectable insulin until a meeting is possible. A member of the study staff (within their scope of practice and under the supervision of the site principal investigator) may advise them on how to manage their diabetes in the interim. If necessary, the iLet device may be replaced. This should occur in most cases within 12 hours. Staff will not go into subjects' houses or other non-public places; nor will they go to any place to meet the subject that is not public or where they do not feel safe.
    - If there is a complete failure of iLet operation and it is anticipated that restarting it will take more than an hour, participants may revert to usual care using their own insulin pump or with insulin injections until the iLet can be brought back online with the help of study staff. Every effort should be made to correct the problem as soon as possible, which should almost always be possible within 12 hours.
- Participants will not change the default glucose target of the bionic pancreas without consulting with the study staff.
- Participants may use the temporary target feature of the bionic pancreas but should use for for limited periods and for a specific purpose, such as exercise.

Local Alarms

- All alarm settings will be the same in both study arms.
- Alarms will sound and a visual alert will appear on the screen of the G5 mobile app when the CGM glucose is ≤ 70 mg/dl and the iLet will alarm when the CGM glucose is ≤ 50 mg/dl.
    - Participants will be trained to test their BG with the study glucometer in response to such an alarm and take any necessary measures to treat hypoglycemia.

- Participants will be trained on troubleshooting for various scenarios that could lead to low threshold alarms. For instance, a threshold alarm could be due to true hypoglycemia, inaccurate CGM readings, or a compression artifact at the site of the sensor.
    - The first step for all glucose-related alarms will be to perform a fingerstick BG measurement with the study provided glucometer (provided).
    - If the BG measurement is not consistent with the fact that a threshold alarm has occurred, then the participant will assess the accuracy of the CGM, and the possibility of a compression artifact (they will be trained in the causes and recognition of these events). If a compression artifact is suspected, they will take steps to relieve the pressure on the transmitter. If no compression is suspected, the participant will calibrate the CGM.
    - If the BG measurement is consistent with a low threshold alarm, the participant will treat hypoglycemia with carbohydrate ingestion according to their usual practice. Study staff will recommend the standard of care, 15 grams of rapid acting carbohydrates and re-testing BG in 15 minutes. Study staff will recommend the participant continue to monitor their BG until it returns to normoglycemia, and to contact study staff with any questions or concerns.
    - Participants will be asked to investigate their dasiglucagon infusion set and consider replacing it, check for any occlusions along the fluid path, and check to make sure that the cartridge is not empty.

- Alarms will sound and a visual alert will appear on the Dexcom CGM app if CGM glucose is > 250 mg/dl.
    - Participants will be trained to test their BG with the study glucometer and the ketone level with the study ketone meter (provided) in response to such an alarm and take necessary measures to treat the hyperglycemia.
    - Participants will be trained on troubleshooting for various scenarios that could lead to persistent or severe hyperglycemia. For instance, hyperglycemia could be due to true hyperglycemia (caused by a missed insulin dose or a failed infusion set for example) or inaccurate CGM readings.
    - The first step in responding to severe or persistent hyperglycemia according to the CGM will be to perform a fingerstick BG and, if necessary, ketone measurement.
        - If the BG measurement is not consistent with the CGM readings, the participant will calibrate the CGM.
        - If the BG measurement is consistent with the CGM readings:
            - Participants will be asked to investigate their insulin infusion set and consider replacing it, check for any occlusions along the fluid path, and check to make sure that the cartridge is not empty.
            - Study staff will recommend the participant continue to monitor their BG until it returns to normoglycemia, and to contact study staff with any questions or concerns.
    - If ketones $\geq$0.6 mmol/L are present:
        - Participants will be advised to change their pump infusion set and will be reminded that the BP should dose insulin accordingly.
        - Study staff will recommend the participant continue to monitor their ketone levels and BG every 60 minutes until ketones return to < 0.6 mmol/L and BG is < 180 mg/dl, and to contact study staff with any questions.
    - If participants experience persistent hyperglycemia lasting more than 2 hours, they will be instructed to contact study staff for consideration of infusion set replacement and/or correction insulin according to the above protocol.

Remote Monitoring Protocol

- All remote monitoring will be the same in both study arms.
- Real-time remote telemetric monitoring for biochemically severe hypoglycemia or persistent hyperglycemia will be performed by the study staff 24 hours a day. There will be at least one provider (MD, NP, or PA) on call at all times. Additional study staff members may assist with on call duties. A staff member will make contact with participants as necessary and help them troubleshoot any issues that may arise.
- When an alert comes in, a study staff member will call the participant. Depending on the circumstances, they may call locations the participant is known to frequent (e.g. usual work location) or they may be dispatched to make contact with the participant (if the location is nearby and reaching the location would be no risk to the safety of staff member or violate employment rules).
- If study staff are not able to get in contact with the participant, they may contact the designated contact. These designated contacts should have access to where the participant may be sleeping if necessary.
- Remote monitoring is only possible when the participant has Verizon network coverage and data can be transmitted to the cloud service. There may be times when a participant enters an area where Verizon coverage is not available. We may also encourage participants to connect to public but secure wireless networks if they are having trouble connecting to cellular service.
- An alert will be generated if remote monitoring indicates that a participant is offline.
    - If there are no indications of device malfunction as the cause for lost connectivity, the glucose level is in safe range, and a participant chooses to remain in an area with poor network coverage, we will instruct the participant to check the iLet display or CGM at least every 20 minutes for alert icons and to be aware that we are unable to monitor for severe lows or highs at this time.
    - We will call the participant every 2 hours to check on safety and device function until remote monitoring is restored.
    - The same rules will be used for checking in when the participant in both study periods.
- The remote monitoring system will generate an alarm if the CGM glucose is < 50 mg/dl for 15 minutes.
    - Study staff will verify the participants are aware of the hypoglycemia and taking action to treat it. Participants will be reminded of the protocol for hypoglycemia, and the study provider will ensure they understand and will follow study procedures. Participants will be encouraged to follow up with any questions or concerns. All contact with the participants in response to hypoglycemia alarms will be documented.
    - In the case of a low threshold alarm with no response from the participant and no success in locating them or their designated contact, the site principal investigator will be immediately informed. If remote monitoring shows ongoing hypoglycemia, a decision may be made to dispatch emergency medical services to the locations the participant is known to frequent.
    - Remote monitoring for hypoglycemia will be the same in both study periods.
- The remote monitoring system will generate an alarm if the CGM glucose is > 300 mg/dl for 90 minutes.
    - Participants will be reminded of the protocol for prolonged hyperglycemia, and the study provider will ensure they understand and will follow study procedures. Participants will be encouraged to follow up with any questions or concerns. All contact with the participants in response to hyperglycemia alarms will be documented.

- Remote monitoring for hyperglycemia will be the same in both study periods.

VI. d. iv. Visit Procedures

Day 1 Visit:

Participants may be trained on the operation of the all study devices in a group setting or may be trained one-on-one. Both the participant and the study staff must be satisfied that the participant is comfortable with the operation of all study devices before he/she begins the study. Additional training sessions may be arranged as needed.

- Participants will complete their baseline psychosocial questionnaires
- Their body weight, heart rate and blood pressure will be documented
- Blood will be drawn for a chemistry panel and complete blood count, and a baseline assessment of anti-drug antibodies.
- Study staff will review any changes in the participant's medical history or medications to ensure continued eligibility, and any adverse events that may have occurred since their screening visit. Study staff will specifically ask subjects if they had any infusion site reactions or other skin irritation in the past 7 days, and will document any reactions.
- Participants will be trained on the insertion and use of the Dexcom G5 CGM. Participants will insert their own CGM sensor and study staff will confirm they are doing it correctly.
- Study staff will review all study procedures and policies (including the use of the Dexcom G5, the Contour Next One, the Precision Xtra ketone meter, and the iLet device) and the upcoming visit schedule.
- The Dexcom G5 will be calibrated by the participant using the Ascensia Contour Next One SMBG glucometer, and study staff will confirm they are doing it properly.
- Participants will be trained on the use of the iLet.
- The control algorithm will be initialized with the participant's current weight
- The participant will remove his/her own insulin infusion pump and the participant will set up and start the iLet under the supervision of study staff. Participants will be instructed to not take any further insulin outside of the iLet throughout the study.
- The staff will confirm that the iLet is functioning properly prior to discharging the participant.
- All participants will be given Dexcom G5 CGM sensors, an Ascensia Contour Next One SMBG meter with test strips, ketone meter with strips, insulin, dasiglucagon for the bihormonal study arm, a glucagon emergency kit, an iLet, iLet pigtail adapters and Contact Detach infusion sets (an alternate infusion set may be supplied if the subject is known not to do well with the Contact Detach).

Day 7/Day 1 Visit:

- There may be no washout between the two study arms or the washout period may be up to 7 days.
- At the end of the first 7-day arm, subjects will return to the clinic and answer the post questionnaires for the study arm.
- The body weight, heart rate and blood pressure of the subject will be documented
- Blood will be drawn for a chemistry panel and complete blood count.
- Study staff will review any changes in the participant's medical history or medications to ensure continued eligibility, and any adverse events that may have occurred since their last study visit. Study staff will specifically ask subjects if they had any infusion site reactions or other skin irritation in the past 7 days, and will document any reactions.
- The iLet and the study glucometer will be downloaded. The memory of the iLet will be wiped and

it will be re-initialized for the next study period.
- Participants will replace their CGM sensor and calibrate when prompted if they haven't already.
- Study staff will review all study procedures and policies (including the use of the Dexcom G5, the Contour Next One, and the iLet) and the upcoming visit schedule.
- The control algorithm will be initialized with the participant's current weight. The participant will set up and start the iLet under the supervision of study staff. Participants will be instructed to not take any further insulin outside of the iLet throughout the study.
- The staff will confirm that the iLet is functioning properly prior to discharging the participant.
- All participants will be given additional supplies as needed.

Final Day 7 Visit:
- At the end of the second 7-day arm, subjects will return to the clinic and answer the post questionnaires for the study arm.
- The body weight, heart rate and blood pressure of the subject will be documented.
- Blood will be drawn for a chemistry panel and complete blood count.
- An EKG will be performed. This EKG will be assessed relative to the baseline EKG to identify any potential changes.
- Any changes to medications or medical history and any adverse events that may have occurred since their last study visit will be documented. Study staff will specifically ask subjects if they had any infusion site reactions or other skin irritation in the past 7 days, and will document any reactions.
- The iLet and the study meters will be downloaded.
- All equipment will be collected and cleaned. The Dexcom G5 transmitter will be cleaned using the validated cleaning and disinfecting procedures.
- A study provider will review the last few hours of glucose trend data and insulin and dasiglucagon on board from the iLet, and assist the participants in resuming their usual diabetes management.

Follow Up Visits
- Subjects will return for a follow up visit 10 days (± 3 days) after the final day 7.
    - Any changes to medications or medical history and any adverse events that may have occurred since their last study visit will be documented. Study staff will specifically ask subjects if they had any infusion site reactions or other skin irritation since their last study visit, and will document any reactions.
    - Blood will be drawn for anti-drug antibodies.
    - If the EKG on the final Day 7 visit showed any changes relative to the baseline EKG, an additional EKG will be performed at this follow up visit.
- Subjects will return for a second follow up visit 25 days (± 4 days) after the final day 7.
    - Any changes to medications or medical history and any adverse events that may have occurred since their last study visit will be documented. Study staff will specifically ask subjects if they had any infusion site reactions or other skin irritation since their last study visit, and will document any reactions.
    - Blood will be drawn for a chemistry panel, complete blood count, and anti-drug antibodies.

VI. d. v. Response to Hypoglycemia
- The response to hypoglycemia will be the same in both arms. Subjects will be reminded that the iLet decreases insulin dosing in response to decreasing CGMG in both the insulin-only and bihormonal configurations.

- The Dexcom G5 app will generate a local low glucose threshold alarm if the subject's CGM value is ≤70 mg/dl and the iLet will generate an alarm for a CGMG < 50 mg/dl.
- In all study arms subjects are encouraged to check their BG for any symptoms of hypoglycemia.
- Subjects are encouraged to treat hypoglycemia according their usual practice or according to the "rule of 15s": take 15 grams of rapid acting carbohydrate and recheck in 15 minutes, then repeat as needed. Subjects will be reminded to check their glucagon infusion site and their bionic pancreas for normal operation anytime they experience hypoglycemia during the bihormonal arm. If there is any suspicion of glucagon infusion set malfunction, the site should be replaced.
- Subjects may contact a study provider (RN, MD or NP) for advice at any time, and may contact the troubleshooting support team, as they wish. Subjects will be assisted in checking the bionic pancreas for any malfunction and correcting any problems that are found.
    - If no correctable fault is found, but there is doubt regarding the correct function of the bionic pancreas system, an entirely new backup bionic pancreas system may be brought to the subject's location by study staff.
- If a subject experiences severe hypoglycemia in the study, his or her participation in the study will be discontinued.

VI. d. vi. Response to Hyperglycemia

- The response to hyperglycemia will be the same in both arms.
- The Dexcom G5 app will generate a local high glucose threshold alarm if the subject's CGM value is > 250 mg/dl. Subjects will be instructed to check their insulin infusion site and their bionic pancreas for normal operation any time they get this alarm, or BG is greater than 250 mg/dl. If there is any suspicion of insulin infusion set malfunction, the site should be replaced.
- Subjects may contact a study provider (RN, MD or NP) for advice at any time, and may contact the troubleshooting support team, as they wish. Subjects will be assisted in checking the bionic pancreas for any malfunction and correcting any problems that are found.
- If no correctable fault is found, but there is doubt regarding the correct function of the bionic pancreas system, an entirely new backup bionic pancreas system may be brought to the subject's location by study staff.
- If a subject experiences diabetic ketoacidosis requiring hospitalization during the study, his or her participation in the study will be discontinued.

VI. d. vii. Response to Nausea and Vomiting

- If significant nausea, nausea that prevents the subject from eating normally, or any vomiting occurs during either arm of the study, subjects will be encouraged to contact a study provider (MD or NP). They will document the report of nausea or vomiting.
- If this occurs during the bihormonal bionic pancreas arm, they may assist the subject in troubleshooting, such as checking the BG and the calibration of the Dexcom CGM (excessive glucagon dosing may occur if the Dexcom CGM is reading lower than the true BG). If a subject experiences persistent nausea and vomiting thought to be related to glucagon dosing, his or her participation in the study will be discontinued.

VI. d. viii. Response to Other Medical Needs

If the subject experiences any non-emergent medical concerns outside the scope of diabetes care, he or she will see their personal physician. If the subject experiences urgent or emergent medical concerns outside the scope of diabetes care and their primary care physicians, they should visit a walk-in clinic or emergency room, or if necessary call 911.

VI. d. ix. Monitoring of Bionic Pancreas Performance
A Beta Bionics engineer will be readily available by phone for consultation at all times during the course of each experiment to assist with any needed troubleshooting.

VI. d. x. Supervision by study staff
A study provider (MD or NP) will be on call at all times during the course of all study arms. All trained staff will have the capability of remotely viewing diagnostic information to facilitate phone troubleshooting with subjects and decide whether additional assistance is needed.

VII. Statistical Considerations
VII.a Statistical Hypotheses
The primary endpoint evaluates if the iLet system performs as designed, receiving CGM signals and delivering drug doses (insulin and, when applicable, glucagon) calculated by the control algorithm with an acceptable degree of reliability and accuracy. No formal test of the primary endpoint is planned.

The null hypothesis tested for the key secondary endpoint is:

> *The amount of hypoglycemia using the bihormonal configuration of the iLet is equal to the amount of hypoglycemia using the insulin-only configuration of the iLet.*

The proportion of time with CGM glucose < 54 mg/dl across days 2-7 estimates the amount of hypoglycemia.

VII.b Sample Size Determination
No formal statistical comparisons have been done for this study. This study is primarily intended to verify that the iLet is operating as expected (based on its design and on bench testing) in each configuration in the outpatient setting. A sample size of 10 subjects in a crossover design is considered adequate for this purpose.

VII.c Populations for Analyses
Adult subjects (≥ 18 years old) with type 1 diabetes.

VII.d Statistical Analyses
VII.d.i General Approach
An intention-to-treat analysis will be performed. Descriptive statistics will be used to summarize patient characteristics and assessments. No formal testing will be conducted to establish superiority. In general, all data will be summarized with descriptive statistics for each treatment group. Unless otherwise specified, continuous data will be summarized by presenting the number of non-missing observations, mean, standard deviation (SD), median, minimum, and maximum. Categorical data will be summarized by presenting the number of patients and percentage for each category. P-values can be displayed to aid interpretation, not for inference. Tables, figures and listings of all primary, secondary and safety endpoints will be created.

Baseline and demographic data will be summarized using descriptive statistics.All comparisons will be between treatments within patients. Listings of individual patient data will be presented.

Data from periods when the bionic pancreas was not in use will be included, if available (Dexcom CGM data may not be available in some failure modes). In cases where an arm was not completed we will use the available data from that arm in the data analysis.

VII.d.ii Analysis of the Primary Efficacy Endpoint
The primary endpoint is whether the iLet operates as designed during the study arms. An arm will be considered to have successfully met its primary endpoint if each of the following is true during the time that the iLet is powered on over the entire treatment period (days 1–7):

1. The percentage of time that valid CGM glucose readings are captured by the iLet is ≥80%. Sensor warm-up periods, occasions of failed communication between the iLet and sensor–transmitter unit, or other sensor failure conditions are all counted as times when valid CGM glucose reading are not captured.
2. The percentage of the time that each drug channel (insulin, and if applicable, glucagon) is available is ≥95%. A channel is considered to be available if a cartridge is successfully loaded, there is no pending occlusion alarm or other system failure to prevent attempting a delivery on that channel, and the cartridge is not yet considered empty.
3. The ratio of cumulative drug doses delivered to cumulative drug doses attempted is between 0.95 and 1.05, inclusive, for insulin and, if applicable, for glucagon. Attempted doses refer to calculated doses that are issued for delivery when the channel is available. Doses delivered refers to the dose amounts reported back by the motor controller after the delivery attempts are completed.

If all three evaluations hold true for a patient's treatment period, the patient-period is operationally successful. The proportion of patients that are operationally successful will be presented by pump type.

VII.d.iii Analysis of the Secondary Endpoints
The proportion of time spent with CGMG <54 mg/dl and the mean CGMG are analyzed using methods for paired data. The treatment means and mean difference between treatments will be estimated together with the corresponding 95% confidence interval and compared using a paired t-test for normally distributed data or Wilcoxon signed-rank test for non-normally distributed data. Time <54 mg/dl and mean glucose will be computed from all available CGM data during days 2-7 for both study arms.

All questionnaire data for patients who have completed a minimum of 4 days with a device will be included. Patients who do not complete a questionnaire will be excluded from the patient analyses. Results will be compared by Wilcoxon signed-rank test.

VII.d.iv Safety Analyses
No inferential tests of safety data will be performed. Descriptive summaries of safety data will be presented. Adverse events (treatment-emergent unless otherwise specified) will be presented by system organ class (SOC) and preferred term (PT) for each treatment group. Treatment-emergent AEs are defined as AEs with onset date on or after the first day of exposure to randomized treatment.

VII.d.v Baseline Descriptive Statistics
The number and percentage of patients who were screened, randomized, discontinued (with reason for discontinuation), and completed the trial will be summarized descriptively for all screened patients.

VII.d.viii Tabulation of Individual participant Data
Listings of individual patient data will be presented.

VII.d.ix Exploratory Analyses
The performance of the iLet in each period can be compared with historical data from previous bionic pancreas trials using previous generations of hardware. If the performance of the iLet in this study is

comparable to our previous data from trials using the iPhone-based bionic pancreas that will support the hypothesis that both configurations of the iLet are effective and safe implementations of the bionic pancreas. All participants with at least 24 hours of CGM data during days 2-7 of any treatments periods will be included in these analyses.

We may, in exploratory analyses, also stratify subjects for secondary analyses of the pre-specified endpoints by subject characteristics including: sex, age, baseline A1c, usual care insulin total daily dose, body mass index, phase of menstrual cycle (follicular vs. luteal).

VII. e Data Collected

VII. e. i. Prior to the start of the experiment:

- Age
- Sex
- Race and ethnicity
- Date of last menstrual period in female subjects
- Date of diabetes diagnosis
- Medical, surgical, and social history, allergies, and review of systems relevant to inclusion and exclusion criteria
- Medications (prescription and non-prescription) and date of last change in medication regimen
- Duration of insulin pump use
- Type of insulin used in pump
- Average total daily dose of insulin in the last 30 days as available
- Usage of CGM (type of CGM, days per month worn, usage of data, whether insulin is dosed based on CGM alone, alarm settings)
- Height and weight
- Blood pressure
- Heart rate
- EKG
- Hemoglobin A1c
- Fractionated metanephrines, if applicable
- Urine HCG (pre-menopausal females)

VII. e. ii. During study arms

- CGMG (CGM glucose) every five minutes from the Dexcom CGM
- All fingerstick BG measurements taken by the subject (meter download)
- Average glucose target used
- Insulin total daily dose from bionic pancreas download
- Glucagon total daily dose from bionic pancreas download
- Timing of meal announcements and size of meals announced from bionic pancreas download
- Time subjects were not under bionic pancreas control
- List of technical faults associated with the bionic pancreas including cause and resolution
- Body weight after each study arm
- Blood pressure after each arm
- Heart rate after each arm
- Skin reactions, if any, after each arm
- EKG after completion of the second arm, and at the first follow up visit if applicable
- Information collected from the daily email survey:

- Number of reported hypoglycemic events requiring carbohydrate intervention
- Total grams of carbohydrates taken to treat hypoglycemia
- Number of severe hypoglycemic events (defined as subject required assistance to treat)
- Mean nausea severity from VAS
- Number of unscheduled insulin cartridge/infusion set changes
- Number of unscheduled glucagon cartridge/infusion set changes
- Alcohol use (mean drinks per day)
- Exercise duration and exposure (duration X intensity)

- Data from questionnaires about attitudes and expectations regarding the bionic pancreas at baseline and on day 7 of each arm.

VIII. Risks and Discomforts

Subjects may experience mild discomfort associated with the insertion of infusion sets and the Dexcom CGM sensor into the SC tissues. The risk of discomfort due to insertion of infusion sets and sensors may be greater than in their lives outside the trial because more infusion sets and sensors are required than are used in usual care.

There is a risk of hypoglycemia. This risk is expected to be less than the risk during the subjects' lives outside of the trial based on data from earlier trials. All of our previous studies have shown that hypoglycemia is significantly reduced in all configurations of the bionic pancreas when compared with usual care.

There is a risk of hyperglycemia. This risk is expected to be less than the risk during the subjects' lives outside of the trial based on data from earlier trials. All of our previous studies have shown that hyperglycemia is significantly reduced in all configurations of the bionic pancreas when compared with usual care.

There is a risk of headache, nausea or vomiting due to the administration of exogenous glucagon. There is a possible risk of skin rash due to administration of exogenous glucagon. There may be risks of daily, low-level glucagon administration that have not become apparent during trials lasting up to 11 days. Possible risks include changes in body weight, blood pressure or blood chemistries or blood counts, or development of anti-drug antibodies. The magnitude of the other possible risks due to daily administration of small amounts of glucagon are unknown, but are not expected to be high because mean glucagon levels have been in the fasted range in previous trials. Of note, the risk of nausea and vomiting has been low in prior studies. There may be other unknown risks associated with dasiglucagon.

IX. Potential Benefits

It is expected that this protocol will yield increased knowledge about using an automated closed-loop to control the glucose level. This research is one step on the path towards development of a fully closed-loop system, and is particularly important for establishing the safety and efficacy of a bihormonal system for larger and longer future studies. The individual participant may not benefit from study participation.

Based on evidence from previous trials of the bionic pancreas and the design of this trial, subjects enrolled in the study may benefit from a reduction in risk of hypoglycemia and hyperglycemia and a better mean glucose than they typically achieve.

Subjects will be financially compensated for participating in the study.

X. Data and Safety Monitoring

X. a. Monitoring of Source Data

During the experiment, CGM data, insulin dosing data, and glucagon dosing data will be automatically stored in the bionic pancreas device (from which it will be downloaded at intervals). All of the data will be combined in a single database that will be compared against the primary data files for integrity. The computer database will be backed up at least monthly and the backup media stored in a secure location.

Study staff will be encouraged to raise any concerns they may have or problems they have identified at any time. The PI, in consultation with the co-investigators, will decide a course of corrective action, and resolution or progress will be assessed no later than the next meeting.

An audit of procedures, regulatory documentation, and a sample of subject files will be performed by a member of the Diabetes Research Center at least biannually. The audit will be conducted by a staff member who is not directly involved in the conduct of the study. This audit will include a review of regulatory documentation, such as IRB and FDA correspondence, and a review of subject files, including a review of consents, case report forms, and other data from study visits.

A numeric code will be substituted for the subjects personal identifying information in the study database, which will be password protected. The key linking the medical record number of the subject with the numeric code, along with case report forms, and all information that is personally identifiable, will be kept in a locked filing cabinet in an investigator's locked office. All electronic records will be kept in a password protected computer database. All printed computer data will be disposed of confidentially when no longer needed. Only the study staff will have access to the study database. Subjects may not withdraw from the de-identified database, but they may elect to have the key linking their medical record to the de-identified database destroyed.

The study data will be shared with collaborators at Beta Bionics, Inc. and Zealand Pharma, but only in a form in which all personally identifiable information has been removed (e.g. combined database including BG values, record of insulin and glucagon delivered by the device, and blood insulin and glucagon levels). Shared data will be in the form of a database in which only a number identifies subjects.

Subjects may not withdraw their data, as it will be stored in non-personally identifiable form.

X. b. Safety Monitoring

This study is considered moderate risk. An external Data and Safety Monitoring Board (DSMB) will oversee the conduct of the study and review its results on a regular basis. Additionally, they will be informed of all serious adverse events and any unanticipated adverse device events that occur during the study and will review compiled safety data at periodic intervals. The DSMB will be informed if there are any changes to the study protocol that could significantly impact the safety or scientific validity of the study. A final DSMB meeting will convene after the completion of the study. Safety and efficacy data will also be reported to the IRB and FDA in compliance with applicable regulations.

The participation of individual subjects in the study will be discontinued if:

- Subjects experience one episode of diabetic ketoacidosis (DKA) requiring hospitalization
    - Hyperglycemic events will be classified as DKA if the following are present:
        - Symptoms including but not limited to polyuria, polydipsia, nausea or vomiting
        - Serum ketones > 1.5 mmol/L or large/moderate urine ketones
        - Either arterial blood pH <7.30 or venous pH < 7.24, or serum bicarbonate < 15
        - Treatment provided in a healthcare facility
- Subjects experience one episode of severe hypoglycemia, defined as requiring the assistance of

another person due to altered consciousness, and required another person to actively administer carbohydrate, glucagon or other resuscitative actions. This means the participant was impaired cognitively to the point that he/she was unable to treat himself/herself, was unable to verbalize his/her needs, was incoherent, disoriented, and/or combative, or experienced seizure or coma. If plasma glucose measurements are not available during such an event, neurological recovery attributable to the restoration of plasma glucose to normal is sufficient evidence that the event was induced by a low plasma glucose concentration.

- Subjects experience persistent nausea and vomiting thought to be related to glucagon dosing in the bi-hormonal bionic pancreas arm
- The investigator believes it is unsafe for the participant to continue in the study
- A participant becomes pregnant
- The participant requests that the study be stopped

If more than 1 subject must be withdrawn from the study for severe hypoglycemia or DKA, the study will stop and a vote of the DSMB will be required to restart it. All serious and unexpected events will be reported to the DSMB within 72 hours.

Study participation is voluntary, and participants may withdraw at any time. The investigator may withdraw a participant who is not complying with the protocol. For participants who withdraw, their data will be used up until the time of withdrawal. For participants using the iLet who withdraw, a study provider will help them transition to their own therapy safely.

In case of a recurring system malfunction or participant safety issue observed with multiple participants, the overall study will be suspended while the problem is diagnosed. The study may resume if the underlying problem can be corrected by a protocol or system modification that will not invalidate the results obtained prior to suspension.

X. c. Adverse Event Reporting Guidelines

Definitions

An **adverse event** is defined as any untoward or unfavorable medical occurrence in a human subject including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice).

Hypoglycemic events are recorded as Adverse Events (severe hypoglycemic event) if the event required assistance of another person due to altered consciousness, and required another person to actively administer carbohydrate, glucagon, or other resuscitative actions. This means that the participant was impaired cognitively to the point that he/she was unable to treat himself/herself, was unable to verbalize his/ her needs, was incoherent, disoriented, and/or combative, or experienced seizure or coma. These episodes may be associated with sufficient neuroglycopenia to induce seizure or coma. If plasma glucose measurements are not available during such an event, neurological recovery attributable to the restoration of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration.

Hyperglycemic events are recorded as Adverse Events (severe hyperglycemic event) if the event involved DKA, as defined by the Diabetes Control and Complications Trial (DCCT) and described below, or in the absence of DKA if evaluation or treatment was obtained at a health care provider facility for an acute event involving hyperglycemia or ketosis.

Hyperglycemic events are classified as DKA if the following are present:

- Symptoms such as polyuria, polydipsia, nausea, or vomiting;
- Serum ketones >1.5 mmol/L or large/moderate urine ketones;
- Either arterial blood pH <7.30 or venous pH <7.24 or serum bicarbonate <15; and
- Treatment provided in a health care facility

The PI and co-investigators will review any adverse events after each experiment to determine severity (serious or non-serious), expectedness (expected or unexpected) and relatedness (related, possibly related or unrelated). The study investigator will assess the relationship of any adverse event to be related or unrelated by determining if there is a reasonable possibility that the adverse event may have been caused by the study intervention.

To ensure consistency of adverse event causality assessments, investigators should apply the following general guideline when determining whether an adverse event is related:

Related: There is a plausible temporal relationship between the onset of the adverse event and the study intervention, and the adverse event cannot be readily explained by the participant's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study intervention; and/or the adverse event abates or resolves upon discontinuation of the study intervention or dose reduction and, if applicable, reappears upon re-challenge.

Possibly Related: Possibly related to the research means there is a reasonable possibility that the adverse event, incident, experience or outcome may have been caused by the procedures involved in the research (modified from the definition of associated with use of the drug in FDA regulations at 21 CFR 312.32(a)). Reasonable possibility means that the event is more likely than not related to participation in the research or, in other words, there is a >50% likelihood that the event is related to the research procedures

Not Related: Evidence exists that the adverse event has an etiology other than the study intervention (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to study intervention.

Serious adverse event means any event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death
- is life threatening (places the subject at immediate risk of death from the event as it occurred)
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity
- results in a congenital anomaly/birth defect
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Reporting Guidelines

Any serious adverse events or unexpected but related or possibly related adverse events will be communicated to the PI as soon as possible and within 48 hours of the time they are detected. Adverse

events will be reported promptly to the Partner's IRB and to the BU IRB, the DSMB, Beta Bionics and Zealand Pharma. Collaborator Ed Damiano is the sponsor of the Investigational Device Exception (IDE) for the bionic pancreas to be used in this trial. Zealand Pharma is the sponsor for the Investigational New Drug (IND) application for dasiglucagon to be used in this trial. Reports of adverse events will be made to the FDA in compliance with the terms of IDE and IND.

XI. Subject Compensation

Financial compensation will be provided to all subjects who complete the screening visit. Subjects will be paid $25 for completing the screening visit whether or not they are eligible to participate in the study.

Study participants will be compensated $50 for completing each study visit. Thus, the total compensation for a subject who completed screening and all five study visits would be $275.

Parking expenses will be paid for up to $30 per subject for each visit. Subjects who are unable to complete the study or chose to stop participation will receive prorated compensation for the portion of the study visits that they complete.

Designated contacts will be compensated $25 for completing the screening visit, $25 for completing the study, and $25 for completing their questionnaires. The total compensation available for designated contacts is $75.

XII. References

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