Effect of Metabolic Control at Onset of Diabetes on Progression of Type 1 Diabetes

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PREFACE

The Protocol *Effect of Metabolic Control at Onset of Diabetes on Progression of Type 1 Diabetes*, describes the background, design, and organization of the study. The protocol will be maintained by the Coordinating Center over the course of the study though new releases of the protocol, or issuance of updates either in the form of revisions of complete chapters or pages thereof, or in the form of supplemental protocol memoranda.

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CHAPTER 1
INTRODUCTION

1.1 Background and Rationale

Metabolic control at the onset of diabetes can have a major impact on preserving residual islet cell function. Two weeks of islet cell rest after clinical diagnosis of diabetes resulted in stimulated C-peptide levels 1 year post diagnosis of 0.51 nmol[1], greater than that seen after a year of cyclosporine treatment (peak c-peptide of 0.45 nmol[2]). As new technologies become available, such as the recently FDA approved real-time continuous glucose monitoring, it will be important to standardize diabetes management from the onset of diabetes in immune intervention trials. The purpose of this study is to test the impact of intensive metabolic control from the onset of diabetes on preservation of C-peptide secretion. These studies will also test the feasibility and acceptance of this therapy so that it could be considered in future immune intervention studies. The therapy consists of a short course of sub-cutaneous closed-loop diabetic control at the onset of diabetes followed by real-time continuous glucose monitoring (rtCGM) associated with continuous subcutaneous insulin infusion therapy (CSII).

Specific Aim: To determine if early restoration of metabolic control will improve C-peptide production compared to children receiving routine diabetes management.
Secondary Aim: To determine if allowing the islet cells to be less metabolically active will have an impact on the underlying autoimmune process.

1.2 Background

1.2.1 Human Studies

At the clinical diagnosis of diabetes most patients have residual pancreatic islet cells which can continue to secrete insulin for several additional years. In the DCCT[3], 35% of participants with diabetes duration of 1-5 years had persistent islet cell function (meal stimulated C-peptide levels of 0.2 to 0.5 pmol/ml). Assignment to the intensively managed group reduced the risk for loss of C-peptide by 57% over the mean 6.5 years of study. This was very clear proof that metabolic control had a significant effect on preservation of islet cell function. Unfortunately no immunologic studies were conducted as part of the DCCT to further understand if improved metabolic control had an effect on the immune response. Within the intensively treated group, those retaining some residual islet cell function had a 50% decrease in the risk of retinopathy progression and a 65% lower risk of severe hypoglycemia when compared to intensively treated participants without residual beta cell function.[3, 4]

In histologic descriptions of the human pancreas specimens obtained near the onset of diabetes, the inflammatory process involving the islet cells is patchy, with some islets showing significant infiltration of inflammatory cells, whereas others do not. [5] One explanation for this finding is that islets not showing inflammation are less metabolically active. To test the hypothesis that "functioning islet cells may activate the process that causes their destruction," Shah and Malone used an artificial pancreas, the Biostater, to rest islet cells for 2 weeks following clinical diagnosis of diabetes.[1] The artificial pancreas was programmed to maintain glucose levels between 65 to 80 mg/dl, and blood glucose levels peaked between 110 to 150 mg/dl in the hour following a meal, but returned to target levels by the second hour following a meal. In the year of follow-up, no attempt was made to decrease insulin doses to the minimal possible dose, and the average insulin dose (0.7 units/kg-k) was the same for those participants who had used the

Biostater (n = 12) and those who had received conventional therapy (n = 14). At the end of 1 year, the mixed meal peak stimulated c-peptide was 0.51 pmol/ml in the Biostater treated group, substantially higher than found in the conventionally treated group (0.27 pmol/ml). Again, no immunologic studies were done, so it is unknown if the islet cell rest at the onset of diabetes caused any change in the underlying autoimmune process.

In an earlier study, Mirouze et.al.[6] used an artificial pancreas for 1 to 10 days (average of 5 days) in 12 participants within 7 to 90 days of diagnosis of diabetes (average 30 days from diagnosis). Of those using the artificial pancreas, 75% had a remission (defined as good glucose control using oral agents only for at least 3 months) as compared to 11% in the 28 participants receiving traditional treatment.

Intensive diabetes management using multiple daily injections[7], continuous subcutaneous insulin infusions (CSII)[8, 9] or even intravenous insulin for 2 to 8 weeks[10, 11] has resulted in a transient and earlier increase in C-peptide levels, but by 1 year C-peptide levels were the same in the treatment as in the control groups. In these studies traditional blood glucose monitoring was done, so early post-prandial hyperglycemia was probably not detected and aggressively treated, as it was in the closed-loop studies.

Diazoxide inhibits insulin secretion by opening ATP-sensitive K^+ channels of the β-cell. Since previous studies have demonstrated a relationship between glucose stimulation of islet cell activity and the amount of islet cell autoantigen expression[12-16], and diazoxide inhibition of insulin secretion also reduces autoantigen expression[12], diazoxide was given for 3 months to 27 children (mean age 11) with newly diagnosed diabetes.[17] The diazoxide treatment resulted in higher meal stimulated C-peptide levels at 12 months (0.43 nmol/L) compared to control participants (0.32 nmol/L) but by 24 months both groups had equal C-peptide levels.

In contrast to these studies is the negative result of parenteral intervention in the Diabetes Prevention Trial (DPT). In this study islet cell antibody positive participants with a low first phase insulin response to an intravenous glucose tolerance test were randomized to receive 4 days of intravenous insulin infusion once a year which suppressed endogenous insulin production[18], and twice daily subcutaneous injections of ultralente (0.25 units/kg-day). This dose of insulin did not suppress endogenous insulin production.[19] It is possible that the 4 days of islet cell rest once a year was insufficient to delay the onset of diabetes, and that post prandial hyperglycemia had a significant impact on the rate of diabetes progression. As part of this study, oral glucose tolerance tests were obtained every 6 months. It was clear that glycemia begins to increase at least 2 years before diagnosis, and within 6 months of diagnosis there is a steeper rise in glucose levels.[20] The postprandial hyperglycemia may initiate an increased metabolic rate in islet cells, making them more susceptible to an autoimmune attack. Insulin therapy in the prediabetic state may therefore need to target postprandial hyperglycemia, which was not done in the DPT since only basal ultralente insulin was given for 361 days each year. Although beginning after the clinical diagnosis of diabetes, continuous real-time glucose monitoring offers an opportunity to closely regulate post-prandial hyperglycemia, which is not closely monitored with routine blood glucose testing. Limiting post-prandial hyperglycemia may protect islets from "glucotoxicity", allowing islets to be less metabolically active, and perhaps allow new islet formation.

In summary, substantial evidence exists that intensive diabetes management at the onset of diabetes does help preserve C-peptide secretion. A significant increase in C-peptide secretion appears to be achieved when islet cell activity is significantly decreased (islet cell rest) with closed loop systems which have been used for several weeks after the onset of diabetes[1], or even for 1 day within the first 7 to 90 days following diagnosis.[6] Intensive insulin therapy with MDI, CSII, or intravenous insulin has also been effective in transiently improving C-peptide secretion, but this effect generally diminishes in 1 year. There are no data in humans on how islet cell rest may affect cellular immunity.

1.2.2 Animal Studies

There are no large animal models of type 1 diabetes where a closed loop system has been tried. In rodents there are no published studies on a closed loop system in NOD mice or the BB rat, however improving glycemic control has delayed progression of diabetes in both models of diabetes, and in rodent islet cell transplant studies.

1.2.2.1 BB Rat

Providing insulin to the BB rat protects against diabetes and insulitis.[21-23] It would appear that at least part of the protection is due to the metabolic effect of insulin since protection from diabetes required doses of insulin that caused hypoglycemia[24], and diazoxide also protected against diabetes.[25] It is of interest that the effect of insulin was specifically protective to the islet cell since there was no effect on the development of thyroiditis in these animals.[26]

1.2.2.2 NOD Mouse

Insulin therapy in the NOD mouse appears to have both immunologic and metabolic effects.[27] In the NOD-scid/scid adoptive transfer model of IDDM both glucose lowering doses of insulin as well as non-metabolic doses of insulin, when given prophylactically, were able to equally delay the onset of diabetes. When endogenous insulin production was suppressed with somatostatin, there was again a marked delay in the onset of diabetes, indicating that suppressing endogenous insulin production was one mode of action of insulin therapy. When somatostatin therapy was delayed until after the onset of insulitis, it was still effective in delaying the onset of diabetes, although with less effect than when treated was initiated before onset of insulitis.

There are few therapies which will reverse diabetes in the NOD mouse once hyperglycemia has occurred. In one study, the use of CFA to induce TNF-α and exposure to MHC class I molecules was used to interrupt autoimmunity and restore euglycemia.[28] The success of this treatment was greatly enhanced by the restoration of euglycemia for 40-50 days at the time of the immune intervention by implantation of alginate-encapsulated islets.

1.2.2.3 Islet Transplantation in Non-autoimmune Animal Models

In streptozocin-induced diabetes, if transplanted islets were engrafted into a normoglycemic environment then the number of islets required to restore euglycemia was reduced by 50% (from 400 to 200 islets).[29]

1.2.2.4 Hyperglycemia Induction of β-cell Apoptosis in Animal Models of Type 2 Diabetes

The *Psammomys obesus* gerbil provides an animal model for type 2 diabetes. With the onset of hyperglycemia, these animals have a progressive decline in their pancreatic β-cells. To test the "glucotoxicity" hypothesis, islets from diabetes prone animals were exposed to increasing

glucose levels in vitro which resulted in a dose-dependent increase in DNA fragmentation in β-cells consistent with apoptosis.[30] In other animal models of type 2 diabetes, minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion and progression to diabetes.[31, 32]

1.3. In Vitro Studies

1.3.1 Effect of hyperglycemia on Expression of β-cell Antigens

When β-cells are stimulated by hyperglycemia they express increased levels of β-cell antigens. In using the rat pancreas as a substrate for islet cell antibody assays, it was found that rats fed a high-sucrose/high fat diet had significantly increased binding when exposed to ICA-positive serum.[14] Hyperglycemia increases expression of GAD-65 from islets isolated from Sprague-Dawley rats[33] and from *Macaca nemestrina*.[15] The expression of a β-cell antigen reacting with the monoclonal IC-2 antibody was significantly influenced by the functional state of the islet cell and expression decreased in islets isolated from both rats and mice after one week of insulin treatment. [34] In vitro expression of IC-2 was significantly increased when isolated islets were cultured with increasing glucose concentrations.[35] The expression of the 64-K β-cell antigen is also increased when islets from rats[12, 16] and humans[36] are cultured in high glucose concentrations.

1.3.2 Effect of Metabolic State of the Islet on Islet Survival Following Exposure to Cytokines

IL-1 and TNF individually and in combination cause rat islet cytotoxicity which progressively increases as the glucose concentration in the media increases from 60 to 100 to 200 mg/dl.[37] β-cell apoptosis has been confirmed using TUNEL-staining and marked apoptosis only occurred when high glucose and cytokines (IL-1, THF, IFN) or streptozotocin were simultaneously present in the culture media.[38, 39] Human islets are also more susceptible to IL-1 mediated cytotoxicity in hyperglycemic media, but the deleterious effects of glucose and IL-1β were blocked when insulin secretion was blocked by diazoxide.[40] Mouse islets are also more susceptible to damage from streptozocin if they are cultured in media containing 200 mg/dl of glucose instead of 100 mg/dl.[41] On the other hand, if cultured islets are put in a state of metabolic rest by administration of diazoxide, a K_{ATP} channel opener, they were much less susceptible to damage from strepozotocin.[42] Glucose itself may be toxic to islet cells and in vitro exposure of human islets to progressively higher glucose concentrations (100 to 200 to 600 mg/dl) induces Fas expression and β-cell apoptosis.[43] Human islets cultured on an extracellular matrix were reported to have increased IL-1 production as glucose concentrations were increased[44], however studies using free-floating human islets were unable to confirm islet cell production of IL-1.[45] It is intriguing that nonendocrine cells such as duct cells or fibroblasts may be stimulated to release increased levels of IL-1 locally when glucose concentrations are increased, providing another mechanism whereby hyperglycemia is toxic to islet cells.

In summary, human, animal and *in vitro* data provide strong evidence that hyperglycemia is toxic to islet cells and makes them more susceptible to cytokine mediated cytotoxicity. Hyperglycemia may also cause increased IL-1 secretion from non-endocrine pancreatic tissue, creating a vicious cycle of islet susceptibility to cytokines and increased local production of cytokines.

1.4 Preliminary Data

1.4.1 Current Blood Glucose Control from the Onset of Diabetes: Data from Continuous Glucose Monitoring (CGM) over the First 5 Days of Therapy

1.4.1.1 Case 1

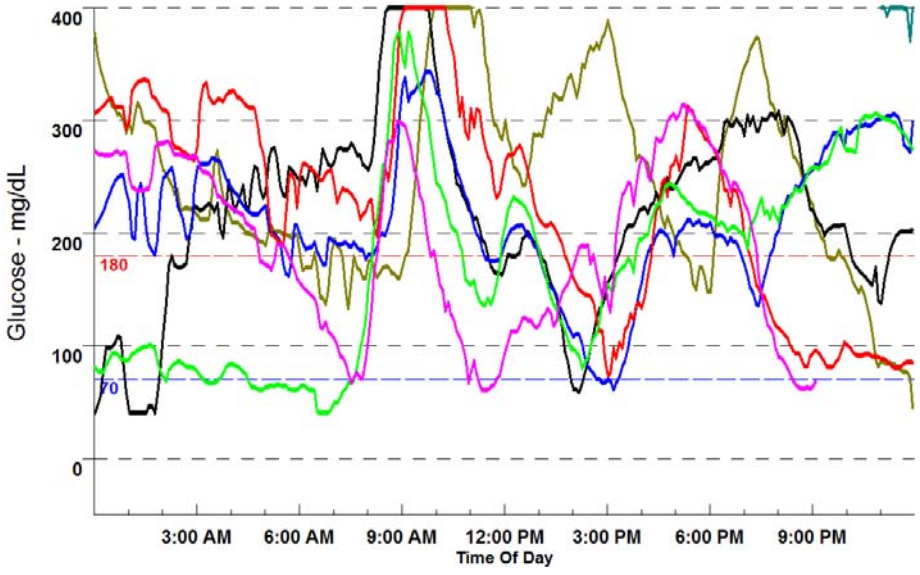
A 5 year old girl was admitted with a BG = 944, and CO_2 = 19 and treated from the onset with subcutaneous insulin therapy with Humalog before breakfast, lunch and dinner and glargine at dinnertime with NPH in the morning. After initial diabetes education was completed, she was discharged to home 2 days after diagnosis, and continued to wear the rtCGM sensor for a total of 6 days. Results of glucose values are given in the table below. The sensor showed excellent function with an overall r = 0.95 and a mean absolute relative difference of 8.3%. Overall 67% of her glucose values were above 170 mg/dl over the first 6 days of treatment, and only 28% were between 70-180 mg/dl. She had persistent nocturnal hyperglycemia until her 5th day of treatment when she developed nocturnal hypoglycemia, and she has consistent post-prandial hyperglycemia. When seen at a 2 month follow-up visit she was in remission with a total daily insulin dose of 0.34 units/kg-day. A sensor modal day graph is presented below.

Table 1: CGM data for Case 1

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Average
Location	Hospital	Hospital	Home	Home	Home	Home	
Average BG	248	220	213	229	172	189	214
Hours above 180 mg/dl	17:55 (75%)	17:10 (72%)	18:45 (78%)	16:45 (70%)	12:00 (50%)	11:20 (54%)	67%
Hours below 70 mg/dl	0:05 (0%)	1:40 (7%)	0:25 (2%)	0	3:40 (15%)	1:25 (7%)	5%
MARD%	18	10	1.3	5.4	5.4	5.7	8.3
R	0.44	N/A*	N/A	0.99	0.99	1.00	0.95

* If the range of glucose values is < 100 mg/dl, the r is not calculated

Figure 1: CGM modal day for Case 1



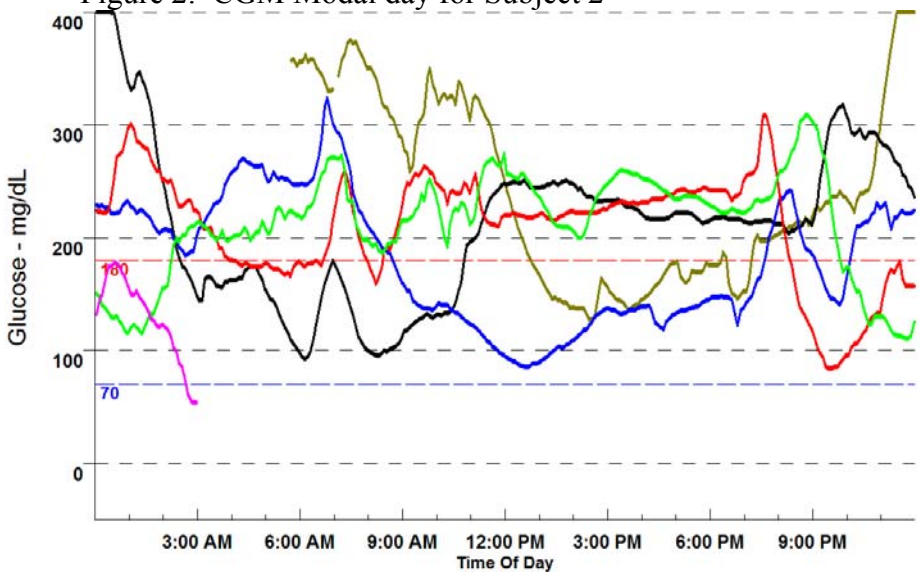
1.4.1.2 Case 2

A 16 year old female was admitted with a blood glucose of 459 mg/dl and a CO_2 = 29. She was initially treated with subcutaneous Humalog before meals and glargine at bedtime. Beginning 2 hours after diagnosis she was started on a continuous glucose sensor. Over 5 days her average blood glucose was 208 mg/dl with 65% of values > 180 mg/dl. There were no episodes of hypoglycemia. The sensor demonstrated excellent function with an r = 0.98 and a mean absolute relative difference (MARD) = 5.3%.

Table 2: CGM data for Case 2

	Day 1	Day 2	Day 3	Day 4	Day 5	Average
Location	Hospital	Hospital	Home	Home	Home	
Average BG	243	212	180	210	213	208
Hours above 180 mg/dl	12:05 (66%)	15:45 (66%)	12:05 (50%)	17:05 (71%)	19:35 (82%)	65%
Hours below 70 mg/dl	0	0	0	0	0	0%
MARD%	2.6	6.8	5.6	4.9	6.8	5.3
R	0.99	0.99	0.98	0.96	0.96	0.98

Figure 2: CGM Modal day for Subject 2



From these two cases it is clear that there is substantial hyperglycemia occurring with routine diabetes management at the onset of diabetes. A closed-loop system would significantly decrease the number of hours each day that islets are exposed to hyperglycemia, thereby decreasing "glucotoxicity" and allowing earlier restoration of islet cell function, and perhaps altering islet antigen presentation to the immune system.

1.4.1.3 Use of Proportional Integral Derivative (PID) Algorithm Automated Closed-Loop Insulin Delivery to Achieve Glucose Control with SC Insulin Delivery

The Yale Pediatric group has recently published data using SC-glucose sensing and SC insulin delivery (Figure 3). [46] While we do not anticipate ambulatory closed-loop insulin delivery being

performed outside the CRC, where patients are well monitored, we anticipate that the closed-loop control can be utilized in the CRC as an aid in determining initial pump settings. These settings include basal profiles, meal carbohydrate to insulin ratio (CIR) and an insulin sensitivity factor (ISF) for using corrective boluses.

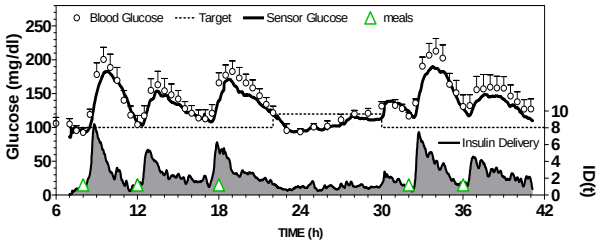


Figure 3 Ambulatory closed-loop profile in pediatrics undergoing continuous automated closed-loop insulin delivery (Yale pediatric study).

1.5 Summary of Design of Randomized Trial

A. Major Eligibility Criteria

- Age 10 to <46 years
- Be within 7 days of initiation of insulin therapy for newly diagnosed type 1 diabetes

B. Sample Size

The study will include approximately 72 participants in order to enroll approximately 66 participants who are autoantibody positive (based on prior TrialNet studies, it is expected that there will be approximately 6 subjects who are antibody negative). Due to the short time window between diagnosis of type 1 diabetes and randomization, the autoantibody test results will not be available until after randomization. Antibody-negative participants will not count towards the recruitment goal of 66 but will be continued in the study.

C. Treatment Groups

Participants will be randomly assigned to the following 2 groups:

- Intensive Treatment Group (2/3 of participants will be assigned to this group)
- Standard Treatment Group (1/3 of participants will be assigned to this group)

D. Duration of Follow-up

- Primary outcome at 1 year
- Follow-up for all participants for 2 years
- Follow-up for participants who still have beta cell function after 2 years may be continued for up to 2 additional years (4 years total)

E. Main Outcome Measures

The primary outcome is C-peptide area under the curve in response to a mixed meal at 1 year following enrollment.

The study will also examine the effect of metabolic control on immunologic assays relevant to type 1 diabetes.

Flow Chart of Study

Screening:

- Assess eligibility and sign informed consent form
- Insert blinded CGM sensor to obtain baseline CGM data

Randomization:

- Randomize participant to intensive treatment (2/3) or standard treatment (1/3) groups

Intensive Treatment Group:

- Admission to CRC for up to 4 days of closed loop therapy followed by up to 1-2 days of training on use of the insulin pump and rtCGM (if not completed during the first 4 days)

Year 1 (All participants)

- Visits at 2,6,13,26,39,52 weeks

Year 2 (All participants)

- Visits at 15 months, 18 months, 21 months and 24 months

Year 3 and 4 (Participants with beta cell function at 30 months after study start may be continued in follow-up)

- Visits every 6 months as long as participant is in the study

1.6 General Considerations

The study is being conducted in compliance with the policies described in the study policies document, with the ethical principles that have their origin in the Declaration of Helsinki, with the protocol described herein, and with the standards of Good Clinical Practice.

Data will be collected in electronic case report forms, which will be considered the source data when data have been directly entered (i.e., not transcribed from existing records).

There are expected to be 4 centers in the study initially. Additional centers may be added at a later time if needed to reach the study's recruitment goal. There is no restriction on the number of participants to be enrolled by a site.

1.7 Schedule of Study Visits and Examination and Laboratory Procedures[1]

Visit Number:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Study Time:	**0**	**2w**	**6w**	**3m**	**6m**	**9m**	**12m**	**15m**	**18m**	**21m**	**24m**	**30m**	**36m**	**42m**	**48m**
Hematocrit	X														
History	X														
Physical exam[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blinded rtCGM[3]	X			X	X	X	X	X	X	X	X	X	X	X	X
CGM downloads[4]		X	X	X	X	X	X	X	X	X	X				
CSII downloads		X	X	X	X	X	X	X	X	X	X				
Local HbA1c	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Central Lab HbA1c	X			X	X	X	X		X		X	X	X	X	X
Mixed Meal Tolerance Test[5]	X	X	X	X	X	X	X		X		X	X	X	X	X
Urine pregnancy test (for females with reproductive potential)	X						X								
Blood samples for autoantibodies and mechanistic outcomes[6]	X	X	X	X	X	X	X		X		X	X	X	X	X
DNA	X														

[1] Follow-up may be continued for up to 4 years for participants with persistence of beta cell function after 30 months.

[2] Full exam at baseline, 12, and 24 months; other visits limited/directed exam only

[3] After signing consent, all participants will use a blinded rtCGM inserted by clinic personnel. Participants in the standard treatment group will again use a blinded rtCGM inserted by clinic personnel during follow-up; after the 12m visit, if a participant is using a rtCGM, data will be collected from that device and a blinded rtCGM will not be used

[4] Data from rtCGM will be reviewed at week 1, 2, 4, 6, 8, and then monthly to allow adjustments to be made in the basal profile, carbohydrate to insulin ratio and insulin sensitivity factor

[5] Initial MMTT consists of baseline and 90 minute samples. Other MMTT are two hour tests. Participants with an undetectable level of C-peptide at the 30-month visit will not undergo any further MMTTs for assessment of Cpeptide levels at subsequent visits.

[6] Autoantibodies will be processed at baseline; autoantibodies, PBMC, RNA and additional plasma and serum samples collected during follow-up will be stored for possible future analysis.

CHAPTER 2
ENROLLMENT AND STUDY INITIATION

2.1 Study Population

Participants diagnosed with T1DM will present to the research team in one of three ways; (1) they were admitted to the hospital for diabetic ketoacidosis (DKA), (2) they were non-acidotic and therefore admitted to a regular hospital floor, (3) they were non-acidotic and diabetes treatment was initiated as an outpatient.

Approximately 72 participants are expected to be enrolled in the study in order to enroll approximately 66 participants who are autoantibody positive (based on prior TrialNet studies, it is expected that there will be approximately 6 subjects who are antibody negative). Due to the short time window between diagnosis of type 1 diabetes and randomization, the autoantibody test results will not be available until after randomization. Antibody-negative participants will not count towards the recruitment goal of 66 but will be continued in the study. As the enrollment goal approaches, sites will be notified of the end date for recruitment. Participants who have signed an informed consent form can be randomized up until the end date, which means the recruitment goal might be exceeded.

2.2 Eligibility and Exclusion Criteria

2.2.1 Eligibility

Potential participants must meet all of the following inclusion criteria:

1. Age 10.0 to <46.0 years.
2. Diagnosis of type 1 diabetes with initiation of insulin therapy within past 7 days (day 1 being the first day of insulin therapy)
3. If participant is female with reproductive potential, willing to avoid pregnancy and pregnancy test negative.
4. Willing to accept randomization to either the intensive diabetes management group or the standard care group.
5. Willing to complete the planned 2 years of follow-up.
6. Able to electronically transmit data monthly.
7. Investigator believes that the participant (and parent/guardian for children) understands and agrees to comply with the study protocol and is capable of undertaking all necessary testing.

2.2.2 Exclusion Criteria

Potential participants must **not** meet any of the following exclusion criteria:

1. Currently pregnant or lactating, or anticipate getting pregnant in the next one year.
2. Currently anemic (hematocrit level will be obtained at the screening visit).
3. Chronic use of systemic steroids or other noninsulin pharmaceuticals that might affect glycemic control or the presence of a disease that is likely to be treated with such medications during the first two years of the study.
4. Complicating medical issues that might interfere with study conduct.

5. Inpatient psychiatric treatment in the past 6 months (if the participant is a minor, for either the participant or the participant's primary care giver).
6. Currently participating in another type 1 diabetes treatment study, including an intervention trial for treatment of diabetic ketoacidosis.

2.3 Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written communication. Written material will include a Volunteer Handbook and written consent forms. The consent form will be reviewed with participants (and their guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants under 18 years of age (unless local IRB requirements differ in procedure). As part of the informed consent process, the participant and/or parent or guardian (if the participant is less than 18 years of age) will also be required to complete a short, written Volunteer Understanding Assessment that is designed to ensure that the participant understands the study, as well as what is being asked of him/her. The participant will be given a copy of his/her signed consent/assent forms.

2.4 Age Distribution

In order to maintain similar proportions in this study to other TrialNet studies, enrollment of those age 16 or above may be closed when about 40 such participants have been enrolled, or 55% of the planned sample size for this trial. Then the remaining participants would be limited to those under age 16 years.

2.5 Screening Assessments

1) History, including recording of medications
2) Physical exam, including neurocognitive evaluation
3) Urine pregnancy test (for females with reproductive potential)
4) Blood sample for evaluation of hematocrit level

2.6 Baseline Assessments

1) Blood samples for local and central laboratory HbA1c assessment, autoantibodies and additional volume for storage for possible future analysis of mechanistic outcomes (PBMC, RNA, DNA and others)
2) Abbreviated MMTT
    - Consists of blood samples before and 90 minutes after the standard liquid mixed meal is consumed.
    - For those presenting in DKA, ketoacidosis must be resolved (defined as CO2>15 or pH >7.3), and the participant ready to begin eating before baseline studies. In this case, the abbreviated MMTT should be their first meal.
3) Use of blinded rtCGM
    - A sensor will be inserted by clinic personnel and participants will be asked to wear the rtCGM device blinded to the data

2.7 Randomization

Study participants will be consented and randomized at the clinical sites as soon as possible after diagnosis of diabetes. The goal is to have randomization occur within 48 hours of diagnosis of

diabetes; however, enrollment up to seven days after initiation of insulin therapy will be acceptable. Participants admitted for treatment of DKA with IV insulin and fluids will be asked to consent to the study and will be randomized before their first meal. Participants who were diagnosed as an outpatient and did not necessarily require hospital admission will come to the CRC for a morning admission for a mixed meal tolerance test, and will be randomized at the time of that admission.

Participants will be randomly assigned to one of two treatment groups

- Two-thirds assigned to experimental treatment consisting of initiation of insulin delivery via a subcutaneous closed-loop system in a monitored setting, and then rtCGM and a CSII in an outpatient setting.
- One-third assigned to standard diabetes management.

The randomization method will be stratified by clinical center and by whether or not the participant presented in diabetic ketoacidosis.

Participants assigned to the intensive treatment group will be transferred to the clinical research center for initiation of the closed loop therapy. Participants assigned to standard diabetes management will have an abbreviated mixed meal test on the floor if admitted to the hospital for DKA or in the CRC if randomized as an outpatient.

2.8 Masking

Investigators and participants will not be masked to treatment assignment, but will be masked to primary outcome data. Laboratories performing assays for this protocol will be masked as to the treatment assignment and the identity of each participant whose biological material is to be studied.

CHAPTER 3
TREATMENT GROUPS

3.1 Standard Care Treatment Group

Participants randomized to the standard care treatment group will receive standard of care management of their diabetes. Their care will be provided by a physician not involved in the management of participants in the intensive treatment group.

Participants will continue to wear the blinded rtCGM inserted at the time consent was obtained with the goal of collecting 72 hours of data.

The study will provide the standard care treatment group with a One Touch Ultra2 home glucose meter, control testing solution, and test strips, and the participant will be asked to use this meter and bring it to each study visit.

3.1.1 Use of Blinded rtCGM by Standard Treatment Group

At the 3, 6, 9, 12, 15, 18, 21, and 24-month visits and every 6 months up to 4 years if the participant's c-peptide is positive, the Standard Treatment Group will use a blinded rtCGM with the goal to obtain 72 hours of data. Clinic personnel will insert a sensor during the visit and instruct the participant on use of the device, including calibration.

Participants who obtain less than 48 hours or less than 10 hours during overnight hours (10 p.m. to 6 a.m.) will be asked to return to the clinical center to have another sensor inserted in order to repeat the blinded sensor wear. If the second attempt also is unsuccessful in obtaining the requisite amount of data, an additional attempt does not need to be made. The results of the "blinded" rtCGM will be transmitted to the Coordinating Center and provided to the participant's treating diabetes health care provider for use in their clinical care.

3.2 Intensive Treatment Group

3.2.1 Sub-cutaneous Closed-Loop System in Monitored Setting

The initiation of sub-cutaneous closed-loop therapy will begin in a 24-hour monitored inpatient setting such as a clinical research center as soon as possible after completion of the abbreviated MMTT. The blinded rtCGM sensor inserted at the time consent was obtained will be switched over to an unblinded sensor and a second unblinded sensor will also be inserted.

Participants will be treated with up to 96 hours (a minimum of 72 hours) of sub-cutaneous closed-loop insulin delivery based on the SC-glucose sensing and SC-insulin delivery in a monitored setting. Supplemental pre-meal insulin is allowed to achieve the target glucose. The closed-loop results will be used to estimate initial CSII settings including: 1) an initial basal profile, 2) a carbohydrate to insulin ratio, and 3) an insulin sensitivity factor. Once these are established the participant may be discharged or observed in a monitored setting for up to 1-2 days of rtCGM and CSII prior to being discharged.

3.2.2 Real-time Continuous Glucose Monitoring (rtCGM) and Continuous Sub-cutaneous Insulin Infusion (CSII) ("pump") Therapy as Outpatient

The device will be started prior to discharge. Education on the use of the system will be provided by study staff. The target glucose will be:

a) 70-140 before meals, < 180 post prandial, hs 80-150

Correction doses will be targeted to glucose values of

a) day =100; night = 120

The above values are targets; adjustments may be made according to clinical judgment. Guidance for CSII settings will be provided in the manual of operations.

Participants will be instructed in how to download their pump, home glucose meter and rtCGM data. They will be expected to download their data at least every 2 weeks. Data from the rtCGM will be periodically reviewed by clinical staff at 1, 2, 4, 6, and 8 weeks, and then monthly. Feedback will be provided to the participant (parent) via phone or email. This will allow adjustments to be made in the basal profile, carbohydrate to insulin ration, and insulin sensitivity factor. Guidelines for therapy will be used that were recently published by the DirecNet study group. [47]

The goal will be to use the pump-CGM on a daily basis for two years.

CHAPTER 4
INPATIENT CLOSED LOOP THERAPY

4.1 Overview

Following completion of the baseline procedures, participants randomized to the intensive management group will have an inpatient CRC admission of approximately 4-6 days. For up to 4 days, participants will have closed loop therapy; for up to 1-2 additional days (if not completed during the first 4 days), participants will be taught how to manage their diabetes at home using the insulin pump and rtCGM and home insulin needs off of the closed-loop system can be assessed. A member of the research team (physician or nurse) will be present while the closed loops system is being used.

- An intravenous catheter will be inserted.
- A second rtCGM sensor will be inserted and will send interstitial glucose readings to a laptop computer which will also be running the algorithm to determine insulin infusion rates.
- An infusion set will be started at the time of admission and the insulin reservoir will be filled with insulin.
- After some meals, blood glucose measurements may be made every 10 minutes for one hour when indicated to allow for algorithm tuning.
- After the closed loop is initiated, blood glucose measurements will be obtained every 30 minutes (reference measurement using a YSI, GlucoScout, HemoCue, Beckman clinical laboratory analyzer, or an iStat that uses cuvettes and not test strips). Laboratory glucose measurements also may be used. If this value is <70 or >180 then study personnel will be notified.
- When the GlucoScout is used, a reading will be obtained every 2 hours using another reference method (YSI, HemoCue, Beckman clinical laboratory analyzer, iStat (using cuvettes, not test strips), or laboratory) to confirm consistency between the two devices.
- Participants can choose their meals and snacks during the admission.
- Following completion of at least 72 hours of closed loop therapy, participants may remain in the CRC for up to 1-2 days and will be taught to use the insulin pump and rtCGM at home to manage their diabetes.

4.2 rtCGM Management and Procedures

4.2.1 Sensor Placement

The rtCGM sensor inserted at the time of consent will remain in place but will no longer be blinded. A second sensor will be placed following completion of the baseline procedures. Calibrations will be performed as needed using the One Touch Ultra2 meter.

4.3 Discrete Blood Glucose Measurements

An intravenous catheter will be inserted in an arm vein. The area where the catheter will be inserted may be numbed with Elamax or EMLA cream prior to catheter insertion.

The discrete blood glucose measurements will be made using a YSI, GlucoScout, HemoCue, Beckman clinical laboratory analyzer, iStat (using cuvettes not test strips) or laboratory testing that is rapidly available. Measurements will be obtained every 30 minutes around the clock and every 15 minutes if the glucose is less than 70 mg/dl. An additional goal will be to obtain

glucose values every 10 minutes for one hour following some meals. This is a secondary goal and will be decided by the investigator based on the need to modify the algorithm, the participant's blood volume and catheter function. If the catheter stops functioning after 72 hours of closed loop therapy has been completed, it may be replaced at the discretion of the investigator. If it is not replaced, the closed loop therapy will be discontinued.

4.3.1 Volume of Blood Draws

Each blood glucose determination may require a blood volume of approximately 0.3 ml depending on the method used for glucose determination. If the GlucoScout, is used there is no loss of blood volume for blood glucose determination. The maximum number of blood draws based on the participant's weight will be calculated at the time of admission so that the maximum blood volume drawn will not exceed 5% of the participant's blood volume.

4.4 Diabetes Management

Standard hypoglycemia treatment will be given for glucose values $\leq$70 mg/dl (approximately 15 grams of carbohydrate, with a recheck of the blood glucose 10-15 minutes later).

For two consecutive glucose values >300 mg/dl, a serum ketone level will be determined.

4.5 Algorithms for Diabetes Management

The algorithm used by the closed loop system to calculate insulin delivery is designed to emulate the plasma insulin response obtained in normal glucose tolerant (NGT) individuals during normal day-to-day glucose excursions. *In vivo,* the β-cells responds to changes in glucose with a characteristic "first" and "second" phase insulin release. For a NGT individual the β-cell is known to adapt itself such that the magnitude of these responses is proportional to the individual's insulin sensitivity. That is, the product of insulin release *times* insulin sensitivity remains constant (this constant has been called by the disposition index and is expressed as $DI=S_I \times \phi_1$ where S_I is insulin sensitivity and ϕ_1 is the first phase release). In the present application, algorithm tuning is desired to be consistent with this index. The algorithm in the closed loop system used for calculating insulin delivery emulates the biphasic insulin response using the elements of Proportional *plus* Integral *plus* Derivative control. Tuning of the algorithm is achieved by adjusting the relative proportion of each component to compensate for the known delay in subcutaneous (SC) insulin absorption kinetics. The overall gain is then adjusted to the individual's insulin clearance/sensitivity.

Prior to discharge, participants will be provided with algorithms for making diabetes management decisions at home based on the rtCGM and HGM readings.

4.6 Daily Activities

Participants will be permitted to perform their usual indoor activities during the hospitalization.

4.7 Diet

The diet during the admission will be at the discretion of the participant and the treating medical team.

4.8 Hospital Discharge

Participants may remain in the CRC for up to 1-2 additional days following completion of the

closed loop therapy to learn how to use the insulin pump and rtCGM at home to manage their diabetes. At the time of discharge, participants will be given infusion sets, reservoirs and rtCGM sensors to last until their next visit.

CHAPTER 5
FOLLOW UP VISITS AND PROCEDURES

5.1 Visit Schedule

Study visits for both groups will occur at baseline, 2 weeks, 6 weeks, 13 weeks (3 months), 26 weeks (6 months), 39 weeks (9 months), 52 weeks (12 months), 65 weeks (15 months), 78 weeks (18 months), 91 weeks (21 months) and 104 weeks (24 months) post randomization.

The 2-week visit has a window of ±4 days. Follow-up visits during the first 6 months should be within ±1 week of the scheduled visit, between 6 months and 2 years ±2 weeks, and ±4 weeks thereafter.

All participants will be followed for a 2-year period. Participants may subsequently be asked to undergo additional follow up for an additional two years with a visit every 6 months until the study end. Participants with an undetectable level of Cpeptide at the 30-month visit will not undergo any further MMTTs for assessment of Cpeptide levels at subsequent visits

5.2 Visit Procedures and Testing

The following will be performed at every visit, unless otherwise stated:

1) History, including recording of medications and adverse events
2) Physical exam (full exam at annual visits and limited/directed exam at other visits)
3) Urine pregnancy test (for females with reproductive potential) at the 12-month visit (at each visit, female subjects with reproductive potential will be questioned about their last menstrual period and pregnancy testing will be performed if a period has been missed)
4) Blood sample for local HbA1c assessment at all visits except 2 weeks
5) Blood sample for central laboratory HbA1c assessment at all visits beginning with the 13-week visit except the 65 and 91-week visits
6) Blood samples for autoantibodies, PBMC, RNA and extra plasma and serum to be stored for possible future analyses
7) Mixed Meal Tolerance Test (see above regarding testing post 30-month visit)

The collection of blood samples will vary if needed to assure that no more than 3 cc/kg is drawn from a child at a single time or 7 cc/kg within any 6-week period.

For the intensive treatment group, the rtCGM, pump, and home glucose meter will be downloaded at each visit. For the standard care treatment group, the home glucose meter will be downloaded at each visit.

As noted in section 3.1.1, a sensor for a blinded CGM will be inserted at each visit through the 2-year visit, beginning with the 13-week visit and may be inserted every 6 months beyond 2 years if the participant is c-peptide positive up until 4 years after their enrollment. If beyond year 2, the participant is wearing a rtCGM, data will be collected from this device and the participant will not be asked to wear a blinded rtCGM.

CHAPTER 6
ADVERSE EVENT REPORTING AND SAFETY MONITORING

6.1 Adverse Event Reporting and Monitoring

6.1.1 Definition

Reportable adverse events in this study include any untoward medical occurrence that meets criteria for a serious adverse event or any medical occurrence (expected or unexpected) in a study participant that is study or device-related.

Skin irritation from sensor wear will be recorded in specific sections of the case report forms. An adverse event form is only completed if skin irritation is severe.

Hypoglycemic events are recorded as Adverse Events if the event required assistance of another person due to altered consciousness 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 his or herself, was unable to verbalize his or 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 if the event involved DKA, as defined by the DCCT, and had all of the following:

- Symptoms such as polyuria, polydipsia, nausea, or vomiting;
- Serum ketones 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

6.1.2 Recording of Adverse Events

Throughout the course of the study, all efforts will be made to remain alert to possible adverse events or untoward findings. The first concern will be the safety of the participant, and appropriate medical intervention will be made.

The investigator will elicit reports of adverse events from the participant at each visit and phone call and complete all adverse event forms online. Each adverse event form is reviewed by the Coordinating Center to verify the coding and the reporting that is required.

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 device or study procedures.

The intensity of adverse events will be rated on a three-point scale: (1) mild, (2) moderate, or (3) severe. It is emphasized that the term severe is a measure of intensity: thus a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

Adverse events that continue after the participant's discontinuation or completion of the study will be followed until their medical outcome is determined or until no further change in the condition is expected.

6.2 Reporting Serious or Unexpected Device-related Adverse Events

A serious adverse event is any untoward occurrence that:

- Results in death
- Is life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in significant disability/incapacity
- Is a congenital anomaly/birth defect

An *Unanticipated Adverse Device Event* is defined as an adverse event caused by, or associated with, a device, if that effect or problem was not previously identified in nature, severity, or degree of incidence.

Serious or unexpected adverse events must be reported to the Coordinating Center immediately via completion of the online serious adverse event form.

The Coordinating Center will notify all participating investigators of any adverse device event that is both serious and unexpected. Notification will be made within 10 days after the Coordinating Center becomes aware of the event. Such events will be reported to the FDA according to regulatory requirements.

Each principal investigator is responsible for informing his/her IRB of serious study-related adverse events and abiding by any other reporting requirements specific to their IRB.

6.3 Reporting of Adverse Events

The FDA and an independent Data and Safety Monitoring Board will be informed of all serious adverse events and any unanticipated adverse device events that occur during the study and will review compiled adverse event data at periodic intervals.

CHAPTER 7
MISCELLANEOUS CONSIDERATIONS

7.1 Risks, Benefits, and Inclusion of Children

The risks of this study are presented below and in the informed consent form and volunteer handbook. This study will examine whether aggressive metabolic control from the clinical onset of diabetes will preserve beta cell function, but there is no guarantee that this will occur.

There is the prospect of direct benefit to the individual participants for their participation in the study. These potential benefits include the recognized benefits of being in a clinical study, including close monitoring and additional resources available to maintain tight glycemic control. Further, the intervention has the prospect of direct benefit to a given participant and is likely to yield general knowledge about type 1 diabetes which is of importance for the understanding and amelioration of type 1 diabetes in children.

The inpatient tight control phase is closely monitored for safety, and while greater than minimal risk, presents the prospect of direct benefit to the individual participants. The other study procedures are minimal risk.

Assent of the children along with consent of the parents will be obtained prior to any study procedures. This research proposal in children is consistent with United States Department of Health and Human Services, Protection of Human Subjects, Subpart D, Section 46.405 (Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual participants) and with Subpart D 50.52 (Clinical Investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual participants).

7.2 Potential Risks and Side Effects

7.2.1 Failure of Closed Loop System

There could be a failure of communication between the components of the closed loop system consisting of the rtCGM, the computer, and the CSII or the function of each individual component. Additionally, the algorithms employed to keep glucose in normal range may not work well for all participants in the age groups to be studied. These failures could result in either hypoglycemia or hyperglycemia.

7.2.2 Hypoglycemia

Hypoglycemia is a recognized consequence of intensive diabetes management.

As outpatients, participants in the experimental treatment arm of the study may have a higher incidence of hypoglycemia, since the goal is to avoid hyperglycemia. They will be wearing a rtCGM which may allow earlier detection of hypoglycemia and treatment to prevent hypoglycemia before it occurs (based on the rate of change of glucose and predicted glucose levels). They will also have real-time alarms to warn of hypo or hyperglycemic events when the rtCGM system is on and functioning.

7.2.3 Ketosis

Participants in the experimental treatment arm of the study may have a higher incidence of ketosis associated with CSII interruption. However, they will also be asked to wear a rtCGM

with alarms which when functional should aid in the recognition of hyperglycemia before ketosis occurs.

7.2.4 Skin Reactions to Adhesives

Some participants will develop skin irritation or allergic reactions to the adhesives used to secure the rtCGM, or to secure the insulin infusion sets for the CSII. If these reactions occur, different adhesives or "under-taping" (such as with IV 3000, Tegaderm, etc.) will be tried, sites will be rotated frequently, and a mild topical steroid cream or other medication may be required.

7.2.5 Infections at rtCGM or CSII Insertion Sites

Whenever the skin is broken there is the possibility of an infection. The rtCGM and CSII infusion sites are inserted under the skin. It is possible that any part of what is inserted under the skin may cause an infection. These occur very infrequently, but if an infection was to occur, oral and/or topical antibiotics can be used. The risk of skin problems could be greater if you use a sensor for longer than it is supposed to be used. Therefore participants will be carefully instructed about proper use of the sensor.

7.2.6 Burden of rtCGM and CSII

Participants in the intensive treatment group may find the daily use of these devices burdensome or overwhelming and may contribute to feelings of being "burned-out".

7.2.7 Loss of Privacy

Data downloaded from the CSII, rtCGM and the home glucose meter will be collected for the study as measures of diabetes self management behaviors. Some people may be uncomfortable with the researchers' having such detailed information about their daily diabetes habits. The downloads will be performed on the Medtronic website, and therefore, Medtronic may have access to study data.

7.2.8 Storage of Samples

During the course of the study, samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at clinical centers for future analysis. These samples will be collected only with the participant's permission. Participants who decline consent for these sample collections will still be eligible to participate in this study.

7.3 Protecting Against or Minimizing Potential Treatment Risks

To protect against hyper or hypoglycemia due to failure of the individual components or their communication, during the use of the closed loop a clinical research nurse and a physician who is either an attending specializing in diabetes or an endocrine fellow or a nurse practitioner who is a CDE trained in diabetes will be available at all times to assist in participant management. The functioning of the closed loop system will be assessed every 30 minutes. Sensor function will be assessed with discrete blood glucose measurements at least every hour, and more frequently if there are sensor alarms, or rapidly occurring changes in blood glucose levels.

An individual participant on the closed loop will stop using the system if the participant has > 3 episodes of hypoglycemia defined as a blood glucose ≤ 50 mg/dL in a 24 hour period or > 4 episodes of hypoglycemia (≤ 50 mg/dL) at anytime during the use of the system. An individual will also discontinue use of the closed loop if they experience DKA or meet the criteria for

severe hypoglycemia defined by seizure, loss of consciousness, or requiring assistance of another due to altered state of consciousness. If DKA develops, the participant will be transferred from the CRC to a hospital unit that routinely manages patients with DKA.

Participants will not be enrolled who have other active serious medical problems. Frequent monitoring of participants with history, physical examination, and laboratory studies will allow for early identification of adverse events. Every attempt will be made to minimize the number of venipunctures.

7.4 Participant Reimbursement and Compensation

The study will provide the intervention group with an insulin pump, rtCGM, sensors and related supplies, and a One Touch Ultra2 home glucose meter, control solution and test strips for the first two years of the study. Medtronic MiniMed, the company that makes the CGM will be loaning a pump to participants for use in the study. When a subject's participation in the study ends, the pump will have to be returned. Participants who complete the study will be able to keep the transmitter for the CGM. The study will provide the standard care group with a One Touch Ultra2 home glucose meter, control solution and test strips. The study will be paying for the costs of the research procedures that are part of the study. Costs of standard medical care for diabetes, including insulin that would occur even if the participant were not in this study will be the participant's responsibility.

The study will pay the participant $50 per completed protocol-required visit for their time and to cover travel and other visit-related expenses. Additional assistance may be available to cover excessive travel expenses. There will be no compensation for completing telephone calls or downloading the study devices at home.

7.5 Quality Assurance

During the study, duplicate collections of blood samples for assays may be obtained for the purpose of external quality surveillance of the performance of the central laboratories.

7.6 Withdrawal from Treatment

The study will be conducted according to the modified intent-to-treat principle ('modified' due to exclusion from primary analysis of antibody-negative cases, since results of antibody testing will not be known until after randomization). This means that once randomized into the study, a participant will be expected to undergo all scheduled follow-up assessments and will remain in the assigned treatment group for purposes of statistical analysis regardless of the actual course of treatment administered. Withdrawal from treatment does not automatically entail withdrawal from the study. Withdrawal from the study will only occur if the participant dies or withdraws consent. Participants who withdraw consent are classified as inactive but may again become active upon re-entry into the study, if they so choose.

Withdrawal from treatment can occur for a number of reasons, some of which are outlined below. A participant may elect to discontinue study CSII and rtCGM, may be unable to continue using them, or may be withdrawn (temporarily or permanently) at the discretion of the Principal Investigator if s/he determines that it is unsafe to continue or there is a significant change in the risk/benefit.

7.7 Re-Entry into Study Treatment

In some circumstances, a participant may temporarily discontinue the study CSII and/or rtCGM and/or not return to the study clinic for follow-up visits. If the participant decides to return for study treatment and/or follow-up assessments at a later date, he or she will be allowed and encouraged to do so.

CHAPTER 8
STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address the primary and secondary objectives of the trial, other stated objectives, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Such analyses may also entail the use of data from other studies in combination with data from this study. Likewise, data from this study may be used in combination with data from another study to address objectives of that study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

The approach to sample size and statistical analyses are summarized below. A detailed statistical analysis plan will be written and finalized prior to the completion of the study.

8.1 Primary Outcome and Analyses

The primary analysis will include all participants with autoantibodies. The primary outcome of each participant is the area under the stimulated C-peptide curve (AUC) of a 2-hour mixed meal glucose tolerance test conducted at the 12 month visit. The AUC is computed using the trapezoidal rule that is a weighted sum of the C-peptide values over the 120 minutes. By the mean value theorem of integral calculus, the weighted mean C-peptide in pmol/mL is simply AUC/120.

The primary statistical hypothesis to be assessed in the primary stratum of the study is whether:

- The mean C-peptide value for study participants in the experimental treatment arm differs significantly from the mean value for participants in the standard treatment arm.

The primary analyses will employ the weighted mean derived from the 2 hour AUC for each participant transformed as *log (mean C-peptide+1)*. The comparison between the two treatment arms will be based on a t-test of treatment effect in an ANCOVA model adjusting for gender, presence or absence of DKA, age and baseline *log(C-peptide+1)* [48] from an abbreviated MMTT as described in section 5.2. The adequacy of the model will be evaluated using the Shapiro-Wilk[49] test for normality of the residuals and the White[50] test for homoscedasticity.

Rubin's method for multiple imputation will be used for any participants lost to follow-up prior to the primary outcome at 12 months. Sensitivity analyses will be conducted to assess whether results are similar when using alternate methods for missing data. This will include last observation carried forward, available cases only, counting all missing cases as failures (i.e., imputing a zero) and counting cases with serious device-related adverse events as failures.

8.2 Secondary Outcome and Analyses

Additional analyses will include:

- A log rank test of the difference in the hazard function between groups in the incidence of the loss of the 2 hour peak C-peptide < 0.2 pmol/ml on a semi-annual MMTT, [51]
- Longitudinal analyses [52] using mixed effects models with a random intercept and slope of the C-peptide values over the post-treatment period, adjusted for baseline level of C-

peptide. The average intercept and slope will be compared between groups adjusting for age, gender, and the *log(C-peptide+1)*.

Analyses will also be conducted to adjust for the baseline C-peptide and HbA1c levels, and by age, clinical presentation, BMI, gender and race/ethnicity, as appropriate. A center-effect will be explored in the analyses by evaluating for interaction between center and treatment group on outcome.

The secondary objectives are to examine how intensive diabetes management affects the following:

- Mean area under the stimulated C-peptide curve (AUC) curve at 2 years.
- HbA1c levels over time.
- Insulin dose (units/kg) over time.
- Number and severity of adverse events (including hospitalization for DKA).
- Hypoglycemia:
  - Number of major hypoglycemic events (defined as loss of consciousness, seizure, or requiring assistance from another person because of altered state of consciousness).
  - Area under the curve and number of events less than 70 mg/dl on the rtCGM record prior to each study visit.
- Hyperglycemia events as measured as the area under the curve and number of events greater than 180 mg/dl on the rtCGM record prior to each study visit.

Various measures of glycemia and glycemic variability will be computed from the rtCGM and HGM data based on available data:

- The daily mean level of glucose, as well as the levels before and after meals.
- Measures of diurnal variability including the J-value, standard deviation of glucose values, and the mean amplitude of glycemic excursion (MAGE).[53]
- Mean and SD of fasting glucose values. A SD of greater than 50 mg/dl in the fasting glucose level over a two week period in the absence of illness will be considered as indicative of a metabolic derangement possibly associated with the end of the "honeymoon" period.
- SD for two week intervals.

The mean levels of quantitative variables (e.g. HbA1c and insulin dose) over all follow-up values will be compared between groups using a normal errors longitudinal analysis.

The rate of hypoglycemic events will be computed (total number of events divided by total participant years of follow-up) and the rates compared using a Poisson regression model, allowing for over-dispersion using a quasi-likelihood model as appropriate. Analyses will be adjusted for age, gender, *log(C-peptide+1)* and HbA1c.

Secondary analyses will be completed using first the primary stratum only, and then using the combined primary and secondary strata. A per-protocol analysis will be defined in the detailed Statistical Analysis Plan.

8.3 Additional Metabolic Outcomes and Analyses

The two treatment arms will be compared combining the data of participants who were antibody positive and antibody negative. This will entail the same analyses as in section 8.1 for the primary analyses with the additional antibody negative participants, adjusting for the stratum effect. Data from this study may be used in conjunction with other DirecNet or Diabetes TrialNet data for additional exploratory analyses.

8.4 Additional Outcomes and Analyses

The goal of the immunologic studies will be to distinguish between experimental and standard group participants. These studies are exploratory in nature. If the treatment group achieves "metabolic rest" for the islet cell, it may dampen the immune response. There may be changes in immune markers in intensively treated participants as a result of decreased metabolic activity of their islet cells or a direct effect of improved glycemic control.

This study will also accrue additional information about immunologic, genetic, and metabolic factors associated with type 1 diabetes by analyzing stored blood samples. New insights into immunological and genetic mechanisms controlling beta-cell loss in type 1 diabetes may lead to more effective strategies to more effectively treat (or prevent) the disease. Mechanistic studies will be conducted to compare mechanistic variables for participants at baseline and over time between the treatment groups. Stored samples could also be utilized to examine potential determinants of the complications of diabetes and of other conditions for which patients with type 1 diabetes could be at increased risk.

The analyses of each quantitative outcome will be conducted using a normal errors longitudinal regression model and of each event using a Poisson regression model.

8.5 Sample Size and Power Estimates

The primary analysis will compare the difference between groups in the levels of the 2-hour AUC-mean using the *log (mean C-peptide+1)* in an ANCOVA model adjusting for gender, age, and *log(C-peptide+1)*. Estimates of *log(mean C-peptide+1)* and root mean square error (RMSE) in the standard treatment group were obtained from prior studies [54] and were assumed to apply in the sample size estimation. Using combined one year data from the MMF/DZB study (collected through September 12, 2008), and all Anti-CD20 control data through year 1 of follow-up the lower 90% confidence limit for the mean log(C-peptide + 1) value is 0.315 and the upper 90% confidence limit for the RMSE is 0.167. Using the lower and upper confidence limits for the mean and RSME, respectively, rather than the point estimates gives a conservative estimate of the necessary sample size.

The corresponding Geometric-like Mean C-peptide value is 0.370 pmol/mL obtained using the inverse transformation exp (0.315) – 1. The expected Geometric-like Mean C-peptide value in the treatment arm is 0.370*1.50 = 0.555 pmol/mL. Using standard equations for the comparison of two means, [51] a total sample size of 63 participants would provide power of 85% to detect a 50% increase in the geometric-like mean C-peptide relative to the standard treatment group using a test at the 0.05 level (one-sided), with an assumed 10% loss to follow-up and a 2:1 allocation to intensive diabetes management versus control. This has been increased by an additional 5% to account for some participants in the intensive group not completing the closed-loop component of the protocol, not using intensive pump/CGM management, or both. In addition, it is expected

that 6 participants will be randomized who are antibody-negative and thus not included in the primary analysis. With these adjustments, the planned sample size will be 72 participants.

8.6 Interim Monitoring Plan

Since it is expected that recruitment will be completed by the time there are sufficient 1-year data to assess efficacy, an interim efficacy analysis is not planned. The DSMB will review study data at periodic intervals to assess whether there are any safety issues that warrant discontinuation of the study and to review conditional power analyses conducted both under the study hypotheses and under the current trend of the data [56] to allow early termination due to futility – i.e. lack of beneficial treatment effect.

CHAPTER 9
ETHICAL CONSIDERATIONS

9.1 Statement of Compliance

This study will be conducted in compliance with the protocol and consistent with current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements. Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Independent Ethics Committee (IEC) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented. Wherever possible, data will be entered into the database in real-time using computers in the clinical centers. The electronic data capture serves as the source document for the study.

9.2 Participating Centers

Participating clinical centers must have an appropriate assurance, such as a Federal-wide Assurance (FWA) or an Unaffiliated Investigators Agreement (UIA), with the Office for Human Research Protections (OHRP), since they are actively engaged in research and provide informed consent. The protocol and consent forms will be approved by Institutional Review Boards at each of the participating clinical sites. HIPAA regulations will be followed by each participating institution in accordance with each institution's requirements. The participating international sites will obtain approval from their corresponding review boards in accordance with their local procedures and institutional requirements.

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all case report forms are completed for every participant entered in the trial.

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

9.3 Informed Consent

The consent process will be conducted by an investigator with the assistance of the study coordinator and other qualified staff as indicated. All participants (or their legally acceptable representative) must read, sign and date a consent form prior to participation in the study, and/or undergoing any study-specific procedures.

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

9.4 Study Participant Confidentiality

For security purposes, participants will be assigned an identifier that will be used instead of their name. Protected health information gathered for this study will be shared with the DirecNet coordinating center, the Jaeb Center for Health Research in Tampa, FL. Data may also be shared with the TrialNet coordinating center also located in Tampa, FL. Information given to the coordinating center will include: diagnosis, general physical exam information, insulin, questionnaire results, hemoglobin A_{1C} results, continuous glucose monitor results, blood work results, HGM blood glucose measurements, information pertaining to hypoglycemic excursions and the treatment given, as well as all other study related data gathered during study visits and phone calls.

During each visit, the study devices will be downloaded to a computer that is secured and password protected, the files will be sent directly to the Coordinating Center via email. All files will include only the participant's identifier; no names or personal information will be included.

Laboratory specimens will be sent to the central laboratories being used for the study.

During the study, participants with a home computer will be asked to download the pump, rtCGM, and study HGM data to their home computer. The downloaded data from the closed loop therapy may be provided to Medtronic MiniMed. The data provided to the company will include only the participant's identifier; no names or personal information will be included. Medtronic MiniMed may be provided with a full dataset at the end of the study.

HLA genotyping is for research purposes only. The HLA genotyping result will not be made available to the participant and his or her physician. DNA will be stored for future use with the permission of the study participant.

Stored samples could be utilized to learn more about causes of type 1 diabetes, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment. The results of these future analyses will not be made known to the participant.

9.5 Sample and Data Storage

Samples to be stored for research purposes will be located at the NIDDK Repository and at the clinical centers. The use of the samples will be restricted to the study researchers unless researchers from outside of the study obtain approval from the Steering Committee and the NIDDK to utilize the samples. The samples will be coded with unique study numbers, but the researchers will be able to identify samples if it is necessary to contact participants for reasons of health or for notification to them about future studies. Approval from the Steering Committee and the NIDDK would be required before such linkage could occur. Researchers from outside of the study will not be permitted to identify samples.

Data collected for this study will be sent to the study Coordinating Center. After the study is completed, de-identified data will be stored at the NIDDK Repository, under the supervision of the NIDDK/NIH, for use by researchers including those outside of the study. When the study is completed, samples will continue to be stored at the NIDDK Repository Sites. Since the stored data will be fully de-identified upon the completion of the study, it will no longer be possible to identify samples. Thus, whereas a sample can be destroyed upon a participant's request during the existence of the study, it can no longer be destroyed once the study is completed. However, there will still be the potential to link data derived from the samples with data that had been derived from the study. Once the study is completed, researchers will only obtain access to samples through grant proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

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