Clinical Study Protocol

Amendment 03

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Sponsors

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This study will be conducted in compliance with the protocol, IND regulations and other applicable regulatory requirements.

PROTOCOL SIGNATURE PAGE

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and pertinent information to the study personnel under my supervision and my hospital ethics committee/institutional review board (EC/IRB). I will discuss this material with them and ensure they are fully informed regarding the study medication and the conduct of the study according to this protocol, applicable law, applicable regulatory requirements including 21 CFR parts, 50, 54, 56 and 812, general standards of good clinical practice and hospital EC/IRB requirements.

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Principal Investigator

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Date

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Definitions

Abbreviation	Definition
Adverse event (AE)	Any undesirable patient experience that may include but is not limited to an abnormal sign, symptom, illness, abnormal laboratory value, or other medical event.
Application (app)	A piece of software downloaded to a person's smartphone, used to record, display and transmit data
Blood brain barrier (BBB)	The layer of tissue separating the central nervous system from the periphery
Columbia-Suicide Severity Rating Scale (C-SSRS)	A scale designed to quantify the severity of suicidal ideation and behavior.
Continuous glucose monitor (CGM)	A device worn on the skin which measures interstitial glucose and displays by a transmitter to a receiver
Data Safety Monitoring Board (DSMB)	An independent group assigned to review safety data to monitor for incidence of trends that would warrant termination of the trial.
Intranasal (IN)	A method of drug delivery that is particularly applicable to delivering centrally acting medications into the central nervous system.
Investigator Brochure (IB)	A comprehensive document summarizing the body of information about an investigational product
Michigan Neuropathy Screening Instrument (MNSI)	A standardized physical exam technique using light touch, vibratory sensation and ankle reflexes to establish risk for peripheral neuropathy
Repeated Battery for the Assessment of Neuropsychological Status (RBANS)	A standardized neuropsychological assessment with five scores for each of five domains (immediate memory, visuospatial/constructional, language, attention, and delayed memory).
Serious adverse events (SAE)	Any symptom, sign, illness or experience that develops during the study and results in a life-threatening situation, hospitalization, significant disability, and other events determined by the investigator to be significant.
Trail Making Test (TMT) Parts A & B	A standardized neuropsychological test of visual attention and task switching.

Protocol Summary

PROTOCOL TITLE	**Intranasal Insulin: A Novel Therapy for Hypoglycemia Unawareness in Type 1 Diabetes**
SHORT TITLE	IN Insulin in Type 1 Diabetes (T1D) Hypoglycemia Unawareness: Safety Only Phase
STUDY PHASE	Phase II
STUDY OBJECTIVES AND PURPOSE	
Primary Objective: Demonstrate no significant increase in dangerous hypoglycemia with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia	
Secondary Objectives: 1. Describe changes in overall glycemic control with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia 2. Describe changes in hypoglycemia awareness with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia 3. Describe changes in safety endpoints with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia ***Exploratory Objective:*** Describe changes in memory, attention and executive function with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia	
STUDY DESIGN	
Study Type	Safety
Control Type	Placebo Crossover
Study Indication Type	Treatment
Randomization Schema	Insulin vs Placebo First
Device	SipNose intranasal device
Study Design	Prospective, randomized, cross-over, placebo controlled
Planned Duration of Subject Participation	10 weeks
Planned Duration of Treatment with IN insulin	14-20 days
PRIMARY ENDPOINTS	
Dangerous hypoglycemia defined by percent time below range (<54 mg/dL), from real-time continuous glucose monitoring (CGM)	
SECONDARY ENDPOINTS	
1. Overall metrics and glycemic control from real-time CGM data during each treatment period defined as: a. Percent time in range (70-180 mg/dL) b. Percent time below range (<70 mg/dL)	

c. Percent time above range (>180 mg/dL, >250 mg/dL)
d. Mean glucose
e. Coefficient of variation (%CV)
f. Glucose management indicator (GMI)
g. Percent time of active sensor wear

2. Hypoglycemia awareness measured three ways using:
   a. 3 hypoglycemia unawareness questionnaires/scores (Gold Score, Clarke survey, HypoA-Q)
   b. Time from CGM reading of, <70 mg/dL, 54-<70 mg/dL and <54 mg/dL to time of participant hypoglycemia awareness obtained from participant journals
   c. Glucose value from real-time CGM at onset time of hypoglycemia awareness obtained from participant journals
3. Safety outcomes include:
   a. Adverse events
   b. Neuropathy test changes
   c. Review of patient non-study medications
   d. HbA1c
   e. EKG
   f. Smell test changes
   g. C-SSRS assessment

EXPLORATORY ENDPOINTS

1. Memory and attention using Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)
2. Executive function using Trail Making Parts A and B

INVESTIGATIONAL PRODUCTS, DOSE AND MODE OF ADMINISTRATION

Investigational Product	Humulin-R 20 IU/IN (0.1ml/10 units IN in each nostril) BID

SUBJECT SELECTION

Targeted Accrual	10 subjects are anticipated to complete this study. We estimate a need to consent up to 20 subjects in order to reach this goal.

INCLUSION CRITERIA

- Adult patients (age ≥18) with type 1 diabetes diagnosis and a duration of diabetes of at least 10 years
- Gold score <4
- HbA1c ≥6.5% within the last 3 months or at screen visit
- Stable insulin regimen (MDI or insulin pump) for at least 3 months, as deemed stable by principal investigator
- Depression/anxiety medications stable for at least 3 months
- Ability and willingness to wear CGM continuously during study participation
- Participants must use their own smartphone, and have the ability and willingness to use CGM smartphone applications compatible with their smartphone
- Ability and willingness to check SMBG using own supplies, as instructed by study staff
- Ability and willingness to document when having symptoms of hypoglycemia in the CGM smartphone app or in a diary
- If using insulin pump, willing to operate insulin pump without threshold suspend feature or hybrid closed-loop, if applicable
- Proficient in speaking, reading and understanding English in order to complete surveys and testing of cognitive function
- Women of child-bearing age must agree to procure and use contraception throughout the study

EXCLUSION CRITERIA

- Pregnancy or planning pregnancy
- eGFR ≤ 30 mL/min/1.73m^2, if available from medical record
- Currently participating in a research study or completed any other research study within 6 months of screening date
- Current or recent use within 3 months of an insulin delivery system that adjusts insulin in response to continuous glucose monitoring (CGM) data (such as an automated insulin delivery system like hybrid closed-loop insulin pump therapy)
- Known dementia or mild cognitive impairment diagnosis
- Diabetic ketoacidosis within the last 6 months
- Use of non-insulin medications to treat diabetes
- Those planning to change diet or exercise regimen during the study
- History of trans-sphenoidal surgery or surgery to the upper part of the nasal cavity, chronic sinusitis, severe deviated septum, or difficulty with smell and/or taste
- Severe psychiatric illness
- Allergy to adhesives, insulin or any components of insulin product
- Subject cannot adequately demonstrate ability to use and deploy the devices as determined by investigator
- Evidence of suicidality using the Columbia-Suicide Severity Rating Scale (C-SSRS)
- Subject has history of any of the following: moderate to severe pulmonary disease, poorly controlled congestive heart failure, significant cardiovascular and/or cerebrovascular events within previous 6 months, condition known to affect absorption, distribution, metabolism, or excretion of drugs such as any hepatic, renal or gastrointestinal disease or any other clinically relevant abnormality or illness that inclusion would pose a safety risk to the subject as determined by investigator.

1 Introduction

1.1 Background/Rationale

Hypoglycemia, or the fear of it, is one of the most significant limits to current management of type 1 diabetes (T1D). Consequences of hypoglycemia, both physical and cognitive, can be severe and in some cases fatal.[1,2] Unfortunately, hypoglycemia occurs with high frequency among patients with T1D. Occurrence estimates put the frequency of mild hypoglycemia at 1 to 2 events per patient per week and severe hypoglycemia (requiring assistance from another person) at 0.2 to 3.2 events per patient per year.[1] Data from the T1D Exchange Clinic Registry (77 clinics in 35 states collecting common data on children and adults with T1D), has shown 18.6% of patients with T1D for ≥40 years have had 1 or more severe hypoglycemia episodes (resulting in seizure or loss of consciousness) within the last year.[3] With repeated episodes of hypoglycemia, the counter-regulatory pathways to restore normal glucose are blunted, and patients can become unaware of the hypoglycemia. It is estimated that 40% of patients with T1D have hypoglycemia unawareness.[4] Patients with long-standing T1D, intensive therapy at advanced age, and frequent hypoglycemia are at the highest risk for hypoglycemia unawareness.[5,6]

Guidelines suggest treating older patients with T1D and those with longer duration of disease with less aggressive HbA1c targets, with the assumption that this will help reduce the risk of hypoglycemia.[7] Despite these more conservative glycemic targets, they do not appear to protect patients from severe hypoglycemia.[3] Indeed, even those patients with T1D who think they can tell when they are hypoglycemic are still at risk for hypoglycemia. Taking patients ≥60 years old who have had T1D ≥20 years, and comparing those who have had at least 1 severe hypoglycemia episode in the last year to a case-control group with no severe hypoglycemia in the last 3 years, it was shown that those with severe hypoglycemia had more glucose variability (P = 0.008) and had continuous glucose monitoring (CGM) levels <60 mg/dL for ≥20 min on 46% of days compared with 33% of days in control subjects (P = 0.10). This was despite there being no appreciable difference in HbA1c levels.[6] It is also of note that the group with perceived "awareness" to hypoglycemia had surprisingly high numbers of hypoglycemic episodes. In T1D patients ≥60 years old, time spent in hypoglycemia (<70 mg/dL) was highest in younger patients, those with shorter diabetes duration, and lower HbA1c levels, and was strongly correlated to glycemic variability based on CGM.[8]

The use of CGM technology can reduce the frequency of hypoglycemia and allows for more aggressive insulin titration to lower HbA1c without increasing hypoglycemia events.[9-11] Indeed, higher HbA1c goals may help reduce the amount of time in hypoglycemia for patients with longer duration of T1D, however higher HbA1c targets are not enough to prevent severe hypoglycemia.[8] The use of CGM, however, does not reliably restore hypoglycemia awareness.[11] Advancements in insulin pump therapy and CGM using hybrid closed-loop artificial pancreas systems holds promise as well for better glycemic control without severe hypoglycemia.[12] Despite these benefits, only 15-20% of patients with T1D use CGM with insulin injections or insulin pump therapy.[13]

While some of the factors associated with hypoglycemia unawareness have been identified, as discussed above, the actual pathophysiology of hypoglycemia unawareness is multifactorial and not well understood. Possible mechanisms include reliance on alternative fuels in the brain (such as lactate), so that with frequent hypoglycemia, the brain adapts to use non-glucose fuels like lactate, which then leads to blunting of awareness to hypoglycemia. Alterations of the hypothalamic-pituitary axis have also been proposed, as well as decreased neuronal communication.[14] Further, cerebral blood flow to key areas of the brain responsible for hypoglycemia awareness appears to be decreased in hypoglycemia unawareness.[15] It has also been noted that recurrent moderate hypoglycemia may precondition the brain to limit the extent of severe hypoglycemia.[16] Mice with a knockout of the insulin receptor in the brain have a blunted response to hypoglycemia.[17] Moreover, there is evidence that the brain plays a role in glucose homeostasis via pancreatic islet innervation resulting in peripheral insulin and glucagon changes.[18] While it is becoming clear that there is a connection between the brain and glucose homeostasis, the precise mechanisms in T1D patients remain unknown.

Evidence is mounting that diabetes is associated with cognitive dysfunction, and the relationship appears to go in both directions. Hyperinsulinemia in the fasting state predicts nearly a doubling in risk for Alzheimer's disease and decline in memory, even in patients without known diabetes.[19] In patients with type 2 diabetes (T2D), there is higher incidence of dementia, and dementia onset occurs at a younger age.[20] There is active research attempting to elucidate further the connection between insulin and cognition.[21] One possible mechanism involves amyloid beta oligomers, a hallmark of Alzheimer's disease pathogenesis. These amyloid deposits have been shown to reduce insulin receptors in hippocampal

neurons, and by delivering insulin *in vitro* to these cells, the insulin receptors were maintained, and the amyloid oligomers reduced.[22] Insulin has also been reported to enhance amyloid beta clearance from the brain.[23] Further, the activity of glycogen-synthase kinase-3-beta, the enzyme that phosphorylates tau to create Alzheimer's neurofibrillary tangles, has been reported to be down-regulated in response to insulin. Also insulin receptor signaling increases synaptic density, and loss of synapses is key to the neuropathology of Alzheimer's disease.

The novel therapy of intranasal insulin, invented and patented by Dr. Frey, one of our investigators (U.S. Patents 5,624,898; 6,313,093), has been proposed as a therapy for Alzheimer's disease[21,24] and PTSD,[25] and is known to improve memory, attention and functioning in patients with mild cognitive impairment and Alzheimer's disease without any major adverse side effects[24,26,27] (Table 1). Intranasal delivery sends insulin directly from the nose to the brain along the olfactory and trigeminal nerves[28] and does not alter the blood levels of insulin or glucose.[21,26] In normal healthy adults[24] and those with type 2 diabetes[29], intranasal insulin improves memory without altering the blood levels of insulin or glucose (although to date this has not been evaluated using CGM as we are proposing). FDG-PET imaging has shown that patients with Alzheimer's disease have a marked global decrease in the cerebral metabolic rate of glucose metabolism leaving brain cells starved for energy.[30] Glucose is the primary energy source for brain cells. Intranasal insulin has been found to rapidly target the brain, help maintain glucose uptake and utilization, and increase brain cell energy (ATP).[31,32] Further, it also attenuates the HPA axis elevation of cortisol (which inhibits glucose uptake to the brain) resulting in improved attention, memory, and function.[25,33] Finally, in rodent models, central insulin signaling directly alters glucose sensing in hypothalamic neurons.[17] Taking all these mechanisms together, intranasal insulin may play a beneficial role in the counter-regulatory response to recurrent hypoglycemia.

In addition to co-authoring the first clinical trials to demonstrate that improved intranasal insulin improves memory in patients with mild cognitive impairment (MCI) and Alzheimer's,[34,35] Dr. Frey's team is currently conducting two trials using intranasal insulin. The first titled, "Investigation of the Safety of Intranasal Glulisine in Down Syndrome" (ClinicalTrials.gov, Identifier: NCT02432716), is a single center, randomized, double-blind, placebo-controlled, cross-over pilot study designed to assess the safety of intranasally delivered glulisine versus placebo in older patients with Down Syndrome who would be expected to have Alzheimer's brain pathology. The second titled, "Intranasal Glulisine in Amnestic Mild Cognitive Impairment and Probable Mild Alzheimer's Disease" (ClinicalTrials.gov Identifier: NCT02503501), is a Phase II, single center, randomized, double-blind, placebo-controlled 6-month study of the safety and the therapeutic effectiveness of intranasal glulisine in amnestic mild cognitive impairment and probable mild Alzheimer's disease. The team also recently completed and published a safety trial of rapid acting insulin in Alzheimer's patients.[36] The study team has expertise in intranasal clinical trial design, investigator-initiated IND submissions to FDA, tracking and dispensing investigational products and intranasal devices, training of patients on the use of intranasal devices, and creation of secure REDcap databases for participant tracking and data entry. From the clinical trials listed in Table 1, 10IU BID of regular insulin have repeatedly shown benefit and safety in healthy adults and patients with either MCI or Alzheimer's disease.

Table 1: Intranasal insulin improves memory function in phase II human clinical trials.

Subjects	Intranasal insulin duration/dose	Main result	References
Healthy Adults	4 × 40 IU/day, for 8 weeks	Intranasal intake of insulin enhanced long-term declarative memory and positively affected mood in humans without causing systemic side effects like hypoglycemia.	Benedict et al.,2004
Healthy Adults	4 × 40 IU/day, for 8 weeks; regular and rapid-acting	Declarative memory was improved in insulin and insulin aspart groups compared to placebo group without altering glucose levels. Insulin aspart treated subjects performed even better that those of insulin treated group.	Benedict et al.,2007
Healthy Adults	Single dose of regular human insulin 160 IU	Hippocampus-dependent memory and working memory were improved in women where as men did not benefit from acute insulin treatment	Benedict et al., 2008
Healthy Adults	Single dose of regular human insulin 160 IU	Enhanced performance in the prefrontal cortex-dependent working memory task in both postmenopausal and young women	Krug et al., 2010
Healthy Adults	4 × 40 IU/day, for 8 weeks	Declarative memory and mood were improved and HPA axis activity as assessed by circulating ACTH and cortisol levels was reduced.	Hallschmid et al., 2008
MCI and mild AD patients	20 or 40 IU of insulin acute treatment	Acute intranasal insulin administration improved verbal memory in AD and MCI subjects without the APOE- ε 4 allele	Reger et al.,2006
MCI and AD patients	10, 20, 40, or 60 IU for 5 days	10, 20, and 40 IU of insulin improved declarative memory only in APOE-ε 4 negative patients. Memory facilitation generally peaked at the 20 IU dose. ↑ Aβ42 levels for memory-impaired adults from saline to 10 IU regardless of APOE-ε 4 status. Intranasal insulin did not affect peripheral glucose or insulin levels.	Reger et al.,2008a
MCI and AD patients	20 IU BID for 21 days	Insulin-treated subjects retained more verbal information and improved attention and functional status. Insulin treatment raised fasting plasma Aβ40/Aβ42 ratio.	Reger et al.,2008b
MCI and mild to moderate AD patients	20 or 40 IU for 4 months	Treatment with 20 IU of insulin improved delayed memory. Both dosages preserved caregiver-rated functional ability and general cognition. Unchanged Aβ42 and tau levels after insulin treatment.	Craft et al.,2012
AD patients with ApoE4 allele	Rapid acting insulin	Rapid acting insulin failed to have an acute impact on cognition in ApoE4 carriers with AD	Rosenbloom et al., 2014
MCI and mild AD patients	20 or 40 IU of insulin detemir for 21 days	High dose (40 IU) improved visuospatial and verbal working memory. High dose improved memory for adults with MCI and AD who were APOE-ε 4 positive patients. APOE-ε 4 carriers taking high dose also improved peripheral insulin resistance. APOE-ε 4 negative patients taking high dose experiences increased peripheral insulin resistance.	Claxton et al.,2015
Older adults with and without type 2 DM	Single dose of 40 IU randomized, crossover design	Intranasal insulin administration appears safe in older adults with type 2 DM, does not affect systemic glucose control. Improvements of visuospatial memory after insulin administration in the DM group and in the verbal fluency test in the control group, potentially through improved regional perfusion and vasoreactivity mechanisms	Novak et al., 2014

Modified from Bedse, Gaurav, et al. "Aberrant insulin signaling in Alzheimer's disease: current knowledge." Frontiers in Neuroscience 9 (2015)

2 Summary of Device Descriptions

2.1 Intranasal SipNose Device Overview

The intranasal SipNose device proposed to be utilized in this protocol is the same device (SP1N1C1) under IND 139885. IND 139885 is an investigator initiated clinical trial, titled "A Single Center Feasibility Study of Intranasal Insulin in Frontotemporal Dementia [NIFT-D (Nasal Insulin in Frontotemporal dementia)]". The Principal Investigator is Dr. Michael H Rosenbloom. In addition, Table 2 summarizes the SipNose devices in MAF311, when compared to the SP1N1C1 device. The next set of sections summarizes the information provided for this protocol, along with updated Instructions for Use (Version 4), Investigator's brochure (Version 3) as attachments. Also letters are provided from SipNose Ltd, and Dr. Michael H Rosenbloom regarding IND 139885 providing permission to review the submitted documents to FDA.

Table 2: SipNose devices in MAF311 compared to SP1N1C1

	HealthPartners - Clinical trials with Insulin- SP1N1C1	MAF311 devices SP1N2C2-NV1 and SP1N3C4
Functional unit: same unit in all devices	SP1	SP1
Nose Piece, "N"	N1: Allows medium volume administration – up to ~250-300ul	N2: Allows medium volume administration – up to ~200ul N3: Allows maximal volume administration – up to ~1000ul
Cover, "C" which serves also as the safety lock	C1: cover is suitable for N1 in length, no drug-filling features,	C2-NV1: Cover includes drug-filling features (needle). C4: cover is suitable for N3 in length, no drug-filling features.
Assembly, Validation	Same process in principle, same verification and validation process.	

2.1.1 Intranasal SipNose Device

The delivery of drugs to the CNS remains a development challenge mainly due to the impenetrable nature of the BBB. SipNose's innovative approach enables broad, consistent drug delivery (either liquids or powders) to the upper area of the nasal cavity, which improves the absorption of the active ingredients when compared to traditional nasal delivery devices (see Figure 1: Nasal Aerosol Deposition - Traditional (Djupesland and Skretting, 2012) and SipNose (99MTC-DTPA) Devices). Currently available nasal devices such as droppers, sprays, or pumps deliver aerosol mainly to the lower and mid turbinate of the nasal cavity and only minor amounts, about 5% to the upper turbinate of the nasal cavity [37]. SipNose has conducted animal studies using an intranasal device with the same technology that is designed for humans. To address the different nasal cavity size and structure, adaptations were used for the device to fit each animal model.

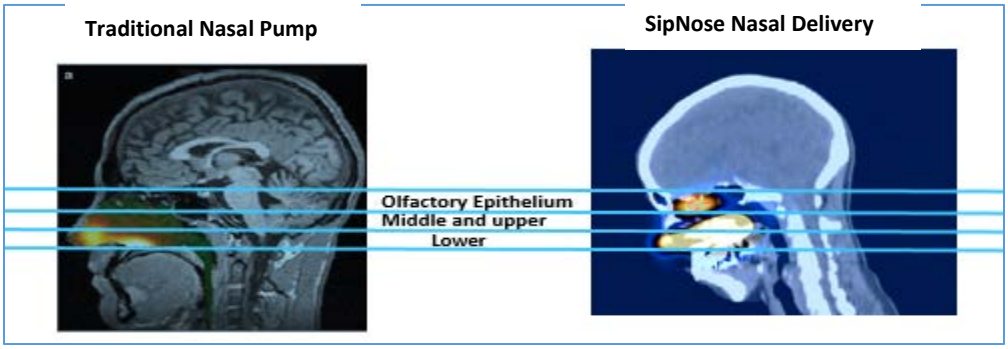

Figure 1: Nasal Aerosol Deposition - Traditional (Djupesland and Skretting, 2012) and SipNose (99MTC-DTPA) Devices

The SipNose device is an aerosol nasal delivery platform that uses pressurized delivery through the discharge of compressed air, resulting in an aerosol that delivers the drug in a narrow plume geometry, which targets the olfactory

epithelium in the upper nasal cavity. From the olfactory epithelium, therapeutics rapidly reach the central nervous system, traveling extracellularly along the olfactory nerves. The device is not currently commercially available, but numerous studies have demonstrated its ability to intranasally deliver radiolabeled and therapeutic compounds to the brain.

SipNose has successfully completed a set of experiments that demonstrate the advantages of its nasal delivery system. A set of in vitro tests, in vivo tests, and small clinical studies have been conducted and described below that support the following (for further details see Investigator brochure):

1. Reproducible and non-user dependent nasal delivery (Section 11.4-11.8 in Investigator's brochure)
2. Improved drug dispersion and deposition targeting to the upper third of the nasal cavity when compared to other nasal delivery devices (Figure 9, Sections 5.1 and 5.3 in Investigator's brochure)
3. Significant delivery of aerosolized drug to the upper nasal cavity and olfactory epithelium with minimal lung contamination (Sections 5.2, 5.3 in Investigator's brochure)
4. Increased CNS concentration of drug when compared to other nasal applications (Sections 5.1 and 11.4, Human Study # 3 in Investigator's brochure)
5. Safety and tolerability of the SipNose device (MAF 311)

2.1.2 SipNose Utility, Reliability, and Safety

The SipNose device was specifically designed to facilitate drug loading and user experience particularly suited for the purposes of this trial.

2.1.2.1 Utility

The SipNose device consists of a nose piece, compressed air chamber, activation lock to prevent unwanted activation, and activation button (Figure 2: SipNose Device Components- SP1N1C1). The device also consists of a cover that prevents the device being activated accidently.

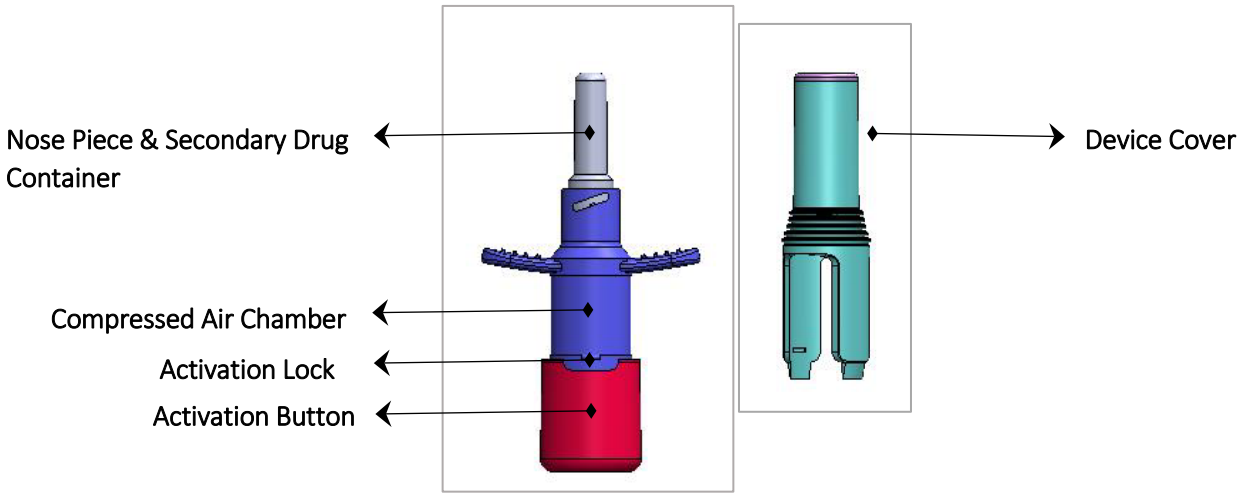

Figure 2: SipNose Device Components- SP1N1C1

The drug is kept within its original commercial bottle or primary container until use and stored under the required conditions as stated in the original drug label. Prior to administration, the drug is drawn out from the primary container using a commercial syringe, and transferred into the Device Nose Piece that serves as a secondary drug container for a short period of time (several minutes). The drug contacts only with the device parts that are made of polypropylene (PP), medical grade USP Class VI compliant. PP is an acceptable material for short term exposure of drugs, including insulin [38]. For example, this material has been used in the following products: MONOJECT SoftPack; GLUCOPRO Insulin Syringe; BD Insulin Syringes, all commonly utilized syringes in clinical settings.

The nostrils come in contact only with the nose piece which is made of the same PP mentioned above. The parts that come in contact with intact skin (subject's hands) are made of PET-G Tritan™ – Estman MX711 (details in sections 3 and 4 of Investigator's brochure).

Drug stability studies have not been conducted for this study as insulin has been shown to be stable at refrigeration and room temperature conditions for up to 31 days [[39]; Section 16.2 –package insert).

2.1.2.2 Reliability

The SipNose device has demonstrated an ability to reliably reproduce the same spray dose volume consistency. The U.S. Food & Drug Administration (FDA) guidance recommends a consistency of ≤1 spray outside 80 – 120% and 0 sprays outside 75 – 125% dose volume. During tests with insulin, SipNose devices expelled an average volume of 105.14ul (Range: 99.6-112ul), all individual spray volumes were within the range of 93%-107% of the target volume 100ul, and mean spray volume was within 95%-105% of the target volume of 100ul. Therefore, both individual and mean results meet the FDA guidelines (See Compatibility Testing document – for details).

Studies have shown that the SipNose device consistently targets the upper nasal cavity, thus delivering the dispelled agent to the region critical for CNS delivery. When using a human nasal model cast, the SipNose more reliably targeted the upper nasal cavity (see section 5.1 in IB) as compared to other commercial devices (LMA MAD NasalTM). In a preclinical rat model, 11C-choline was reliably delivered to the olfactory epithelium and into the brain immediately following administration (detected in brain ~3min following administration). PET-CT scan were measured from ~3 min following administration up to 1 hour. An example of the scan at 40-45 minutes after drug administration is shown in the IB. (See section 5.2 in IB). In addition, midazolam brain concentrations using the intranasal SipNose device were comparable to that obtained with IV administration in a rat model, although blood levels were lower than the IV administration (see section 11 in Investigator's brochure). The SipNose device demonstrated higher anterior and posterior brain concentrations of insulin following IN delivery as compared to IV administration (see section 11.8 in Investigator's brochure).

In a human study using SPECT-CT analysis, it was found that the volume of drug deposition effectively targeted the upper/middle posterior region of the nasal cavity with negligible lung deposition (see section 5.3 in Investigator's brochure). Another human study involving patients presenting with epileptic seizures treated with IN midazolam using the SipNose device showed that the treatment improved electrographic seizures and was well-tolerated in this population (See section 11.4 in Investigator's brochure). Finally, delivery of IN midazolam anesthesia with the SipNose device resulted in a higher level of sedation in patients undergoing a surgical procedure compared to IN drug delivery with a commercial delivery device (see section 11.4 from Investigator's brochure).

2.1.2.3 Safety

SipNose device is an aerosol nasal delivery platform that uses pressurized delivery through the discharge of compressed air. The pressure used in the SipNose is similar to that used in commercial nasal inhalers and is up to 6 bars (1 bar=14.5 Psi). Impact force of the SipNose device is in the range of 1.5-6.5 grams, and is comparable to that found in commercialized inhalers, dry powder inhalers and other nasal delivery devices in the field [40] The actual impact force that is detected following aerosol release via the SipNose device, is found to be 2-5gr, and specifically with 100ul of the drug Insulin is 2.7±0.3gr.

Safety experiments performed have shown no evidence of irritation or local tissue damage following treatment with the SipNose nasal delivery device in both humans and animals (see section 11 in Investigator's brochure).

2.2 Continuous Glucose Monitor (CGM) device

The commercially available Dexcom G6 CGM system (Figure 3) measures the glucose level in the interstitial fluid under the skin. The CGM system includes a sensor which has a small wire that is inserted under the skin, a transmitter that attaches to the sensor and a smartphone app that collects and displays the glucose results. The sensor will be placed under the skin and can stay in place for up to 10 days. The sensor will measure the glucose level in the interstitial fluid every five minutes and transmit that data to the smartphone. The screen will show in real-time the participant's glucose value, most recent glucose trends, and an arrow indicating which way the glucose trend is predicted to go. Alarms can

be set to alert the participant to glucoses that are too high or too low, or if glucose trends are changing quickly. The data collected by the system can be downloaded and evaluated for several CGM-based metrics.

Figure 3: Dexcom G6 CGM System

3 Objectives

3.1 Primary Objective

Demonstrate no significant increase in dangerous hypoglycemia with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia.

3.2 Secondary Objectives

1. Describe changes in overall glycemic control with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia
2. Describe changes in hypoglycemia awareness with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia
3. Describe changes in safety endpoints with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia

3.3 Exploratory Objective

Describe changes in memory, attention and executive function with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia.

4 Endpoint(s)

4.1 Primary Endpoints

Dangerous hypoglycemia defined by percent time below range (<54 mg/dL) from real-time continuous glucose monitoring (CGM).

4.2 Secondary Endpoints

- Overall metrics and glycemic control from real-time CGM data during each treatment period defined as::
  - Percent time in range (70-180 mg/dL) from real-time CGM
  - Percent time below range (<70 mg/dL) from real-time CGM
  - Percent time above range (>180 mg/dL and >250 mg/dL) from real-time CGM
  - Mean glucose from real-time CGM
  - Coefficient of variation (%CV) from real-time CGM
  - Glucose management indicator (GMI) from real-time CGM
  - Percent time of active sensor wear from real-time CGM
- Hypoglycemia awareness measured three ways using:

    - 3 hypoglycemia unawareness questionnaires/scores (Gold Score, Clarke survey, HypoA-Q)
    - Time from CGM reading of, <70 mg/dL, 54-<70 mg/dL and <54 mg/dL to time of participant hypoglycemia awareness obtained from participant journals
    - Glucose value from real-time CGM at onset time of hypoglycemia awareness obtained from participant journals
- Safety outcomes include:
    - Adverse events
    - Neuropathy test changes
    - Review of patient non-research insulin doses and medications
    - HbA1c
    - EKG
    - Smell test changes
    - C-SSRS assessment

4.3 Exploratory Endpoints

- Memory and attention using Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)
- Executive function using Trail Making Parts A and B

5 Study Design

This study is a single center trial designed to assess the safety of administering intranasal (IN) regular insulin in subjects with type 1 diabetes and intact hypoglycemia awareness Figure 4.

After written informed consent has been obtained, subjects will be screened to assess study eligibility based on study inclusion/exclusion criteria. Subjects who are eligible at the end of the screening visit (Visit 1) will be scheduled for a baseline visit to take place 1 to 2 weeks later (Visit 2). During the baseline/randomization visit, subjects will be administered the hypoglycemia and cognitive questionnaires and tests. Patients then are randomized 1:1 to either treatment with intranasal insulin or placebo. They will wear two 10-day real-time CGMs, and then crossover to the other group for another two 10-day period following a 2 week washout period. A final safety/assessment visit will take place up to 1 week after visit 10.

5.1 Drug Administration Training

All subjects will receive training at start of treatment visits regarding home administration of IN insulin using the SipNose device during visits 2 and 7. Subjects will be required to administer the dose twice daily, one in the morning and the other in the evening (at least 8 hours between doses). If the SipNose device is discharged prior to insertion into the nose, then a retrial admission would be indicated. However, if the device is discharged following nasal insertion, then a retrial would not be permitted. The steps to ensure that subjects maintain compliance with the study protocol over 4 weeks include conducting the following:

- In-clinic training sessions: baseline/randomization (Visit 2) and start of treatment period 2 (visit 7)
- Phone call from study coordinator/investigator 1 day and 5 days after baseline visit and after Visit 7.

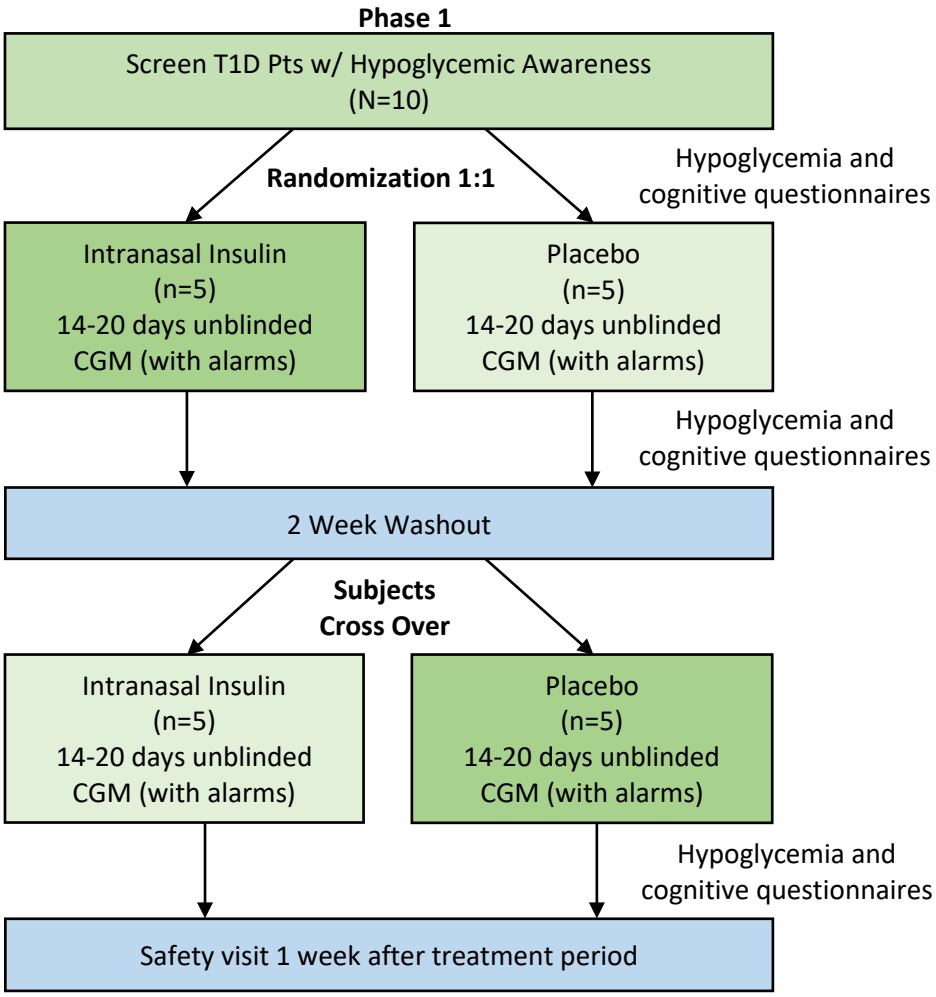

Figure 4: Study Design Flow Diagram

5.2 Drug Administration with SipNose

Each dosing administration will require the following items: a) 2 SipNose Devices (2) 2 nose piece covers; b) device holder; c) study drug vial (10 ml vial of regular Insulin (Humulin-R or Placebo)); d) Insulin syringe; e) alcohol wipe; f) needle clipper and/or sharps container.

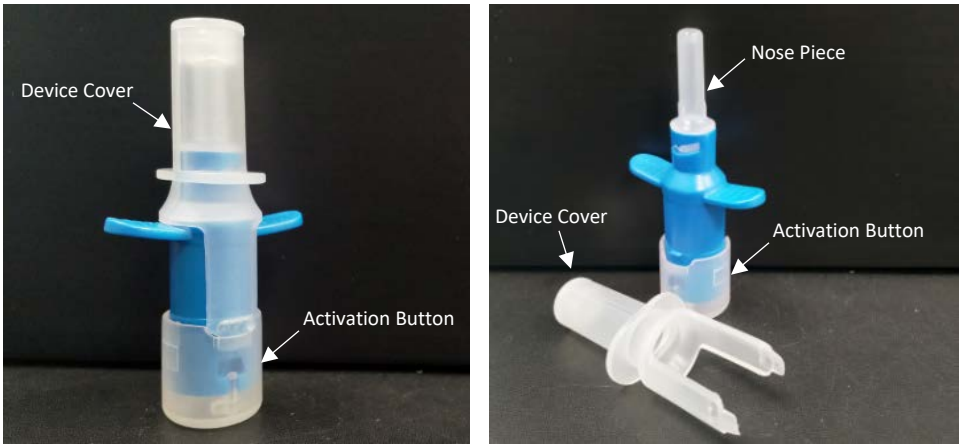

Figure 5: SipNose Device -Sp1N1C1

During the baseline/randomization visit the subject will administer the study drug in front of study personnel to ensure adherence to study protocol for drug administration. To ensure safety and medication compliance, subjects will be followed-up with a routine phone call 1 day and 5 days after baseline/randomization visit, 1 day and 5 days after Visit 7 (start of treatment period 2) and 1 week after final treatment visit.

Devices will be shipped to the clinical study site. One device with a nose piece cover will be supplied for each dose to be administered in the study. Devices are one-time use only. A kit containing all necessary items will be distributed to each subject and replaced at each study visit or as necessary. This will include several extra devices in case there are issues with any devices. Each device is to be loaded with a 10 unit dose of study drug to be used immediately or up to a maximum of 10 minutes prior to IN administration. The Nose Piece and Secondary Drug Container (temporary) are the only components of the device that will come into contact with the study drug and subject's nasal cavity.

5.3 Device Dose Loading and Administration

1. Gather and prepare the needed materials.

*Not the actual syringe and study drug. The above pic for illustration only.

Figure 6: Study Drug Administration Materials

2. Open the devices covers and place the devices in the device holder

Figure 7: Open Device Cover

3. Remove the protective cap from the study drug vial as shown in figure 8 if you have not already done this step.
4. Load the drug formulation into the device.
    a. Remove the cap from the top of the vial.

b. Wipe the top of the vial with an alcohol wipe.

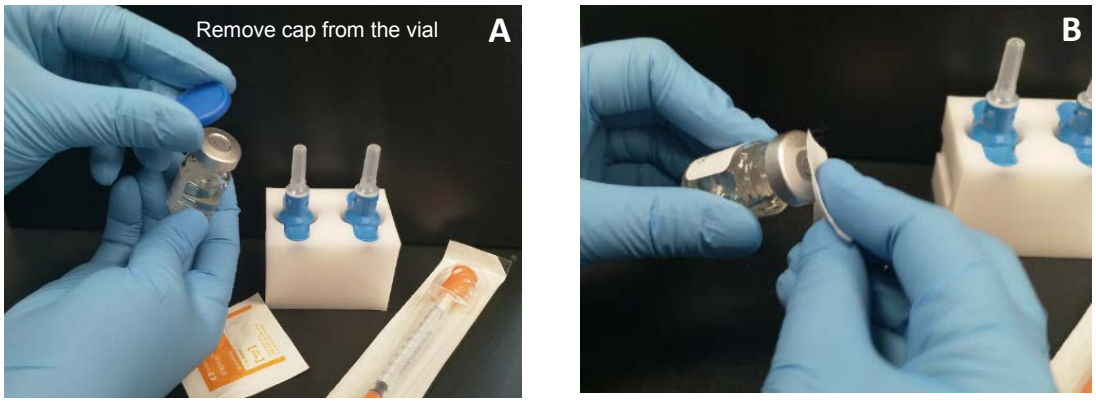

Figure 8: Remove Cap (A) and Cleaning Vial (B)

c. Immediately before (within 10 minutes) of taking dose, load the 0.1 ml (10 Units) desired study drug dose volume from the vial using the Insulin syringe following the instructions provided with the drug formulation.
   i. Pull syringe plunger down to 0.1 ml (10 Units)
   ii. Push air into the vial
   iii. Turn the medication vial upside-down and slowly pull the syringe plunger down, withdrawing the study drug until 0.3ml (30 Units) line is visible just above the plunger. Tap the syringe to move any air bubbles to the top

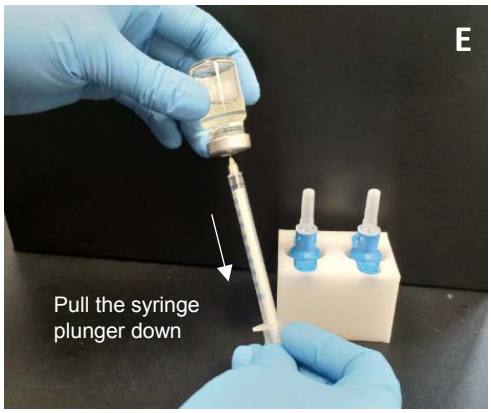

Figure 9: Filling Syringe

   iv. Slowly push the plunger up to the 0.1ml (10 Units) line to push any air bubbles back into the vial.
   v. Turn the medication vial upright and gently pull the syringe without moving the plunger.

d. Insert the syringe with the needle carefully into the top of the device (Figure 10).
e. Slowly depress the syringe plunger, ensuring that no study drug leaks from the device. After the plunger is fully depressed, remove the syringe with the needle carefully from the device and leave the device in the device holder.
f. Dispose of the syringe with the needle by using a needle clipper and/or a sharps container.
g. Repeat these steps, using another syringe for the second device.
h. The study drug must be administered within 10 minutes after loading it into the device.

5. Instruct the subject to blow their nose into tissue prior to administering the first dose.

Figure 11: Filling SipNose Device

6. Instruct the subject to turn the device upside down and flick the nose piece to allow the drug to concentrate at its edge as shown in the figure below (Figure 10). This helps the subject to see if the drug is loaded prior to administration.

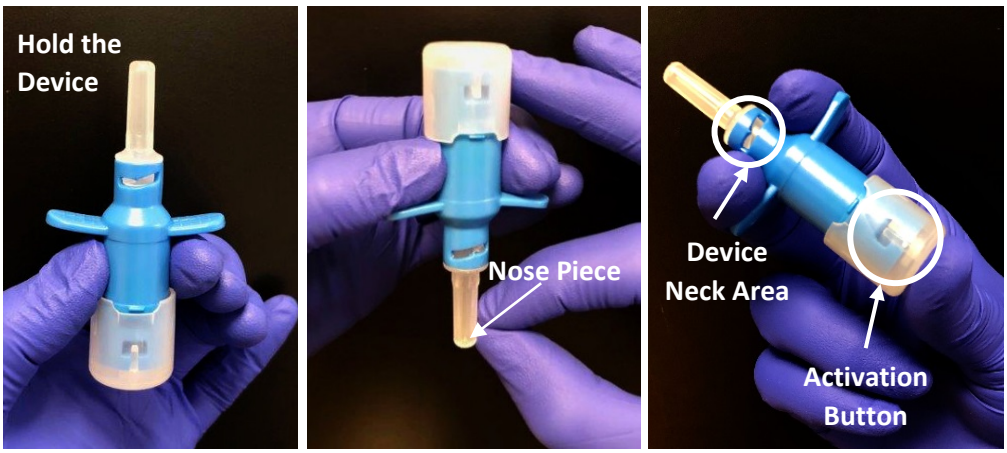

Figure 10: Prepare Device for Administration

7. The research staff will provide guidance to the subject on placement for nasal administration for the first dose.
   a. Place the nose piece of the device comfortably in the nose as shown in the figure below (Figure 12)
   b. After the initial placement, the examiner will make slight adjustments to orient the nose piece in the proper direction (parallel to the nasal septum).

Figure 12: Placement of Device for Nasal Administration

8. Use thumb to press the activation button to administer the drug in the nasal cavity.

9. Without blowing nose, repeat steps 6-8 for the other nostril using the second prepared device.
10. Place used devices into the Ziploc bag in the dosing kit to be returned to the study staff for disposal via biohazard waste. Devices that malfunction will be placed into a separate plastic bag and returned to SipNose for quality inspection.

5.4 CGM Device and Training

CGM device and smartphone app training will occur at the start of both treatment periods, and user guide materials will be provided. Participants will first download the Dexcom G6 Mobile app to their smartphone. After entering their transmitter's serial number into the app, there is a 2 hour warm-up for the CGM. Next they will identify a location on their abdomen as directed by study staff for insertion of the sensor. After cleaning the skin, participants are instructed how to use the applicator to insert the sensor and connect the transmitter to the sensor. After the 2 hour warm up is over, the CGM will start transmitting real-time glucose data. Participants will be allowed to use CGM data for their diabetes management. Blood glucose monitoring can be performed using the participant's own glucose meters if CGM readings do not match a participant's symptoms. CGM alert thresholds will be set at default levels of 70 mg/dL for low glucose, and 180 mg/dL for high glucose. These alerts can be adjusted during the study as needed. The G6 app also has a feature where wearers can mark events such as food or exercise; for the purposes of this study, participants will be instructed to use the "other" activity as a marker of symptomatic hypoglycemia (which they can use as an alternative to the hard-copy participant journal for documentation of symptomatic hypoglycemia)

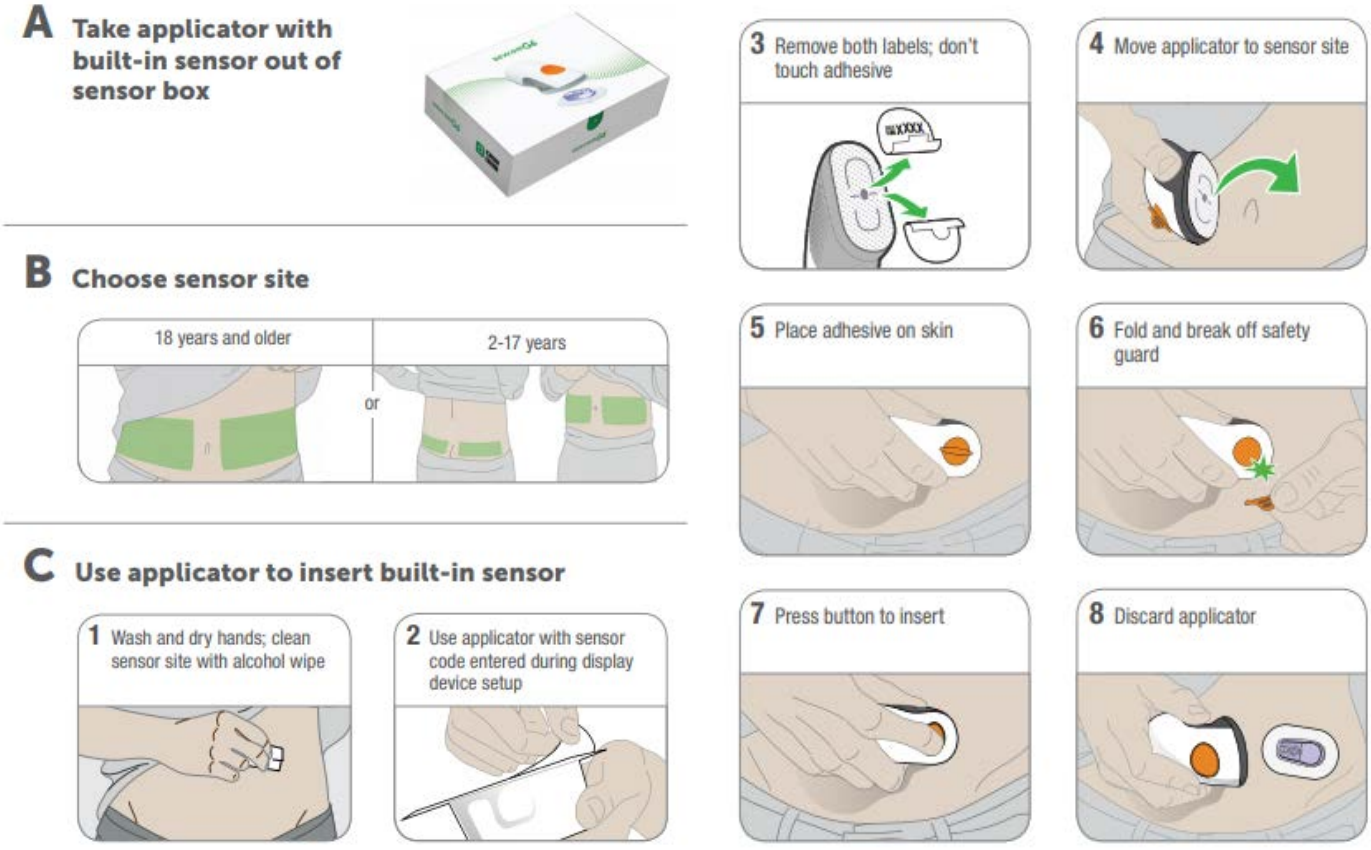

Figure 13: Inserting the CGM Sensor

The Dexcom G6 system allows for the wearer to share their glucose data with up to 5 followers remotely through the Follow app. This "sharing" feature will be downloaded and started with assistance of the study staff. The study staff, including the PI, will have 24 hour access to this data for safety purposes, if participants call with any concerns during the study. A CLARITY mobile account will be set up for participants (using the CLARITY app which links to the G6 Mobile app) and linked to the research site, which can capture device data for analysis.

5.5 Outcome Measures

CGM data will be downloaded at the end of each treatment period. We will capture percent time in each glucose range of interest as well as mean glucose and other glycemic metrics. Hypoglycemia unawareness questionnaires, cognitive tests, and neuropathy tests will be collected at beginning and end of the first treatment period and at the end of the second treatment period. AE's will be assessed throughout the study. The remaining safety outcomes will only be measured at screening and post-study completion.

5.6 Study Duration

Study participation will last approximately 10 weeks, consisting of a screening visit, 4 study phase visits, 1 follow-up safety assessment visit and at least 5 phone call visits. Subjects will receive treatment with IN insulin for a period of 14-20 days. Subjects who discontinue treatment will continue to complete follow-up measures as possible.

6 Study Population

6.1 Eligibility Criteria

6.1.1 Inclusion Criteria

- Adult patients (age ≥18) with type 1 diabetes diagnosis and a duration of diabetes of at least 10 years
- Gold score <4
- HbA1c ≥6.5% within the last 3 months or at screen visit
- Stable insulin regimen (MDI or insulin pump) for at least 3 months, as deemed stable by principal investigator
- Depression/anxiety medications stable for at least 3 months
- Ability and willingness to wear CGM continuously during study participation
- Participants must use their own smartphone, and have the ability and willingness to use CGM smartphone applications compatible with their smartphone
- Ability and willingness to check SMBG using own supplies, as instructed by study staff
- Ability and willingness to document when having symptoms of hypoglycemia in the CGM smartphone app or in a diary
- If using insulin pump, willing to operate insulin pump without threshold suspend feature or hybrid closed-loop, if applicable
- Proficient in speaking, reading and understanding English in order to complete surveys and testing of cognitive function
- Women of child-bearing age must agree to procure and use contraception throughout the study

6.1.2 Exclusion Criteria

A subject will not be included for consideration in this study if any of the following criteria are met:

- Pregnancy or planning pregnancy
- eGFR ≤ 30 mL/min per 1.73 m2, if available from medical record
- Currently participating in a research study or completed any other research study within 6 months of screening date
- Current or recent use within 3 months of an insulin delivery system that adjusts insulin in response to continuous glucose monitoring (CGM) data (such as an automated insulin delivery system like hybrid closed-loop insulin pump therapy)
- Known dementia or mild cognitive impairment diagnosis
- Diabetic ketoacidosis within the last 6 months
- Use of non-insulin medications to treat diabetes
- Those planning to change diet or exercise regimen during the study
- History of trans-sphenoidal surgery or surgery to the upper part of the nasal cavity, chronic sinusitis, severe deviated septum, or difficulty with smell and/or taste
- Severe psychiatric illness
- Allergy to adhesives, insulin or any components of insulin product
- Subject cannot adequately demonstrate ability to use and deploy the devices as determined by investigator

- Evidence of suicidality using the Columbia-Suicide Severity Rating Scale (C-SSRS)
- Subject has history of any of the following: moderate to severe pulmonary disease, poorly controlled congestive heart failure, significant cardiovascular and/or cerebrovascular events within previous 6 months, condition known to affect absorption, distribution, metabolism, or excretion of drugs such as any hepatic, renal or gastrointestinal disease or any other clinically relevant abnormality or illness that inclusion would pose a safety risk to the subject as determined by investigator.

7 Study Assessments and Procedures

A summary of study events and procedures is outlined in the Study Visit Table (see appendix 5). Demographic and Baseline Assessments

7.1.1 Visit 1: Screening (Day -14 to -7)

The following procedures will be performed at this visit:

- Obtain written informed consent from subject prior to any study related procedures
- Obtain Gold score – if <4, can stop here.
- Review inclusion/exclusion criteria
- Review medical history, as it pertains to inclusion/exclusion criteria
- Obtain subject's demographic information (date of birth, gender, race, education, etc.)
- Obtain details of medications, prescription, over the counter and vitamin/nutraceuticals taken over the course of the last 30 days prior to screening
- Collect vital signs (blood pressure & pulse), height, weight and BMI
- Obtain laboratory studies (Na, K, CO2, Creat/GFR, AST/ALT, CBC w/ diff) and HbA1c POC, if not obtained within the past 3 months
- Urine pregnancy test in women of child bearing potential
- Perform a standard 12-lead ECG
- Download participant's blood glucose meter and/or CGM to evaluate recent glucose control
- Provide patients with glucagon self-injection kit, if they do not have a current kit at home
- Provider to see patient at this visit for visit assessment review

7.1.2 Visit 2: Randomization/Start of Treatment Period 1 (Day 0) - This will be scheduled as a morning appointment to accommodate visit length

The following procedures will be performed at this visit before SipNose administration:

- Assess for AE/SAEs
- Collect vital signs (blood pressure & pulse)
- CGM placed and started before testing (requires 2 hour warm up)
- Document BG values before and after cognitive testing
- C-SSRS questionnaire
- MNSI survey
- Hypo surveys (Clarke, HypoAQ)
- Cognitive tests (RBANS, Trail-making)
- Smell test (12-item UPSIT test versions A & B)
- Ensure patient still meets inclusion/exclusion criteria
- Randomize after completion of cognitive testing and hypo surveys
- SipNose device teaching

Administer first dose of intranasal insulin in clinic after above testing done, observe patient for 4 hours (only if randomized to intranasal insulin for treatment period 1).
The following procedures will be performed after the first dose is administered:

- Teach daily diary (or how to document within the Dexcom G6 app that symptomatic hypoglycemia is commencing)
- Teach glucagon and hypoglycemia management
- Teach CGM & apps (Including G6, Clarity, Follow apps); provide user guide materials
- Download participant's SMBG (if used) and/or CGM, and review for hypoglycemia and overall glucose control
- Provider to see patient at this visit for visit assessment review
- Subject instructed to call if any hypoglycemia occurs before the next day phone call
- Subject instructed to return devices (used and unused) to study staff.

7.1.3 Phone Visit 3: Safety Check (visit 2 +1 day)

The following procedures will be performed at this visit:

- Study staff phone call to assess IN insulin compliance and AEs/SAEs
- Reinforce SipNose device training
- Reinforce daily diary training
- Review CGM data in Clarity

7.1.4 Phone Visit 4: Safety Check (Day 6 +/- 2 days)

The following procedures will be performed at this visit:

- Study staff phone call to assess IN insulin compliance and AEs/SAEs
- Reinforce SipNose device training
- Reinforce daily diary training
- Review CGM data in Clarity

7.1.5 Visit 5: End of Treatment Period 1 (Day 17 +/- 3 days)

The following procedures will be performed at this visit:

- Safety check and assess AEs/SAEs
- Gold score (for data collection purposes only)
- Medication review for any changes since screening
- VS (blood pressure/pulse)
- MNSI survey
- Hypo surveys
- Document BG values before and after cognitive testing
- Cognitive tests (study drug must have been taken prior to testing)
- Review daily diary
- Evaluate CGM for adequate data collected
- Inventory returned devices
- Download SMBG for safety, if used
- Download and review CGM data
- Provider to see patient at this visit for visit assessment review
- In the event the study site is recommending no non-essential in person clinic contacts (such as during the COVID-19 pandemic), this visit may be done remotely and study staff will obtain as much of the above procedural information as possible. Procedures such as MNSI survey and VS may not be able to be done.

7.1.6 Phone Visit 6: Safety Check 1 Week into the 2 Week Washout (Day 24 +/-2 days)

The following procedures will be performed at this visit:

- Study staff phone call to assess for AEs/SEs

7.1.7 Visit 7: Crossover/Start of Period Treatment 2 (Day 31 +/- 2 days)

The following procedures will be performed at this visit:

- Safety check and assess AEs/SAEs
- Medication review for any changes since screening

- Vital signs (blood pressure/pulse)

Administer first dose of intranasal insulin in clinic after above testing done, observe patient for 4 hours (only if randomized to intranasal insulin for treatment period 2).
The following procedures will be performed after the first dose is administered:

- SipNose device teaching
- Teach daily diary (or how to document within the G6 app that symptomatic hypoglycemia is commencing)
- Teach glucagon and hypoglycemia management
- Download SMBG for safety (if used)
- CGM placed
- Teach CGM & apps (Including G6, Clarity, Follow apps); provide user guide materials
- Provider to see patient at this visit for visit assessment review
- Subject instructed to call if any hypoglycemia before phone call occurs the next day
- In the event the study site is recommending no non-essential in person clinic contacts (such as during the COVID-19 pandemic), this visit may be done remotely and study staff will obtain as much of the above procedural information as possible, IF the participant is beginning the saline phase of the study only (not for insulin start). Distribution of supplies may be done remotely as well. If the participant is to begin the intranasal insulin part of the study, then the washout will be extended until a time when non-essential clinic visits at the site are permitted.

7.1.8 Phone Visit 8: Safety Check (visit 7 + 1 day)

The following procedures will be performed at this visit:

- Study staff phone call to assess IN insulin compliance and AEs/SAEs
- Reinforce SipNose device training
- Reinforce daily diary training
- Review CGM data in Clarity

7.1.9 Phone Visit 9: Safety Check (Day 37 +/- 2 days)

The following procedures will be performed at this visit:

- Study staff phone call to assess IN insulin compliance and AEs/SAEs
- Reinforce SipNose device training
- Reinforce daily diary training
- Review CGM data in Clarity

7.1.10 Visit 10: End of Treatment Period 2 (Day 48 +/- 3 days

The following procedures will be performed at this visit:

- Safety check and assess AEs/SAEs
- Gold score
- Medication review for any changes since screening
- VS (blood pressure/pulse)
- Height/weight/BMI
- MNSI survey
- Hypo surveys (Clarke, HypoAQ)
- Document BG values before and after cognitive testing
- Cognitive tests (study drug must have been taken prior to testing) (RBANS, Trail-making)
- Smell test (12-item UPSIT test versions A & B)
- Review daily diary
- Download SMBG for safety (if used)
- Evaluate CGM for adequate data collected

- Inventory returned devices
- Review CGM data in Clarity
- Provider to see patient at this visit for visit assessment review
- In the event the study site is recommending no non-essential in person clinic contacts (such as during the COVID-19 pandemic), this visit may be done remotely and study staff will obtain as much of the above procedural information as possible. MSNI, VS, height/weight/BMI may not be able to be obtained. Supplies may need to be returned remotely.
- Study visits 10 and 11 may be combined into 1 visit to conclude the patient's participation in the study, with as many of the data points for both of these visits being obtained at the same visit. Any assessments that are able to be done remotely (such as assessment of AE/SAEs) may be done remotely by phone.

7.1.11 Visit 11: Safety Visit (Day 55 +/- 2 days)

The following procedures will be performed at this visit:

- Safety check and assess AEs/SAEs
- Collect vital signs (blood pressure & pulse)
- Obtain laboratory studies (Na, K, CO2, creat/GFR, AST/ALT, CBC w/ diff) and HbA1c POC
- Perform a standard 12-lead ECG
- C-SSRS questionnaire
- Provider to see patient at this visit for visit assessment review
- In the event the study site is recommending no non-essential in person clinic contacts, this visit may need to be completed as an "essential" visit for safety to document no harm related to the study interventions. Only the VS, laboratory studies and ECG will require in-person evaluation, and the study staff and provider can then follow up with patient by phone to complete the remaining visit items.
- Study visits 10 and 11 may be combined into 1 visit to conclude the patient's participation in the study, with as many of the data points for both of these visits being obtained at the same visit. Any assessments that are able to be done remotely (such as assessment of AE/SAEs) may be done remotely by phone. In the event there are active or unresolved AE/SAEs at the combined visit 10/11, a separate safety phone call 1 week later will be conducted by the study team to assess these AE/SAEs and address as appropriate per investigator.

7.2 Early Withdrawal

If subject withdraws from the study after the screening visit, but before visit 2, no further evaluations are necessary. If subject withdraws from the study after visit 2, all safety assessments will be performed (see section 7.3).

7.3 Safety

For all safety assessments described below, any clinically significant change will be recorded as an AE or SAE (see section 11).

7.3.1 Vital Signs

Vital signs will be recorded at all clinic visits. For within subject consistency, brachial artery pressure will be obtained in the routine fashion. Weight/height/BMI will be recorded at screening and at end of study treatment period 2.

7.3.2 ECG

A standard 12-lead ECG will be performed on subjects at screening and at visit 11 (safety visit).

7.3.3 Laboratory Samples

Laboratory tests will be done at screening and at visit 11 (safety visit).

7.4 Early Withdrawal

If subject withdraws from the study after the screening visit, but before visit 2, no further evaluations are necessary. If subject withdraws from the study after visit 2, all safety assessments will be performed (see section 7.3).

7.5 Safety

For all safety assessments described below, any clinically significant change will be recorded as an AE or SAE (see section 11).

7.5.1 Vital Signs

Vital signs will be recorded at all clinic visits. For within subject consistency, brachial artery pressure will be obtained in the routine fashion. Weight/height/BMI will be recorded at screening and at end of study treatment period 2.

7.5.2 ECG

A standard 12-lead ECG will be performed on subjects at screening and at visit 11 (safety visit).

7.5.3 Laboratory Samples

Laboratory tests will be done at screening and at visit 11 (safety visit).

7.6 Neuropsychological Assessment

7.6.1 Columbia-Suicide Severity Scale (C-SSRS)

The Columbia–Suicide Severity Rating Scale (C-SSRS) was designed to quantify the severity of suicidal ideation and behavior [41]. In this study, suicidal ideation and behavior will be prospectively assessed using the C-SSRS. The C-SSRS will be administered by trained raters at specified time points, as indicated in table 1 as well as when clinically indicated. Any subjects demonstrating evidence of suicidality will prompt immediate consultation with the site's on-call psychiatrist for assistance with decision-making and potential referral to behavioral health services.

7.6.2 Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The RBANS is a brief, individually administered neurocognitive battery measuring immediate and delayed memory, attention, language, and visuospatial skills.[42] The RBANS Update is a "pencil-and-paper" test and requires only a stimulus booklet and record form for administration and scoring. It is specially designed for repeat evaluations and includes alternate forms to control for practice effects related to content. The measure is broadly used for clinical diagnostic purposes and has also been increasingly employed as an endpoint in clinical trial investigations of medications believed to impact neurocognitive status. Three versions (A, B, and C) will be used for this study and randomized to visit for participants.

7.6.3 Trail Making Test (TMT) Parts A & B

The TMT Parts A & B is a brief paper and pencil measure of attention, processing speed, and mental flexibility (or set-shifting). The test has been well validated for use with a wide range of subject populations, is easy to administer, and has been shown to be very sensitive in detecting subtle changes in central nervous system compromise and specifically changes in frontal lobe or "executive" functions. Part A requires subjects to connect a series of numbered dots in order as quickly as possible. Part B requires subjects to connect numbered and lettered dots as quickly as possible by alternating back and forth between the two without making mistakes. Scores include both time to completion and various error types.

8 Investigational Product(s)

8.1 Description of Investigational Product

The study will utilize the following investigational products:

- Regular insulin (Humulin-R), intranasal route
- SipNose Intranasal Device (Sections 2, 5 and Investigator brochure)

8.2 Handling and Storage

The study drug (insulin or saline) will be given to subject after training for administration.

Subjects will be instructed to keep unopened and opened study drug at room temperature according to label recommendations.

The study drugs will be kept at study site per label recommendations and institutional Standard Operational Policy. The unopened regular insulin will be stored in the refrigerator, and the saline stored at room temperature. The refrigerator and storage room used will be connected to the centralized temperature monitoring system, Temp Trak, which monitors

temperatures 24/7 and alerts site staff when out of range. Site staff will review and document temperature in Temp Trak once daily each day department is open. Study staff will be responsible for safeguarding and maintaining the master log.

8.3 Treatment Assignment

Randomization to initial drug treatment will use the permuted block method. Investigators will receive sealed envelopes containing sequence of insulin and saline for each group.

8.4 Packaging and Labeling

All study drug will be labeled according to the following specifications:

- Protocol identifier/IRB approval/account/study number.
- Participant ID # or name.
- "Store at room temperature."
- "Last day to use" date."
- "Caution: New Drug--Limited by Federal (or United States) law to investigational use".
- Study contact # 952-993-3123

8.5 Occupational Safety:

No known significant safety risks exist to site personnel in direct or indirect contact with the study drug.

9 Concomitant Medications and Non-Drug Therapies

9.1 Permitted Medications

Any medication not listed in list of Prohibited Medications (Table 3) will be permitted during this study. A record will be kept by site staff detailing doses and indication of any concomitant medications used by subjects.

9.2 Prohibited Medications

Subjects taking prohibited medications at time of screening will not be allowed to participate in study, unless such treatment is discontinued at least 30 days prior to screening. These medications include opiates, benzodiazepines, and barbiturates. Randomized subjects taking a prohibited medication (episodic or PRN) will be considered for study participation on an individual basis.

Table 3: Prohibited Medications

Barbiturates
Benzodiazepines
Opiates
Any non-insulin therapy to treat diabetes mellitus

10 Subject Completion, Compensation and Withdrawal

10.1 Subject Completion

Subjects completing all 11 study visits (including the 5 phone visits) will be considered to have completed study.

10.2 Subject Compensation

Subjects will be paid $50 for the completion of each of the 6 in-clinic visits, up to $300 for the entire study.

10.3 Subject Withdrawal

Subject may withdraw from study at any time for any reason without penalty or be terminated from the study by the clinical investigator (see provisions for termination by study team.) Investigational team will document the reason(s) for withdrawal. In the event a subject chooses to withdraw from study before Visit 3, the safety procedures described in Section 7.3 will be performed ideally within 3 days following subject's decision to withdraw. For all subjects who

withdraw, all final safety assessments will be collected regardless of time elapsed since previous visit. In addition to the termination visit, subjects who withdraw early will be contacted within 7 days by study staff via telephone to assess development of new and/or ongoing AEs and concomitant medications.

Subject's participation may be terminated at the discretion of the investigator. Individuals may be withdrawn for the following reasons:

- Clinically significant adverse events
- Lost to follow-up
- Protocol violations
- Inability to tolerate study medication
- Other

11 Adverse Events (AE) and Serious Adverse Events (SAE)

11.1 Definition of AE

An adverse event is any symptom, sign, illness or experience which develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- Results in study withdrawal
- Is associated with clinical signs or symptoms
- Leads to treatment or to further diagnostic tests
- Is considered by the investigator to be of clinical significance

11.2 Definition of SAE

Adverse events are classified as either serious or non-serious. A serious adverse event is any event that results in:

- Severe hypoglycemia
- Severe hyperglycemia
- Diabetic ketoacidosis
- Death
- Life-threatening situation
- Hospitalization or prolongation of hospitalization
- Disability or incapacitation
- Congenital abnormality or birth defect
- Other events determined by investigator to be medically significant in which subject's well-being is jeopardized

11.3 Relationship of Adverse Event to Study Treatment

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 treatment.

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

- **Unrelated:** The AE is clearly not related to a study drug/device and a likely alternative etiology exists such as an underlying disease, environmental or toxic factors or other therapy.
- **Unlikely Related:** The AE does not follow a reasonable temporal sequence during or after use of study drug/device and a more likely alternative etiology exists such as an underlying disease, environmental or toxic factors, or other therapy.
- **Possibly Related:** The AE occurred in a reasonable time during or after use of study drug/device; but could be related to another factor such as an underlying disease, environmental or toxic factors, or other therapy; and there is a possible, though weak, scientific basis for establishing a causal association between the AE and the study drug/device.

- **Probably Related:** The AE occurred in a reasonable time during or after use of study drug/device; is unlikely to be related to another factor such as an underlying disease, environmental or toxic factors, or other therapy; and there is a plausible, though not strong, scientific basis for establishing a causal association between the AE and the study drug/device.
- **Definitely Related:** The AE occurred in a reasonable time during or after use of study drug/device; cannot be explained by another factor such as an underlying disease, environmental or toxic factors, or therapy; and there is a strong scientific basis for establishing a causal association between the AE and the study drug/device.
- **Not Assessable:** Causality of an adverse event cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

11.4 Intensity of Adverse Event

The intensity of an adverse event will be rated as (1) mild, (2) moderate, or (3) severe. 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.

- MILD: Usually transient, requires no special treatment, and does not interfere with the participant's daily activities.
- MODERATE: Usually causes a low level of inconvenience or concern to the participant and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures.
- SEVERE: Interrupts a participant's usual daily activities and generally requires systemic drug therapy or other treatment.

11.5 Timing of Event Detection and Reporting

Upon consenting, a subject is considered to be a subject in the study, and until that person either withdraws or completes study, AEs and SAEs will be recorded. The investigational team will promptly report any AE/SAE as required per federal guidelines. Each principal investigator is responsible for reporting serious study-related adverse events and abiding by any other reporting requirements specific to his/her Institutional Review Board or Ethics Committee.

11.6 Glycemic Events

11.6.1 Hypoglycemic Events

Hypoglycemia not associated with an Adverse Device Effect is only reportable as a serious adverse event (SAE) when the following definition for severe hypoglycemia is met: 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 loss of consciousness. These episodes may be associated with sufficient neuroglycopenia to induce seizure or loss of consciousness. 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. Non-severe hypoglycemia will not be reported as an AE (but will be captured by the data analysis).

11.6.2 Hyperglycemic/Ketotic Events

Hyperglycemia/ketoic events not associated with an Adverse Device Effect is only reportable as a serious adverse event (SAE) when either of the following criteria are met:

1. Severe hyperglycemia:
    - Blood glucose >300 mg/dL, urine ketones moderate or large (or blood ketone >1.5 mmol/L), or accompanied by symptoms of nausea, vomiting or abdominal pain

2. Hyperglycemic events are additionally classified as DKA if all of the following are present:
    - Blood glucose >250 mg/dL

- Symptoms such as polyuria, polydipsia, nausea, vomiting or abdominal pain
- 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 mEq/L
- Treatment provided in a health care facility

Non-severe hyperglycemia will not be reported as an AE as mild hyperglycemia is an expected occurrence in the study population.

11.7 Clinical Laboratory Abnormalities & Other Abnormal Assessments as AEs & SAEs

Any new abnormal vital sign, examination, or laboratory finding judged clinically significant by the investigator will be documented as an AE or SAE, if meeting the definitions for such. Abnormal lab findings or other abnormal assessments associated with the disease under study will not be considered AEs or SAEs unless more severe than expected, as judged by the investigator.

11.8 SipNose Failures and Malfunctions

All failures and malfunctions of the device will be recorded. All performance issues and malfunctions will be reported to SipNose during the study and in the clinical results (e.g., final report).

11.9 Pregnancy Reporting

If pregnancy occurs, the participant will be discontinued from the study. Urine pregnancy test may be done at any time if pregnancy is suspected. The occurrence of pregnancy will be reported on an AE Form.

12 Data Analysis and Statistical Considerations

12.1 Analysis Overview

Discrete variables will be summarized using frequencies and percentages, while continuous variables will be summarized by mean and standard deviation or median and interquartile range, as appropriate. Baseline variables will be compared between groups to ensure randomization achieved a balanced design. Distribution of the outcome variables will be assessed to determine if the planned analyses are appropriate. All analyses will be performed on an intention-to-treat basis. Missing data will be considered to be missing at random and those subjects will be excluded from analysis. If a large proportion of data is missing (>10%), we will compare baseline characteristics between subjects with missing and non-missing data. No adjustment for multiple comparisons will be done. All statistical analyses will be performed in SAS Version 9.4.

12.2 Statistical Analysis of Primary Outcomes

The primary objective of this study is to assess non-inferiority of dangerous hypoglycemia with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia. A two-sided 95% confidence interval will be reported for the difference between the two treatment periods based on a least squares model. A non-inferiority margin

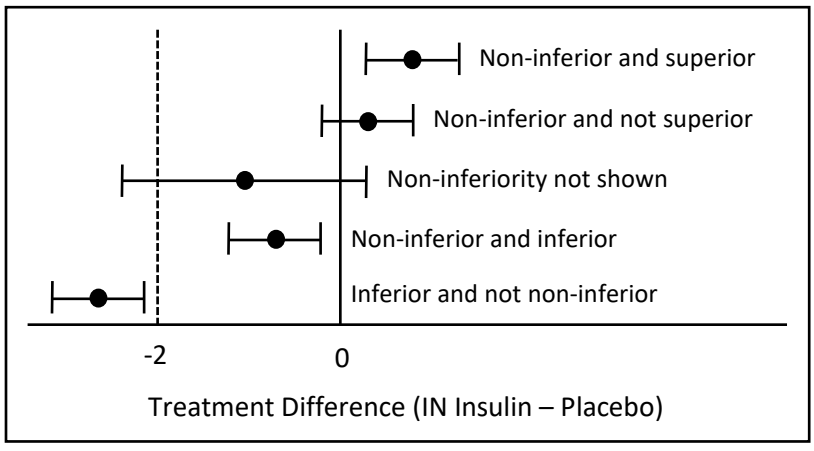

Figure 14: Possible outcomes of non-inferiority analysis

of 2% (approximately 30 minutes per day) will be used (see Section 12.4 for justification). The null hypothesis of inequality (difference is greater than or equal to 2%) is rejected in favor of the alternative hypothesis of equality (difference is less than margin) if the lower limit of the confidence interval is greater than -2%.

Since non-inferiority is typically framed in terms of a one-sided test, it is worth noting that the left half of a two-sided test at alpha = 0.05 gives the same rejection region as a one-sided test at alpha = 0.025. Therefore, reporting a two-sided 95% confidence interval will provide flexibility to test for inferiority if non-inferiority cannot be declared while maintaining the overall type 1 error rate at 5%. Figure 14 shows the inference to be drawn for various scenarios of the two-sided 95% confidence interval.

12.3 Statistical Analysis of Secondary and Exploratory Outcomes

The secondary and exploratory objectives are purely descriptive. No inferential statistics will be performed. All measures of overall glycemic control, hypoglycemia awareness, and safety endpoints will be reported using the appropriate summary statistics. Values will be reported from baseline and conclusion of each dosing period.

12.4 Power Calculation

The study is powered on our primary objective of determining non-inferiority of dangerous hypoglycemia with administration of intranasal insulin in T1D participants with intact awareness of hypoglycemia. The non-inferiority margin of 2% is based solely on clinical judgement. A total sample size of 10 (5 per sequence arm) achieves 79.8% power to detect non-inferiority assuming a true mean difference of 0 and a square root of within mean square error of 1.4%. This power calculation was conducted using the PASS v16 module for non-inferiority test for the difference between two means in a 2x2 cross-over design.

13 Study Conduct Considerations

13.1 Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with GCP. Subject privacy requirements will also be observed as well as the fundamental concepts of the Declaration of Helsinki (e.g. IRB approval of the study, obtaining informed consent from all subjects, and meeting reporting requirements).

13.2 Data Safety Monitoring Board

The DSMB will be an independent group who are not participating in the trial and have no direct affiliation with HealthPartners Center for Memory and Aging or IDC. They will serve as an advisory panel to HealthPartners Center for Memory and Aging/IDC. The DSMB will be comprised of three appropriately qualified individuals. The DSMB Charter will be established prior to initiation of the study. The DSMB responsibilities include but are not limited to the following:

- Monitoring the study for compliance to the protocol.
- Stopping the study if the rate of SAE's raises safety concerns. The details will be specified in the DSMB charter.

The DSMB will convene following the study completion of the 10 subjects in phase 1, or earlier in the case of SAEs. During the course of the trial, the DSMB will review accumulating safety data to monitor for incidence of trends that would warrant termination of the trial. The frequency of the DSMB meetings, responsibilities, membership, and procedures will be documented in the DSMB charter.

13.3 Quality Assurance

In the event of a regulatory agency audit or inspection, site will allow the auditor/inspector access to all records documented and facilities utilized in conducting the study. Site will also make accommodations (e.g. time, schedule) to discuss findings, concerns, and questions with auditor/inspector.

13.4 Study Closure

Upon completion of all subject visits, data entry and analysis, investigator will inform local IRB of study closure.

13.5 Records Retention

All site records will be maintained and stored in a safe and secure location for a minimum of 15 years post study completion.

13.6 Provision of Study Results and Information to Investigators

Study results will be made available by the study statistician once analysis is complete.

13.7 Data Management

Data collection/reporting tools will be developed internally (i.e. CRFs and source documents). Data collected and stored electronically will remain confidential and secure (e.g. secured server, encrypted data, password protected file)

13.8 Device and Drug Accountability

A Device Tracking Log will be maintained at the investigational site. SipNose devices will be recorded on the log upon delivery to the investigational site and will be stored in a secured area. The Device Tracking Log will be updated as each device is delivered, dispensed, returned and the reason for the return. Batch numbers, expiration date and model number of devices delivered to the site will also be recorded. Similarly, a Drug Tracking Log will be maintained at the investigational site. Vials of insulin will be secured in the storage area, and stored per manufacturer label.

14 References

1. Feltbower RG, Bodansky HJ, Patterson CC, et al. Acute complications and drug misuse are important causes of death for children and young adults with type 1 diabetes: results from the Yorkshire Register of diabetes in children and young adults. *Diabetes care.* 2008;31(5):922-926.
2. Frier BM. Hypoglycaemia in diabetes mellitus: epidemiology and clinical implications. *Nature Reviews Endocrinology.* 2014;10(12):711.
3. Weinstock RS, Xing D, Maahs DM, et al. Severe hypoglycemia and diabetic ketoacidosis in adults with type 1 diabetes: results from the T1D Exchange clinic registry. *The Journal of Clinical Endocrinology & Metabolism.* 2013;98(8):3411-3419.
4. Czyzewska K, Czerniawska E, Szadkowska A. Prevalence of hypoglycemia unawareness in patients with type 1 diabetes. *Pediatr Diabet.* 2012;13:77.
5. Cryer PE. The barrier of hypoglycemia in diabetes. *Diabetes.* 2008;57(12):3169-3176.
6. Weinstock RS, DuBose SN, Bergenstal RM, et al. Risk factors associated with severe hypoglycemia in older adults with type 1 diabetes. *Diabetes Care.* 2016;39(4):603-610.
7. Marathe PH, Gao HX, Close KL. American D iabetes A ssociation S tandards of M edical C are in D iabetes 2017. *Journal of diabetes.* 2017;9(4):320-324.
8. DuBose SN, Weinstock RS, Beck RW, et al. Hypoglycemia in older adults with type 1 diabetes. *Diabetes technology & therapeutics.* 2016;18(12):765-771.
9. Choudhary P, Ramasamy S, Green L, et al. Real-time continuous glucose monitoring significantly reduces severe hypoglycemia in hypoglycemia-unaware patients with type 1 diabetes. *Diabetes Care.* 2013;36(12):4160-4162.
10. Pickup JC, Freeman SC, Sutton AJ. Glycaemic control in type 1 diabetes during real time continuous glucose monitoring compared with self monitoring of blood glucose: meta-analysis of randomised controlled trials using individual patient data. *Bmj.* 2011;343:d3805.
11. van Beers CA, DeVries JH, Kleijer SJ, et al. Continuous glucose monitoring for patients with type 1 diabetes and impaired awareness of hypoglycaemia (IN CONTROL): a randomised, open-label, crossover trial. *The lancet Diabetes & endocrinology.* 2016;4(11):893-902.
12. Bergenstal RM, Garg S, Weinzimer SA, et al. Safety of a hybrid closed-loop insulin delivery system in patients with type 1 diabetes. *Jama.* 2016;316(13):1407-1408.
13. Foster NC, Miller KM, Tamborlane WV, Bergenstal RM, Beck RW. Continuous glucose monitoring in patients with type 1 diabetes using insulin injections. *Diabetes Care.* 2016;39(6):e81.
14. Martín-Timón I, del Cañizo-Gómez FJ. Mechanisms of hypoglycemia unawareness and implications in diabetic patients. *World journal of diabetes.* 2015;6(7):912.

15. Mangia S, Tesfaye N, De Martino F, et al. Hypoglycemia-induced increases in thalamic cerebral blood flow are blunted in subjects with type 1 diabetes and hypoglycemia unawareness. *Journal of Cerebral Blood Flow & Metabolism.* 2012;32(11):2084-2090.
16. Puente EC, Silverstein J, Bree AJ, et al. Recurrent moderate hypoglycemia ameliorates brain damage and cognitive dysfunction induced by severe hypoglycemia. *Diabetes.* 2010;59(4):1055-1062.
17. Diggs-Andrews KA, Zhang X, Song Z, Daphna-Iken D, Routh VH, Fisher SJ. Brain insulin action regulates hypothalamic glucose sensing and the counterregulatory response to hypoglycemia. *Diabetes.* 2010;59(9):2271-2280.
18. Rosario W, Singh I, Wautlet A, et al. The brain–to–pancreatic islet neuronal map reveals differential glucose regulation from distinct hypothalamic regions. *Diabetes.* 2016;65(9):2711-2723.
19. Luchsinger JA, Tang M-X, Shea S, Mayeux R. Hyperinsulinemia and risk of Alzheimer disease. *Neurology.* 2004;63(7):1187-1192.
20. Davis WA, Zilkens RR, Starkstein SE, Davis TM, Bruce DG. Dementia onset, incidence and risk in type 2 diabetes: a matched cohort study with the Fremantle Diabetes Study Phase I. *Diabetologia.* 2017;60(1):89-97.
21. Freiherr J, Hallschmid M, Frey WH, et al. Intranasal insulin as a treatment for Alzheimer's disease: a review of basic research and clinical evidence. *CNS drugs.* 2013;27(7):505-514.
22. De Felice FG, Vieira MN, Bomfim TR, et al. Protection of synapses against Alzheimer's-linked toxins: insulin signaling prevents the pathogenic binding of Aβ oligomers. *Proceedings of the National Academy of Sciences.* 2009;106(6):1971-1976.
23. Vandal M, White PJ, Tremblay C, et al. Insulin reverses the high-fat diet–induced increase in brain Aβ and improves memory in an animal model of Alzheimer disease. *Diabetes.* 2014;63(12):4291-4301.
24. Schiöth HB, Craft S, Brooks SJ, Frey WH, Benedict C. Brain insulin signaling and Alzheimer's disease: current evidence and future directions. *Molecular neurobiology.* 2012;46(1):4-10.
25. Frey WH. Intranasal insulin to treat and protect against posttraumatic stress disorder. *The Journal of nervous and mental disease.* 2013;201(7):638-639.
26. Schmid V, Kullmann S, Gfrörer W, et al. Safety of intranasal human insulin: A review. *Diabetes, Obesity and Metabolism.* 2018;20(7):1563-1577.
27. Bedse G, Di Domenico F, Serviddio G, Cassano T. Aberrant insulin signaling in Alzheimer's disease: current knowledge. *Frontiers in neuroscience.* 2015;9:204.
28. Williams G. Intranasal drug delivery bypasses the blood-brain barrier. *Neurol Rev.* 2016;24(4):40-41.
29. Novak V, Milberg W, Hao Y, et al. Enhancement of vasoreactivity and cognition by intranasal insulin in type 2 diabetes. *Diabetes care.* 2014;37(3):751-759.
30. Li Y, Rinne JO, Mosconi L, et al. Regional analysis of FDG and PIB-PET images in normal aging, mild cognitive impairment, and Alzheimer's disease. *European journal of nuclear medicine and molecular imaging.* 2008;35(12):2169-2181.
31. Craft S, Baker LD, Montine TJ, et al. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. *Archives of neurology.* 2012;69(1):29-38.
32. Jauch-Chara K, Friedrich A, Rezmer M, et al. Intranasal insulin suppresses food intake via enhancement of brain energy levels in humans. *Diabetes.* 2012;61(9):2261-2268.
33. Bohringer A, Schwabe L, Richter S, Schachinger H. Intranasal insulin attenuates the hypothalamic–pituitary–adrenal axis response to psychosocial stress. *Psychoneuroendocrinology.* 2008;33(10):1394-1400.
34. Reger M, Watson G, Frey Ii W, et al. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. *Neurobiology of aging.* 2006;27(3):451-458.
35. Reger M, Watson G, Green P, et al. Intranasal insulin improves cognition and modulates β-amyloid in early AD. *Neurology.* 2008;70(6):440-448.
36. Rosenbloom MH, Barclay TR, Pyle M, et al. A single-dose pilot trial of intranasal rapid-acting insulin in apolipoprotein E4 carriers with mild–moderate Alzheimer's disease. *CNS drugs.* 2014;28(12):1185-1189.
37. Djupesland PG, Skretting A. Nasal deposition and clearance in man: comparison of a bidirectional powder device and a traditional liquid spray pump. *Journal of aerosol medicine and pulmonary drug delivery.* 2012;25(5):280-289.
38. Sastri VR. *Plastics in medical devices: properties, requirements, and applications.* Second ed: William Andrew; 2013.

39. Grajower MM, Fraser CG, Holcombe JH, et al. How long should insulin be used once a vial is started? *Diabetes Care.* 2003;26(9):2665-2669.
40. Guo C, Ye W, Kauffman J, Doub WH. Evaluation of Impaction Force of Nasal Sprays and Metered-Dose Inhalers Using the Texture Analyser☆. *Journal of pharmaceutical sciences.* 2009;98(8):2799-2806.
41. Posner K BG, Stanley B, Brent DA, Yershova KV, Oquendo MA, Currier GW, Melvin GA, Greenhill L, Shen S, Mann JJ. The Columbia-Suicide Rating Scale: Initial Validity and Internal Consistency Findings from Three Mulitsite Studies with Adolescents and Adults. *Am J Psychiatry.* 2011;168:1266-1277.
42. Randolph C, Tierney MC, Mohr E, Chase TN. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. *Journal of clinical and experimental neuropsychology.* 1998;20(3):310-319.

15 Appendices

15.1 Appendix 1: SipNose Investigator Brochure

15.2 Appendix 2: SipNose Drug-device Compatibility Testing

15.3 Appendix 3: Insulin Full Prescribing Information

15.4 Appendix 4: Questionnaires and Exam Batteries

15.5 Appendix 5: Visit Schedule - Phase 1