Phase 1, Open Label Dose-Finding Study of Intranasal Insulin in Healthy Participants

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Statement of Compliance

The trial will be conducted in accordance with applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the funding agency and documented approval from the Institutional Review Board (IRB), and the Investigational New Drug (IND) sponsor, if applicable, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training. The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form(s) must be obtained before any participant is consented. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form(s) will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Phase 1, Open Label Dose-Finding Study of Intranasal Insulin in Healthy Participants
Study Description:	The current study will evaluate the tolerability of intranasal insulin in 24 healthy adult volunteers after being serially exposed to escalating doses over 5 visits with a 1-week washout period in between each visit. Regular insulin (Humulin R U500, Eli Lilly, Indianapolis IN) at a concentration of 500 U/mL will be prepared in doses of up to 2 mL containing between 0 – 1000 U. The ≤2 mL doses will be administered intranasally using a 5 mL syringe divided into ≤1 mL per nostril. Intranasal administration will be performed with the participants supine with the neck extended to mimic the position of comatose out-of-hospital cardiac arrest (OHCA) patients. A total of 11 possible doses will be tested, ranging from 0 to 1000 U intranasal insulin. For every 6 participants, iterations of a Bayesian model will be performed to associate the administered dose with the frequency of dose-limiting hypoglycemia or other adverse events. The model will adaptively reallocate the dose increments for the next 6 participants. To provide more precise and reproducible estimates of dose-limiting toxicity, the trial will modify the size of dose increments based on the developing model to allocate more administrations to areas of the model with greatest uncertainty, or to higher doses in the absence of dose-limiting hypoglycemia. This will provide more observations and the greatest information density at doses of greatest interest that are most relevant to the determination of maximally tolerated dose. Safety and tolerability will be assessed for approximately 4 hours after each insulin administration.
Objectives:	Determine the maximum tolerated dose of intranasal insulin in healthy participants
Endpoints:	Primary: The primary safety outcome will be severe hypoglycemia as defined by blood glucose <45mg/dL. Secondary: Secondary endpoints will include the change in blood glucose, insulin, and C-peptide levels for up to 4 hours after drug administration to evaluate for any systemic absorption of intranasal insulin.
Study Population:	24 healthy subjects, male or female, 18 years of age or older
Phase:	I
Study Site:	Single site, Michigan Medicine, University of Michigan

Description of Study Intervention:	HUMULIN R U-500 (insulin human injection, Eli Lily, NDC 0002-8501-01) to be given intranasally at differing doses depending on study day. Sterile diluent to prepare the zero insulin dose and lower concentrations of intranasal insulin will also be acquired through Eli Lily (NDC 0002-0800). A total of 11 possible doses will be tested, ranging from 0 to 1000 U insulin. Each volunteer will come for 5 visits.
Study Duration:	One year
Participant Duration:	Up to 60 days from the day of screening to last visit.

1.2 SCHEMA

Figure 1.2.1. Study visit schedule

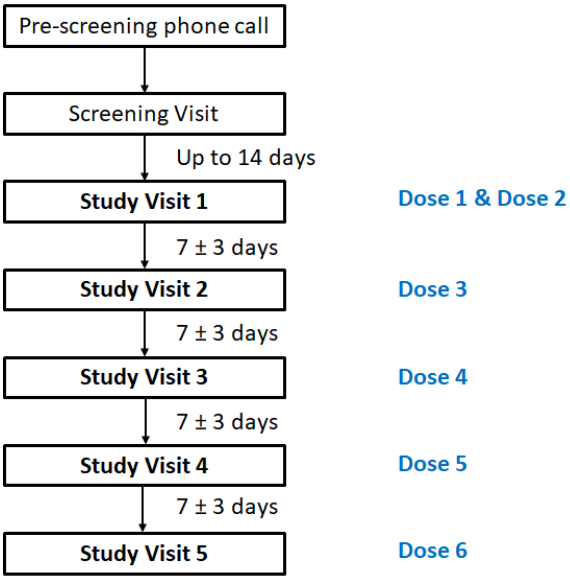
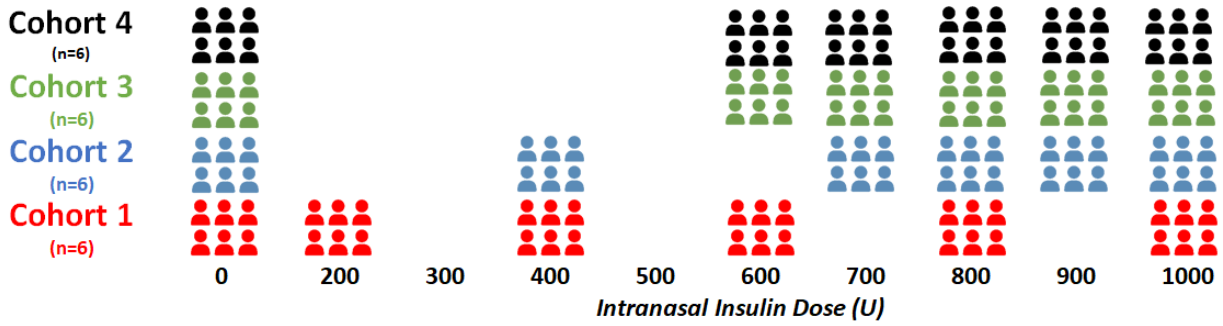


Figure 1.2.2. Intranasal insulin dose allocation if no study participants **experiences severe hypoglycemia**



1.3 SCHEDULE OF ACTIVITIES (SOA)

Schedule of Events table						
Study Visits	**Screening**	**1**	**2**	**3**	**4**	**5**
Study Day(s)	-28	0	7+/-3	14+/-3	21+/-3	28+/-3
Provide study overview and participant responsibilities	X					
Informed consent	X					
Medical history and inclusion/exclusion criteria review	X					
Previous and concomitant medications	X	X	X	X	X	X
Physical examination	X					
Vital signs[1]	X	X	X	X	X	X
Urine pregnancy test (female subjects of child bearing potential)	X	X	X	X	X	X
Insertion of a short term intravenous catheter		X	X	X	X	X
Study drug administration[2]		X	X	X	X	X
Clinical laboratory screening labs[3]	X					
POC serum glucose[4]		X	X	X	X	X
Serum C-Peptide[5]		X	X	X	X	X
Serum Insulin[5]		X	X	X	X	X
Adverse event reporting		X	X	X	X	X

[1] Vital signs include blood pressure, pulse rate, respiration rate, and temperature at supine position.

[2] Study drug will be administered intranasally.

[3] Clinical laboratory screening labs include clinical comprehensive metabolic panel and CBC.

[4] Point-of-care (POC) serum glucose glucometry will be performed at 0, 15, 30, 60, 120, 180, and 240 minutes after each intranasal insulin administration. Sample will be collected +/- 10 minutes of scheduled assessment.

[5] Blood and plasma samples will be collected, aliquoted, and frozen at 0, 15, 30, 60, 120, 180, and 240 minutes after each intranasal insulin administration for blood insulin and C-Peptide measurements by the University of Michigan Pharmacokinetics Core. Sample will be collected +/- 10 minutes of scheduled assessment.

2 INTRODUCTION

2.1 STUDY RATIONALE

Sudden cardiac arrest is responsible for 1 in 5 U.S. deaths (1). Among patients who achieve return of spontaneous circulation after OHCA, less than 1 in 3 survive. Irreversible brain injury is the primary cause of death. Although many pharmacologic therapies are neuroprotective in animal models, none have been successfully translated to clinical cardiac arrest care. A major barrier is the inability to deliver drugs to the brain within the therapeutic window, which is often minutes after cardiac arrest onset. For cardiac arrest occurring outside the hospital, we anticipate this will require bystanders or first responders (police and firefighters) to deliver the therapy in addition to performing cardiopulmonary resuscitation (CPR) and defibrillation. However, the feasibility, safety, and effectiveness of this approach must first be explored.

There is a substantial body of pre-clinical data demonstrating a potential neuroprotective effect of intranasal insulin in varying models of acute ischemic and chronic neurodegenerative diseases in which mechanisms of oxidative stress are implicated (2,3). Human clinical data evaluating neuroprotection by insulin is mostly limited to small studies of chronic administration in patients with Alzheimer's disease. A recent systematic review of these data reported on 7 exploratory clinical trials of intranasal insulin in 293 participants (4). These were predominantly safety and feasibility trials, and they found intranasal insulin to be well tolerated and without safety concerns. Furthermore, they individually and collectively showed signals of efficacy on at least some cognitive outcome parameters. There are 8 recruiting trials on ClinicalTrials.gov of intranasal insulin in 500 planned patients with Alzheimer's disease, stroke, cognitive decline in multiple sclerosis, cognitive decline in HIV, and other cognitive disorders. Thus, a well-planned phase 1 study to explore the safety and tolerability of intranasal insulin in healthy participants is necessary prior to studying its neuroprotective effect in OHCA patients.

This phase 1 study will evaluate the safety and tolerability of intranasal regular humulin insulin in healthy volunteers. The establishment of maximum tolerated dose for intranasal insulin in healthy participants will elucidate the doses to explore in future dose-finding OHCA efficacy studies. Understanding the maximum tolerated dose is also important because the proposed community use of the intervention by bystanders and first responders demands a larger margin of safety than typical drugs, as errors in administration and the potential for misdosing may increase in community use. Therefore, the maximum tolerated dose is needed to estimate the safety margin and therapeutic index of the intervention.

2.2 BACKGROUND

Given the high morbidity rate from sudden cardiac arrest in the U.S. (1), there is a critical need to pursue innovative interventions to improve OHCA survival. Despite modest improvements in the first half of the decade, outcomes have not changed in the past 5 years based on data from the Cardiac Registry to Enhance Survival (5). Similarly, recent reports from the Resuscitation Outcomes Consortium and the Heart Rescue Project show no improvement in survival with good neurologic outcome since 2012 (6, 7).

A novel and disruptive approach is needed to discover, translate, and implement new therapies to improve the outcome of cardiac arrest.

When resuscitation is attempted outside the hospital in the U.S., only 8.5% of patients survive with good neurologic function (8). Sudden cardiac arrest is the most time sensitive human heart condition, and time to treatment is the most critical factor in successful translation of cardiac arrest interventions. In fact, the only therapies during cardiac arrest that are proven to increase survival with good neurologic function are early bystander CPR and early defibrillation, both of which require early, time-sensitive intervention. Moreover, pharmacologic therapies such as epinephrine and antiarrhythmic drugs that are given intravenously or intraosseously by paramedics much later during OHCA (>15-20 min after 911 call) have not improved survival with good neurologic function in double-blinded randomized clinical trials (9,10). These observations coupled with robust pre-clinical mechanistic data support our premise that neuroprotective drug therapies are most effective if given within minutes of brain injury. To do so, it is critical that we design the novel neuroprotective therapies that can be delivered by those who are first on the scene—bystanders and first responders (police, firefighters)—rather than paramedics, who often arrive outside the therapeutic window for maximum neuroprotection.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Anticipated adverse events associated with the use of intranasal insulin may include an allergic reaction, hypoglycemia, nose soreness, nasal side effects (nasal congestion, rhinorrhea, minor bleeding, nasal or pharyngeal stinging, rhinitis, sneezing), headache, dizziness, weakness, aspiration, upper respiratory tract infections, rash, and gastrointestinal symptoms such as nausea or vomiting (4). Other adverse reactions seen with intravenous administration of insulin, including hypokalemia, have not been previously identified with intranasal administration of insulin.

Peripheral intravenous access and collection of blood samples can be associated with presyncope, syncope, bruising, anemia, thrombophlebitis, and in rare instances, infections.

2.3.2 Known Potential Benefits

There are no direct benefits to study participants . The tangible benefit to society is that this study may help bring a neuroprotective agent one step closer to eventual clinical use by first responders to use in OHCA. This can potentially help countless lives.

2.3.3 Assessment of Potential Risks and Benefits

Although hypoglycemia has never been demonstrated with intranasal insulin at lower dose in humans, the potential for this safety concern exists in this study. Mechanisms of insulin transport into the brain through the olfactory epithelium is more efficient than vascular absorption of regular insulin into the systemic circulation, but Kullmann et al have demonstrated small but measurable increases in serum insulin with intranasal administration and have shown these to represent dose-dependent spillage (11).

There is insufficient data to show whether this dose response is linear, and the very small changes in insulin level detected did not manifest as a measurable change in glucose (11). Of note, a minority of the 19 studies in which participants received 160 U of intranasal insulin also reported clinically insignificant but measurable decreases in serum glucose ranging from 0.2 to 0.4 mmol/L. Based on these data, we have developed an initially conservative dose escalation study that has the potential to adapt the magnitude of dose escalation with accumulating data if lower dose tiers demonstrate the same overwhelming safety signals seen in the prior data. Point-of-care (POC) serum glucometry will be performed at 0, 15, 30, 60, 120, 180, and 240 minutes after each intranasal insulin administration. Hypoglycemia as defined by a blood glucose of <45mg/dL will be treated with 50 ml of intravenous D50W and food. The subject will also be monitored until achieving two consecutive measurements of blood glucose > 70mg/dL, measured at 30 minutes apart. If blood glucose falls to ≤70 mg/dL but not <45 mg/dL, the blood glucose will be monitored every 30 minutes until it returns to >70 mg/dL.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Determine the maximum tolerated dose of intranasal insulin in healthy participants	**Primary endpoint:** The primary safety outcome will be severe hypoglycemia as defined by blood glucose <45mg/dL at any post administration glucose check during a study visit.	The American Diabetes Association defined severe hypoglycemia as "severe cognitive impairment requiring external assistance for recovery" without assigning a blood glucose cutoff (12). As such, we chose blood glucose < 45mg/dL as the cutoff for severe hypoglycemia based on prior clinical studies (13).
Secondary		
Characterize the pharmacokinetics and pharmacodynamics (PK-PD) of intranasal insulin in healthy participants	**Secondary endpoints** will include the change in blood glucose, insulin, and C-peptide levels for up to 4 hours after drug administration to evaluate for any systemic absorption of intranasal insulin.	Prior studies have not characterized the PK-PD of conventional insulin formulation doses above 160 U administered intranasally.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a phase 1 study to determine the safety and tolerability of 100 – 1000 U regular short-acting intranasal insulin in 24 healthy adult volunteers. The primary endpoint will be severe hypoglycemia using a numerical cutpoint of blood glucose <45 mg/dL as a binary variable. The secondary endpoints will include the change in blood glucose, serum insulin, and serum C-peptide levels for up to 4 hours after drug administration to evaluate for any systemic absorption of intranasal insulin.

Experiments will be conducted at the Michigan Clinical Research Unit, which offers all the clinical research services necessary to study investigators at Michigan Medicine. Participants will be serially exposed to escalating doses of intranasal regular insulin.

Participants will be asked to participate in 6 administrations over 5 sessions with a 1-week washout interval between each session. The first session will include an initial control administration of 0 U followed by 4 hours of observation, then followed by the first dose-escalation administration and another 4 hours of observation. Subsequent visits will involve a single dose-escalation administration per visit. The starting escalation schedule will use dose increments of 200 units for a schedule over 6 administrations of 0 U, 200 U, 400 U, 600 U, 800 U, and 1000 U. Any subject experiencing a hypoglycemic episode meeting the definition of serum glucose level <45 mg/dL or having any related or possibly related serious adverse event will not be given a subsequent dose escalation.

Adaptive Design: For every 6 participants, iterations of a Bayesian model will be performed to associate the administered dose with the frequency of dose-limiting hypoglycemia or other adverse events. The model will adaptively reallocate the dose increments for the next 6 participants. To provide more precise and reproducible estimates of dose-limiting toxicity, the trial will modify the size of dose increments based on the developing model to allocate more administrations to areas of the model with greatest uncertainty, or to higher doses in the absence of dose-limiting hypoglycemia. This will provide more observations and the greatest information density at doses of greatest interest that are most relevant to the determination of maximally tolerated dose. Safety and tolerability will be assessed for approximately 4 hours after each insulin administration.

Approach: The maximum tolerated dose between 100 – 1000 U of regular short-acting insulin will be determined with an adaptive dose-escalation phase I safety study performed in 24 healthy volunteers. The primary safety outcome will be severe hypoglycemia using a numerical cutpoint of serum glucose <45 mg/dL. Secondary endpoints will include the change in blood glucose, serum insulin, and serum C-peptide levels for up to 4 hours after drug administration to evaluate for any systemic absorption of intranasal insulin.

4.2 Scientific Rationale for Study Design

The most effective cardiac arrest therapies are those delivered within minutes of cardiac arrest onset by bystanders and first responders. By optimizing the time to initiating therapies already proven effective—CPR and defibrillation—and adding safe, effective, and easily administered neuroprotective drug therapy, thousands of lives will be saved each year.

Nose-to-Brain Insulin Delivery: Although the brain is highly vascular, systemic intravenous delivery of neuroprotective therapeutics is problematic, especially for large therapeutic peptides due to the blood-brain barrier. Intravenous administration also creates pragmatic barriers to very early administration in OHCA because vascular access requires skills and equipment not accessible to bystander and most first responders that first attend to these patients, and because blood flow is limited.

The unique physiological properties of direct drug delivery into the brain through novel intranasal pathways offer the potential to address both of these problems. These pathways have been known for years, but are only recently being fully evaluated in clinical trials of neurotherapeutics. These pathways are distinct from the better known use of intranasal administration as a route to drug delivery to the circulation of smaller and lipophilic molecules. Rather, the direct nose-to-brain pathways provide a noninvasive strategy to deliver drugs rapidly across the nasal mucosa directly into the central nervous system (14,15). These direct pathways were first demonstrated in the 1980's and have been confirmed through increasingly elegant methods in recent years. **Figure 1** illustrates the intracellular, intercellular, and perivascular absorption pathways found in the olfactory epithelium. Trigeminal nerve endings and olfactory receptor neurons are interspersed among supporting cells. These convey chemosensory information to the central nervous system but also serve as pathways for direct drug delivery through extracellular or intracellular mechanisms (dashed arrows in **Figure 1**). Drugs can also be transported through the nasal mucosa to the central nervous system by entering perivascular channels (dashed lines surrounding blood vessels in **Figure 1**). After crossing the olfactory epithelium and reaching the lamina propria, drugs enter channels created by olfactory ensheathing cells surrounding the olfactory nerves, where they can access the cerebrospinal fluid and rapidly distribute via bulk flow mechanisms (15).

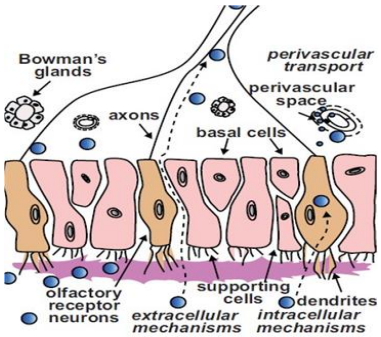


Figure 1: Pathways found in the olfactory epithelium. Figure from a review by Dhuria(4).

Direct confirmation of direct-to-brain drug delivery in the absence of vascular absorption has been demonstrated in humans by delivery of intranasal insulin and tracing subsequent levels in both cerebrospinal fluid and the blood (16). As shown in **Figure 2**, increases in cerebrospinal fluid insulin are seen with nasal administration of insulin but not placebo, whereas concentrations of insulin in the blood are identical for nasal administration of insulin vs. placebo. This indicates that intranasal insulin enters the brain directly without entering the blood, allowing dosing of the target organ without systemic side effects like hypoglycemia. These pathways have also been confirmed by indirect but functional methods. In a study using functional magnetic resonance imaging, intranasal administration of four-fold doses of insulin resulted in a dose-dependent modulation of resting state regional brain activity diffusely in both cortex and midbrain, again without any evidence of clinically-relevant systemic effect (11). This confirms that insulin is present, physiologically relevant, and safe when delivered directly to the brain after nasal administration.

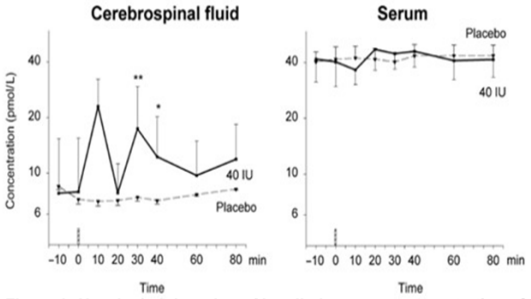


Figure 2: Nasal administration of insulin increases concentration of insulin in cerebrospinal fluid but not in blood. Figure from Born et al.(5) comparing concentration of insulin in the cerebrospinal fluid vs. serum after administration of 40 IU of insulin vs. placebo.

4.3 Justification for Dose

There is substantial clinical safety data for intranasal insulin in human subjects, but there is no data suggesting the maximum tolerated dose. The majority of existing safety data involve doses of intranasal regular insulin in daily repeated administrations from 20 to 160 U. As reviewed recently by Schmid et al, there were no episodes of hypoglycemia in 1,053 subjects in 38 studies in this dose range (17). Although these data are reassuring, there are at least two important reasons to explore higher dose ranges.

The first reason is to understand the upper limits of the dose that might be explored in future dose-finding efficacy studies. Planned animal studies will refine the dose efficacy response in models of OHCA, but existing data demonstrate efficacy and safety in other animal models in doses that scale to human doses ranging from 300 U to 1000 U (depending on the method of scaling and parameters chosen). It is important to determine if the maximum tolerated dose in humans is within or above these doses.

Understanding the maximum tolerated dose is also important because the proposed community use of the intervention by bystanders and first responders demands a larger margin of safety than typical drugs, as errors in administration and the potential for inaccurate dosing may increase in community use. A maximum tolerated dose is needed to estimate the safety margin and therapeutic index of the intervention. The therapeutic dose will be estimated from ongoing animal experiments.

4.4 End of Study Definition

A participant is considered to have completed the study after he/she has completed all study visits as shown in the Schedule of Activities, Section 1.3.

5 Study Population

5.1 Inclusion Criteria

Participants will be healthy adult volunteers who have provided written informed consent. Sex balance will be sought through deliberate equitable selection patterns. There will be no preferential or discriminatory selection of participants on the basis of race.

Each subject must meet the following criteria to be enrolled in this study:

1. Male or female volunteers at least 18 years of age and older, inclusive, in good health based on medical history, physical exam, and routine laboratory testing.
2. Female participants must have negative urine pregnancy test or be surgically sterilized or postmenopausal. Criteria for menopause are surgical menopause (hysterectomy, oophorectomy) or age > 45 years with the absence of menses for greater than 12 months. Tubal ligation with menses within the past 12 months is not considered to be surgical sterilization.
3. Body mass index (BMI) between 18 kg/m^2 and 35 kg/m^2.
4. Willing and able to stay at the clinical research facility as required by the protocol.
5. Willing and able to comply with the investigational nature of the study and able to communicate well with investigators.
6. Ability to comprehend and willing to provide written informed consent in accordance with institutional and regulatory guidelines

5.2 Exclusion Criteria

Participants who meet any of the following criteria will be excluded from the study:

1. Known allergy to insulin.
2. Preexisting diabetes.
3. Current or previous use of diabetes medication or insulin.
4. Any nasal disease or congestion that may interfere with intranasal drug absorption.
5. Baseline hypoglycemia (blood glucose ≤ 65 mg/dL) or hyperglycemia (blood glucose > 200 mg/dL) as evident from the screening labs.
6. Active serious disease, such as liver disease, kidney disease, uncontrolled hypertension, clinically significant hypokalemia, and significant or unstable medical illness
7. Blood donation in excess of 500 mL within 60 days prior to the first dose of study medication.
8. Treated with an investigational drug within 30 days.
9. Individuals with inadequate venous access.

5.3 Lifestyle Considerations

Nothing to eat or drink for at least 2 hours prior to each study visit.
Abstain from alcohol for at least 12 hours before the start of each dosing session.

5.4 Screen Failures

Individuals who do not meet the criteria for participation in this trial (screen failure) will not be rescreened given that this is a healthy participants study.

5.5 Strategies for Recruitment and Retention

The University of Michigan maintains a database of healthy male and female volunteers (*https://www.umhealthresearch.org/*). The database includes approximately 40,000 volunteers. The initial strategy will include a targeting request to volunteers that are likely to meet inclusion criteria based on information stored in this database. Given the study plan to recruit up to 24 volunteers, it is highly likely that this approach will be sufficient. However, other strategies such as the use of Institutional Review Board's (IRB) approved flyers may be posted throughout the University of Michigan, Ann Arbor campus to solicit potential healthy volunteers. The scheduling of procedures will be carefully orchestrated by the study team to improve subject retention.

Participants will be compensated at the local competitive rate for healthy volunteers in phase I investigational drug studies. The full rate will be prorated over a maximum of 6 research visits (one screening visit and 5 study visits). Phone calls, texts, or email reminders will be sent ahead of their appointment dates.

5.6 Considerations related to COVID-19

To reduce risks to participants and study staff related to COVID-19 infection, the study will comply with institutional and governmental precautions and restrictions in place at the time the study is being conducted. In addition, the study will ask that all participants be vaccinated against COVID-19 prior to the first study visit. The study may also perform point-of-care screening for COVID-19 infection using a saliva test at the time of the study visits depending on community prevalence and contemporaneous guidance.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

Regular insulin (Humulin R U500, Eli Lilly, Indianapolis IN) at a concentration of 500 U/mL will be used to create ≤2 mL doses containing between 0 – 1000 U. The ≤2 mL doses will be administered intranasally using a 5 mL syringe divided into ≤1 mL per nostril. Intranasal administration will be performed with the participants supine with the neck extended (a modified Mygind position)(19, 20, 21). to mimic the position of comatose OHCA patients. Participants will be instructed to sniff following intranasal administration and rest in supine position for 15 minutes.

A total of 11 possible doses will be tested, ranging from 0 to 1000 U insulin.
Each volunteer will come for 5 study visits. In the first visit, the volunteer is administered placebo. They will receive the first assigned intranasal insulin dose 4 hours later followed by another 4 hours of observation. During visits 2-5, they will receive additional escalating doses based on the dosing protocol.

6.1.2 DOSING AND ADMINISTRATION

Rules for assigning patients

Within subject rules (subject progressing through tiers for cohort):

1. If a volunteer experiences severe hypoglycemia (blood glucose <45 mg/dL), they will be assigned a dose 100 U less for the next round and for all subsequent rounds.
2. If a volunteer experiences severe hypoglycemia a second time, they will be withdrawn from the study.

Study wide rules (determine which dose tiers are allowable for all future volunteers):

1. All doses from 0 to 1000 U by increments of 100 U will be eligible for testing in the study.
2. If there is greater than 0.9 probability that a dose has a greater than 0.1 toxicity rate, that dose will be removed for all future cohorts (see below).

Allocation Rules

Initial Allocation and Strategy:

Overall, the philosophy of allocation is to assign as many patients as possible to higher doses, while also limiting exposure to toxic doses. The trial will start the first cohort with 200, 400, 600, 800, and 1000 U.

Allocation Adjustment Rules:

After each hypoglycemic event, we will recalculate the toxicity probabilities for that dose to see if a dose removal threshold has been met.

If a dose meets the criteria for removal, that dose and all higher doses will be removed from future participants and cohorts. For the next cohort or patient within a cohort, the highest additional doses available will be used. For example, if 700 U was removed, then 200, 300, 400, 500, 600 U would be the new future allocation.

If a cohort completes with no observed episodes of dose-limiting toxicity (DLT), the smallest (and any smaller) doses will drop, and the highest unused dose will replace it. For example, after zero episodes of DLT in Cohort 1 at any dose, Cohort 2 will use 400, 600, 800, 900, and 1000 U. Doses 100 and 200 U will be dropped.

If a cohort has any DLT, but no dose is dropped (only 1 DLT in two different doses for example), the initial allocation rules will be used for the next cohort (200, 400, 600, 800, 1000 U).

4 Cohort Example - No DLT (Demonstrated by Schema Figure 1.2.2)

Cohort 1: Participants #1 to 6

The doses will be 0, 200, 400, 600, 800, and 1000 U.

Cohort 2: Participants #7 to 12

If there were 0 events in Cohort 1, then the doses for Cohort 2 will be 0, 400, 600, 800, 900, and 1000 U. 200 U will be dropped and 900 U will be entered.

Cohort 3: Participants #13 to 18

If there were 0 events in Cohorts 1 and 2, then the doses for Cohort 3 will be 0, 600, 700, 800, 900, and 1000 U. 400 U will be dropped and 700 U will be entered.

Cohort 4: Participants #19 to 24

Same as Cohort 3.

Other situations (more than one DLT)

Please also refer below to rules for discontinuing a dose.

If a dose (or doses) is dropped during or after a cohort, the highest available unused doses will be used for the next patients within that cohort and the next cohort. For example, if 1000 U is dropped, the future doses will be 200, 400, 600, 800 and 900 U. Or, if 800 U and all higher doses are dropped, then 200, 400, 500, 600, and 700 U will be the next set.

As one example, if there is 1 DLT in a different dose in each of the 4 cohorts, the trial will simply allocate using the initial rules for all cohorts, with the exception of the within subject adjustment rule noted above.

For example, if there are no DLTs in Cohort 1, then Cohort 2 will use 400, 600, 800, 900, and 1000 U. If Cohort 2 also has no DLTs, then 300 and 400 U will drop and Cohort 3 will use 600, 700, 800, 900, and

1000 U. Even if there are no DLTs in Cohort 3 given that allocation, we will continue that allocation as it utilizes the 5 highest available doses.

If higher doses are dropped, they cannot be reconsidered in the study.

If lower doses are dropped, they can re-enter the study. This would occur if higher doses needed to be dropped by having DLTs over the threshold. The lower doses may need to re-enter in order for each subject to have 5 potential doses.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

HUMULIN R U-500 (insulin human injection, NDC 0002-8501-01) will be purchased as commercial supply through Eli Lilly and Company. Storage and accountability will be maintained by Research Pharmacy, Michigan Medicine. Diluent to prepare the 0 U/mL dose and lower concentrations of intranasal insulin will be acquired through Eli Lilly and Company (Sterile Diluent, NDC 0002-0800) or similar manufacturer (lilly_distribution@lilly.com).

6.2.2 Formulation, Appearance, Packaging, and Labeling

HUMULIN R U-500 is available as single 20 mL multi-use vials. The concentration is 500units/mL

6.2.3 Product Storage and Stability

Unopened vials of HUMULIN R U-500 will be stored in a refrigerator (36° to 46°F [2° to 8°C]) protected from freezing and light, with a do not shake notice. Opened vials of HUMULIN R U-500 will also be stored in a refrigerator (36° to 46°F [2° to 8°C]) protected from freezing and light and used within 40 days then subsequently discarded. Syringes containing ≤1 mL volume at doses of 0 (diluent), 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 U (undiluted) will be prepared and stored in a refrigerator for no more than four hours total at (36° to 46°F [2° to 8°C]) and/or room temperature, protected from freezing and light. A prior stability study demonstrated at least 28-day stability at this temperature. (18)

6.2.4 Preparation

No special preparation is necessary for HUMULIN R U-500 and sterile diluent measured to the aforementioned doses under a laminar flow hood by Research Pharmacy.

6.3 Measures to Minimize Bias: Randomization and Blinding

No randomization or masking procedures will be used in this study.

6.4 Study Intervention Compliance

The study related activities, including drug administration and observation, will be performed with support from the Michigan Clinical Research Unit with an extensive track record of data integrity and compliance with regulatory procedures. Given that participants will be seen weekly, study compliance

will be reviewed at each visit. A record of any study queries, discrepancies, or deviations from the protocol will be maintained and provided to the study site and PI to resolve and document as a part of the study record. There are no subject diaries or study related activities that occur outside of subject visits.

6.5 Concomitant Therapy

6.5.1 Rescue Medicine

If a subject's blood glucose level falls <45 mg/dL, he/she will be treated with 50 ml of intravenous D50W and provided food. He/she will be monitored until achieving two consecutive measurements of blood glucose > 70mg/dL, measured at 30 minutes apart. If blood glucose falls to ≤70 mg/dL but not <45 mg/dL, the blood glucose will be monitored every 30 minutes until it returns to >70 mg/dL.

7 Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

7.1 Discontinuation of Study Intervention

The rules for stopping or changing an individual dosing regimen are given above in the Dosing and Administration section.

Rules for discontinuing a dose

We are interested in proceeding with a dose if we remain confident that the Dose Limiting Toxicity (blood glucose < 45 mg/dL) is < 0.1 (1 in 10).

Using the Bayesian Toxicity Monitoring software from MD Anderson (https://ibl.mdanderson.org/BTM/)

We define Toxicity parameters as:
Probability of DLT is represented by θ
Maximum probability of DLT allowed (θ_{max}): 0.1

When calculating the probability of DLT at a dose, we will use a Beta(0.2,0.8) prior. This is a vague prior with a median of 0.2 and a 95% credible interval from 0 to 0.94. There is greater density over probabilities of DLT near zero in this non-symmetric distribution. Each DLT is added to the A parameter of the beta distribution, and each patient who is DLT free is added to the B parameter of the B distribution. Therefore, with the prior included, if 24 participants have DLT on a dose, the probability of DLT can be described with a Beta(0.2,24.8) distribution. In this setting, the posterior estimate for DLT probability is 0.008 (or 0.8%). In addition, the probability that that dose has a DLT proportion over 0.1 is only 0.006 (or 0.6%).

$Prob(\theta > \theta_{max}) \geq 0.9$ (Or that there is 90% or greater probability that the DLT is greater than 0.1)

We define the probability that $Prob(\theta > \theta_{max})$ as the final output of the trial for each dose. For each dose, we will also calculate the probability that toxicity is greater than 0.1 at that dose.

The below table (SB1A) illustrates the probability that DLT is greater than 0.1 based on the number of observed events.

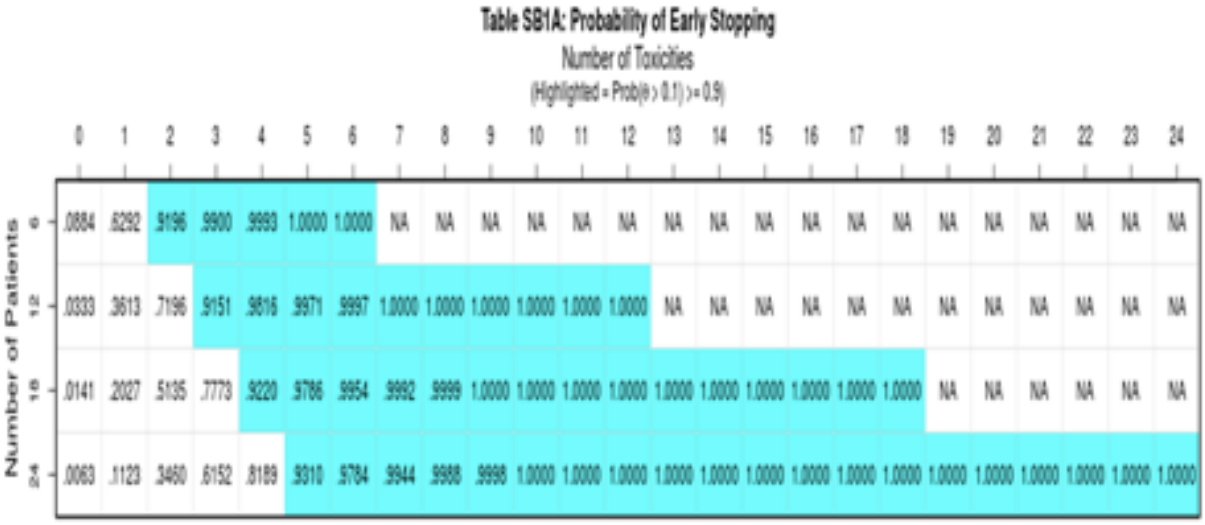


Table SB1A: Probability of Early Stopping

Number of Toxicities

(Highlighted = Prob(θ > 0.1) >= 0.9)

Number of Patients	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
9	.0884	.6292	.9196	.9900	.9993	1.0000	1.0000	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
12	.0333	.3613	.7196	.9151	.9816	.9971	.9997	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
16	.0141	.2027	.5135	.7773	.9220	.9786	.9954	.9992	.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	NA	NA	NA	NA	NA	NA
24	.0063	.1123	.3460	.6152	.8189	.9310	.9784	.9944	.9988	.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

In practice, this means if 2 DLTs are observed for a dose in the first cohort, the dose is dropped. To drop a dose in subsequent cohorts, see the shaded area of the above table. To delete a dose, one must have 3 total observed DLTs in Cohorts 1 and 2, or 4 total DLTs in Cohorts 1-3, or 5 total DLTs in Cohorts 1-4.

Rules for discontinuing or modifying the overall study

There are no binding rules to discontinue the overall study. However, if the 500 U dose tier was dropped, there would only be 100, 200, 300, and 400 U available. In this case, the PI will consult with the other investigators to present an amended plan for future study conduct or overall discontinuation.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants may be withdrawn from the study at any time for voluntary reasons or discontinued from study treatment at the request of the study investigators. Reasons for withdrawal or termination may include but are not limited to: 1) Subject requests discontinuation of study drug or withdrawal; 2) Subject becomes pregnant; 3) Subject requires treatment that is not permitted per protocol due to a risk for a drug interaction.

7.3 Lost to Follow-Up

Participants who do not return to their clinic appointment for a required study visit will be contacted by telephone immediately to reschedule their appointment. They will be counseled on the importance of maintaining the assigned visit schedule and ascertain whether they wish to continue in the study.

Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's medical record or study file.

Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up. Data collected on study participants to the point of withdrawal will remain a part of the study record.

8 ASSESSMENTS

8.1 EFFICACY ASSESSMENTS

Initial screening will be completed by telephone after a potential subject seeks interest in study participation. This initial screening will include review of the study protocol inclusion/exclusion criteria. If the subject meets eligibility criteria, they will be asked to come into the research clinic to start the informed consent process and undergo screening laboratory testing.

8.1.1 Screening Visit

All participants will be informed of the nature and purpose of the study, and their written informed consent will be obtained prior to any study related activities. The investigator or designated qualified individual will perform the physical examinations. A physical examination will include measurement of body weight and height, general assessment of the skin, nose/throat, heart, lungs, and abdomen. The subject's total body weight and supine vital signs (heart rate, blood pressure, temperature, and respiratory rate) will be recorded.

Clinical Laboratory Testing and Urine hCG

- Serum comprehensive metabolic panel will be done including blood levels of albumin, blood urea nitrogen, calcium, carbon dioxide, chloride, creatinine, glucose, potassium, sodium, total bilirubin and protein, and liver enzymes (alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase).
- Serum hemoglobin will be measured.
- Urine human chorionic gonadotropin (hCG) will be measured for females who are not diagnosed as postmenopausal.

8.1.2 Study Visits

Participants (in cohorts of 6) will present to the Michigan Clinical Research Unit on their scheduled dosing day, and will be asked to have not consumed any food for at least 2 hours and any alcohol for at least 12 hours prior to this visit. A urine pregnancy test will be performed to rule out pregnancy prior to administration of the intranasal insulin dose to applicable female participants. The subject's total body weight and supine vital signs (heart rate, blood pressure, temperature, and respiratory rate) will be recorded. Eligible Participants will proceed with the study procedures after review of inclusion and exclusion criteria.

Participants will be asked to participate in 6 administrations over 5 sessions with a 1-week washout interval between each session. The first session will include an initial control administration of 0 U with 4 hours of observation, then followed by the first dose-escalation intranasal insulin administration and another 4 hours of observation. Subsequent visits will involve a single dose-escalation administration per visit as described in 6.1.2 Dosing and Administration. Any subject experiencing severe hypoglycemic episode will be given 100 U less than the last administered dose for the remainder of the study. Any participant having a second event, will be withdrawn from the study.

Participants will have peripheral intravenous access established with the placement of a short-term IV catheter for serial blood sampling and administration of supplemental glucose if needed. 5 mL of venous blood samples (3 mL as waste and 2 mL for blood glucose, insulin, and C-Peptide analyses) will be collected at 0, 15, 30, 60, 120, 180, and 240 minutes after each intranasal drug administration. Blood glucose levels will be measured with a point of care glucometer. The remaining blood samples will be centrifuged, aliquoted, and frozen for serum insulin and C-Peptide analyses by the University's Pharmacokinetics Core.

If a subject's blood glucose level falls <45 mg/dL, he/she will be treated with 50 ml of intravenous D50W and provided food. He/she will be monitored until achieving two consecutive measurements of blood glucose > 70mg/dL, measured at 30 minutes apart. If blood glucose falls to ≤70 mg/dL but not <45 mg/dL, the blood glucose will be monitored every 30 minutes until it returns to >70 mg/dL. Participants will not be released to home until any symptoms of hypoglycemia have resolved.
Participant-reported adverse events will also be tracked as supplemental outcomes.

8.2 Safety and Other Assessments

- Vital Signs (blood pressure, pulse, respiratory rate, temperature)
- Clinical laboratory tests including blood chemistry and hematology parameters
- Urinalysis pregnancy screening test(s) in women
- Adverse events
- Concomitant medication use
- Physical exam

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

The internationally accepted definition of an adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product.

8.3.2 Definition of Serious Adverse Events (SAE)

A serious adverse event (SAE) is any AE occurring at any dose that results in any of the following outcomes: a life-threatening situation, death, inpatient hospitalization or prolongation of existing hospitalization, persistent or significant disability or incapacity, congenital anomaly or birth defect, or important medical events such as those that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when based upon appropriate medical judgment. It jeopardizes the subject or requires medical or surgical intervention to prevent one of the outcomes listed above.

8.3.3 Classification of an Adverse Event

8.3.3.1 Severity of Event

The severity of adverse events will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) – NIH/NCI

Grade 1:	Mild AE
Grade 2:	Moderate AE
Grade 3:	Severe AE
Grade 4:	Life-Threatening or Disabling AE
Grade 5:	Death related to AE

8.3.3.2 Relationship to Study Intervention

The study physician will use clinical judgment to define a necessary medical course. AEs of interest are those that occur after the first dose of study medication. The relationship of AEs to the investigational product should be determined by the Investigator using the definitions below:

- Definitely related: clearly associated with study drug/treatment
- Probably related: likely associated with study drug/treatment
- Possibly related: may be associated with the study drug or other treatment
- Unlikely to be related
- Unrelated to the study drug

8.3.3.3 Expectedness

The investigators will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent in the protocol, informed consent, or labeling for the Humulin R U-500

8.3.4 Time Period and Frequency for Event Assessment and Follow-Up

The intranasal insulin dose administration will be managed by the study personnel. The participant will be directly monitored by study staff for the initial 4 hours after dose administration. AE assessments will occur during each study visit.

8.3.5 Adverse Event Reporting

Out of range clinical laboratory findings (e.g., clinical chemistry, hematology, urinalysis) or findings on other assessments (vital signs) *per se* are not reported as AEs. However, those abnormal findings that are deemed clinically significant by the investigator or are associated with signs and/or symptoms must be recorded as AEs (and recorded as an SAE if they meet the criteria of being serious; see Section 8.3.2). The investigator should exercise medical and scientific judgment in deciding whether an out of range clinical laboratory finding or finding on other assessment is clinically significant.

All AEs occurring within 24 hours of study drug dosing and all SAEs occurring up until the last day of study participation will be recorded on the online AE case report form (CRF) through the study database. The investigators or Study Coordinator or designee is responsible for entering any and all AEs and SAEs into the database as soon as he/she becomes aware of the event and updating the information (e.g., date of resolution, action taken) in a timely manner. All non-serious AEs must be recorded on the electronic AE CRF within 5 days from the time it was discovered by the site study personnel.

Reporting of AEs and SAEs will follow the IRB standard AE reporting timeline.

8.3.6 Serious Adverse Event Reporting

SAEs associated with intranasal insulin will be reported to the IRB in accordance with the University of Michigan IRBMED reporting guidelines and to the FDA according to the regulations found in 21 CFR 312.32.

8.3.7 Reporting Events to Participants

Participants who experience an AE will be told at the time the event is identified.

8.3.8 Events of Special Interest

Not applicable.

8.3.9 Reporting of Pregnancy

Any participant with a positive pregnancy test will be notified of the test result and withdrawn or excluded from the study.

8.3.10 Definitions of Selected or Anticipated Adverse Events

The adverse events identified below are anticipated and will be tracked.

Allergic reaction

Aspiration

Nasal irritation

Sneezing

Warm feeling in nostrils

Congestion/Rhinitis

Lightheadedness

Nausea and vomiting

Nosebleeds

Hypoglycemia

Headache

Weakness

Upper respiratory infection

Rash

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

Any incident, experience or outcome that is unexpected in nature, severity or frequency that is related or possibly related to study participation will be deemed an unanticipated problem. The impetus for actions related to the unanticipated problem will depend on the information that suggests that the research study procedure places participants at greater risk of harm than previously recognized.

8.4.2 Unanticipated Problem Reporting

Any unexpected AE and unanticipated problem will be reported to the University of Michigan IRBMED according to their reporting guidelines. Upon becoming aware of any incident, experience, or outcome (not related to an adverse event) that may represent an unanticipated problem, the investigator should assess whether the incident, experience, or outcome represents an unanticipated problem. The incident, experience, or outcomes is considered unanticipated if it meets all of the following criteria:

Unexpected (in terms of nature, severity, or frequency);
Related or possibly related to participation in the research; and
Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.
Unanticipated problem Reporting: Per 21 CFR 312.66, 312.53 (c)(1)(vii), and 56.108(b)(1), should an Unanticipated problem occur during the investigation, the investigator will promptly report all unanticipated problems involving risks to human subjects or others to the IRB /FDA.

8.4.3 Reporting Unanticipated Problems to Participants

Reporting unanticipated problems to participants will occur as directed by the UM IRBMED.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s): Severe hypoglycemia (DLT) as defined by blood glucose <45mg/dL.

The maximum dose that is under the threshold of a 0.9 probability of having a DLT proportion of greater than 0.1 will be considered the maximum tolerated dose for this study.

- Secondary Efficacy Endpoint(s): Change in blood glucose, insulin, and C-peptide levels for up to 4 hours after drug administration to evaluate for any systemic absorption of intranasal insulin.

Our main secondary hypothesis is the secondary efficacy endpoints are not associated with increasing doses of insulin. For example, change in blood glucose from baseline to 4 hours will be similar in the placebo and highest doses.

9.2 SAMPLE SIZE DETERMINATION

Overall, for any dose we estimated whether the study would end, with different assumptions of the probability of toxicity for a given dose. These ranged from 0.01 to 0.25. It is possible, however, that a dose tier may not be desirable despite having not been dropped. For example, there will be about an 82% probability that the toxicity rate is > 0.1 if 4 out of 24 participants experience a DLT, then the point estimate using the Bayesian model will be that arm has a 0.17 probability of DLT (slightly above the raw observed rate of 0.125 due to the Bayesian prior).

The design has approximately 80% “power” at the margin (scenario 5). That is, if the true rate of DLT is 0.1 at a dose, the DLT will NOT be declared toxic using the pre-specified definition ($Prob(\theta > 0.1) \geq 0.9$ just under 80% of the time.

It is important to note that the table below is for a single dose with 24 observations. As the trial progresses, we will generate independent estimates of the probabilities for each dose. The average number of patients indicates that the dose will stop early some proportion of the time. The average number of toxicities provides the average number of DLTs observed within a simulated trial when the true probability of toxicity is given (for example 0.1 in scenario 5).

Table: Operating Characteristics for a Dose Assuming N of 24

Scenario	Prob.Of.Tox	Prob Early.Stop	ProbDeclare.Tox	Avg.N.Patients	Avg.N.Tox	Obs.Tox.Rate
1	0.01	0.0016	0.0016	23.9724	0.2397	0.0104
2	0.025	0.0105	0.0105	23.8235	0.5956	0.0272
3	0.05	0.0453	0.0467	23.2792	1.164	0.0583
4	0.075	0.1062	0.1125	22.3836	1.6788	0.0921
5	0.1	0.1898	0.2062	21.1965	2.1196	0.127
6	0.125	0.2892	0.3196	19.8036	2.4754	0.1617
7	0.15	0.3962	0.4415	18.2978	2.7447	0.1952
8	0.2	0.6031	0.669	15.277	3.0554	0.2563
9	0.25	0.7684	0.8336	12.6203	3.1551	0.3089

9.3 Populations for Analyses

Male or female volunteers at least 18 years of age and older, who meet study inclusion and exclusion criteria.

9.4 Statistical Analyses

9.4.1 General Approach

For each dose DLT probability θ, we will calculate the (Prob(θ >0.1). The maximum dose that is under the threshold of a 0.9 probability of having a DLT proportion of greater than 0.1 will be considered the maximum tolerated dose for this study.

We will also report the observed toxicity rate for each dose. Depending on the number of DLT observed, we may fit a dose response model that uses data from all doses to fit a dose-toxicity curve. We also will plan to use dose response models for the analyses of the secondary endpoints.

9.4.2 Analysis of the Primary Efficacy Endpoint(s)

Primary analyses of these clinical trial data will be descriptive, including critical event rates reported with 95% confidence intervals.

9.4.3 Analysis of the Secondary Endpoint(s)

First, we will graphically plot the relationship between the secondary endpoint and dose. We will explore these plots to determine a reasonable functional form (i.e. linear, Emax, log transformed, log-logistic) for dose-response. We will consider dose as a linear predictor in addition to the most reasonable functional form. However, we will not fit other models if the linear predictor is reasonable.

We will fit generalized linear models to predict change in glucose from baseline given dose. We will use a random effects model (with a random intercept for each subject). The main predictor variable will be dose. The main covariate will be the baseline value for the covariate for variables that are measured longitudinally (i.e. model predicts 4 hour glucose, and baseline glucose is a covariate in the model).

9.4.4 Safety Analyses

Secondary analyses include modeling of dose-safety curves based on primary and secondary safety outcomes, and modeling of pharmacokinetics/pharmacodynamics based on dose and serial serum insulin measurements if there is sufficient signal. Analyses of the effects of intranasal insulin on indicators of glycemic variability will also be explored.

9.4.5 Baseline Descriptive Statistics

The baseline values of labs, vital signs, and our descriptive characteristics will be summarized in a table with means and 95% confidence intervals or proportion with 95% confidence intervals.

9.4.6 Planned Interim Analyses

The dosing plan for the next cohort will be determined after each cohort completes. Participants within any cohort will have their dosing plan adjusted in the event one of the planned doses for that cohort is dropped due to excess DLTs in the other participants within that cohort.

9.4.7 Sub-Group Analyses

We will conduct stratified analyses by gender for the primary and secondary analyses, unless the gender ratio is greater than 3:1 (i.e. over 75% most common sampled gender).

9.4.8 Tabulation of Individual Participant Data

The full dataset will be de-identified for future use.

9.4.9 Exploratory Analyses

None planned.

10 Supporting Documentation and Operational Considerations

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

All participants will be informed of the volunteer nature and purpose of the study. Their written informed consent will be obtained in a private setting, during the pre-study screening procedures conducted prior to the first dosing. The informed consent form will contain all elements required by the International Conference on Harmonization Good Clinical Practices Guidelines in addition to any other elements required by state, local or institutional policy.

10.1.1.1 Consent/assent and Other Informational Documents Provided to participants

All participants will provide an IRB approved informed consent prior to any research procedures. A signed informed consent document will be obtained during the initial screening visit.

10.1.1.2 Consent Procedures and Documentation

Informed consent procedures will be performed in a private setting. As part of the consent process, participants will be asked to summarize what is being asked of them to ensure they understand the study, have had sufficient time to contemplate the study, have not been coerced, and understand the voluntary nature of their participation. The original copy of the informed consent document will be maintained in the individual subject study binder.

10.1.2 Study Discontinuation and Closure

Participants may withdraw from the study at any time for voluntary reasons or discontinued from study treatment at the request of the study investigators. Reasons for withdrawal or termination may include but are not limited to 1) Subject requests discontinuation of study drug or withdrawal; 2) Subject becomes pregnant; 3) Subject requires treatment that is not permitted by the study protocol.

10.1.3 Confidentiality and Privacy

The investigators will abide by detailed guidance provided by the IRB on data management and security considerations.

10.1.4 Future Use of Stored Specimens and Data

There will be no stored specimens for future use.

Data collected for this study will be analyzed and stored in a secure location in the Emergency Medicine Research Office. After the study completion, study records will be retained for a duration in accordance with relevant regulations and institutional policies. This period of time complies with regulations within 45 CFR 46.115(b), and permits sufficient time for an audit of records if deemed necessary by regulatory authorities. A de-identified dataset will be prepared after study completion and archived in the event further analyses are necessary to develop the treatment in.

10.1.5 Key Roles and Study Governance

Define the roles generically without names.

The roles and responsibilities of the investigators and study coordinators include, but are not limited to assuring that the trial is conducted according to the study protocol, identifying, recruiting, and enrolling participants, obtaining individual informed consent, collecting and entering study data into the study database, following participants through study completion, maintaining essential research documents, and assuring regular IRB approvals and renewals,. Additionally, the principal investigator has the overall responsibility for the study and is responsible for ensuring all participating staff members are adequately trained and competent to perform his/her job.

10.1.6 Safety Oversight

Medical personnel will be present throughout the drug administration and study visits. Safety monitoring and reporting of AEs and SAEs will follow IRB procedures. All observed and volunteered adverse reactions will be documented in the study records. All adverse events will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) – NIH/NCI.

10.1.7 Clinical Monitoring

The study procedures will be performed with support from the Michigan Clinical Research Unit with an extensive track record of data integrity and compliance with regulatory procedures. To assure adequate protection of the rights of human subjects, per 21 CFR §312.50, 312.53, this study will be monitored by the University of Michigan Institute for Clinical and Health Research (MICHR). Routine monitoring will be scheduled at appropriate intervals, with more frequent visits occurring at the beginning of the study. An initiation visit will take place, followed by routine monitoring visits. Additional visits can be scheduled at the request of the Project Manager or the Sponsor-Investigator. The established monitoring plan will ensure the quality and integrity of the data through pre- investigation visits and periodic site visits to verify adherence to the protocol, completeness and accuracy of study data and samples collected, proper storage, dispensing and inventory of study medication, and compliance with regulations.

10.1.8 Quality Assurance and Quality Control

Quality control procedures will be implemented beginning with the data entry system. Data quality control checks will be run on the generated database. Any missing data or data anomalies will be communicated to the investigator or study coordinator for clarification/resolution.

10.1.9.1 Data Collection and Management Responsibilities

Data collection and management will be performed consistent with good clinical practice. The investigator-sponsor of the Investigational New Drug will ensure that all investigators, study coordinators, and nursing staff are appropriately trained. Electronic case report forms will be created and programmed with real time logic and range data entry checks in a RedCap database (Vanderbilt, Nashville, TN). Electronic direct data entry into the electronic case report forms at the bedside will be performed for all clinical data. Send-out laboratory data will be entered later from source documents.

10.1.9.2 Study Records Retention

Study records will be maintained in a study binder in a locked cabinet with access limited to the study staff under the direction of the principal investigator. After the study completion, the archived data will be stored for at least 2 years after approval of an NDA or determination that an NDA will not be pursued for this use of intranasal insulin. This period of time complies with regulations at 21 CFR 312.62(c), and permits sufficient time for an audit of records if deemed necessary by regulatory authorities. Copies of these data will be destroyed after this period of time.

10.1.10 Protocol Deviations

The study will be conducted according to the study protocol. If a protocol deviation occurs, such as a visit being missed, the investigator must decide whether to proceed, for example, whether or not to complete the visit outside of the protocol-defined window. The nature and reasons for the protocol deviation will be recorded in the subject's CRF and submitted to the IRB according to institutional guidelines.

10.1.11 Publication and Data Sharing Policy

All data, results, and intellectual property rights in the data and results derived from the study will be the property of the University of Michigan, who may utilize the data in various ways, such as for submission to government regulatory authorities or disclosure to other investigators. If the results of this study are reported in medical journals or at meetings, the identities of all participants will remain confidential.

10.1.12 Conflict of Interest Policy

The University of Michigan defines a conflict of interest (COI) as a situation where a faculty or staff member is in a position to influence the business, research, or other decisions of the University in relationship to an outside organization that could lead directly or indirectly to financial gain for that individual or the family of that individual, or give improper advantage to others to the detriment of the University. Study personnel will abide by the COI policies outlined in the Standard Practice Guide that detail procedures to guide disclosure of outside interests and managing COI that is identified.

10.2 Additional Considerations

Not applicable.

10.3 ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
COI	Conflict of Interest
CRF	Case Report Form
DLT	Dose Limiting Toxicity
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IND	Investigational New Drug
IRB	Institutional Review Board
OHRP	Office for Human Research Protections
PI	Principal Investigator
SOA	Schedule of Activities
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale

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