

**A Double-Blind, Placebo-Controlled Pilot Investigation of the Safety of Intranasal Glutamine
in Down Syndrome.**

NCT02432716

Clinical Study Protocol

Sponsor

HealthPartners Center for Memory & Aging

IND Number

122626

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

Confidential Information

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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.

Principal Investigator

Date

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PROTOCOL SYNOPSIS

PROTOCOL TITLE	A Double-Blind, Placebo-Controlled Pilot Investigation of the Safety of Intranasal Glulisine in Down Syndrome (DS)	
SHORT TITLE	Down IN Insulin Study	
STUDY PHASE	Pilot	
STUDY OBJECTIVES AND PURPOSE		
Study Purpose The purpose of this study is to provide safety evidence to support the development of intranasal (IN) glulisine as a treatment option for cognitive impairment in DS.		
Primary Objective <ul style="list-style-type: none">To demonstrate the safety of IN glulisine in DS.To demonstrate the feasibility of performing a cognitive performance battery in DS.		
Secondary Objective (s) <ul style="list-style-type: none">To estimate the effects of IN glulisine on cognition and memory in DS.		
STUDY DESIGN		
Study Type	Safety	
Control Type	Placebo	
Study Indication Type	Treatment	
Blinding Schema	Double-Blind	
Device	Pressurized Olfactory Delivery (POD) device	
Study Design	This study is a single center, randomized, double-blind, placebo-controlled, cross-over pilot study designed to assess the safety of intranasally (IN) delivered glulisine versus placebo in patients with DS. Subjects will be randomized into this cross-over study and within subject comparisons conducted between single treatment of intranasal insulin glulisine and single treatment of intranasal placebo. All subjects will also receive a single treatment of placebo prior to randomization to ensure adherence to study procedures.	
Planned Duration of Subject Participation	The duration of study participation for each subject is anticipated to be between 6-7 weeks.	
OUTCOMES	Primary-Safety	<ul style="list-style-type: none">Number of related adverse and/or serious eventsNumber of any adverse and/or serious events
	Secondary-Cognitive Performance	<ul style="list-style-type: none">Fuld Object-Memory EvaluationRivermead Behavioral Memory Test
INVESTIGATIONAL PRODUCTS, DOSE AND MODE OF ADMINISTRATION		
Investigational Product	Glulisine 20 IU/IN (.1ml/10 units IN in each nostril)	
Placebo	Sterile Normal Saline 20 IU/IN placebo (.1ml IN in each nostril)	
SUBJECT SELECTION		

Targeted Accrual	Approximately 12 randomized subjects. We estimate will need to consent 20 participants in order to reach this goal.
<p>Inclusion Criteria</p> <ul style="list-style-type: none"> • Male or female aged 35-80 years with a Down syndrome diagnosis that is confirmed by karyotype. • Vital signs must be within normal limits for their age. (Medically treated hypertension will be allowed). • Must have an electrocardiogram free of clinically significant findings. • Must have an authorized representative to provide written informed consent. • Level of speech and comprehension of verbal commands are sufficient to understand and to answer simple requests. • Must have a reliable caregiver or family member who agrees to accompany the subject to all visits, provide information about the subject as required by this protocol. • Must be independent for activities of daily living. • Must tolerate the initial IN treatment of placebo and adhere to study procedures. 	
<p>Exclusion Criteria</p> <ul style="list-style-type: none"> • Any current psychiatric or neurologic diagnosis other than Down syndrome or Down syndrome with dementia that is judged to impact cognition. • Subjects who currently meet or have within the past five years met DSM-IV (Diagnostic and Statistical Manual) criteria for drug or alcohol abuse or dependence. • Subjects residing in a skilled nursing facility or subjects who are anticipated to enter a nursing home within the next 6 months. (Subjects may reside in group homes, assisted living, or other residential settings where they do not require 24 hour skilled nursing.) • Subjects receiving any experimental drug for Down syndrome within the past 30 days of screening visit. • Subjects with significant allergies to or other significant intolerance insulin. • Presence of active seizure disorder. • Presence of significant aggression or agitation that may impact participation with testing and IN administration. All subjects must have NPI-C aggression and agitation subscore ≤ 4 (severity ≤ 2; frequency ≤ 2). • Significant cerebrovascular disease with Modified Hachinski Score > 4. • Subjects who may not be able to comply with the protocol or perform the outcomes measures due to significant hearing or visual impairment or other issues judged relevant by the investigators. • Subject has been diagnosed with any form of diabetes mellitus, actively takes insulin, or has HbA1c $\geq 6.1\%$ at screening. 	

1. INTRODUCTION

Background and Rational

Down syndrome (DS) is the most common chromosomal anomaly recognized at birth, with an incidence of about 1 in 1000 births in the United States [1]. DS is caused by the presence of all or part of an extra copy of chromosome 21, which can lead to deficits in assimilation and adaptation along with cognitive impairment [2].

Both AD and DS have significant overlaps in clinical phenotype and neuropathology. Virtually all individuals with DS are likely to develop clinical and neuropathological brain changes resembling Alzheimer's dementia by the ages of 35-40 years, which include deposits of extracellular amyloid-beta oligomers (A β) and intracellular neurofibrillary tangles (NFTs) [3, 4]. In addition, both DS and AD are associated with a similar hierarchy and distribution of amyloid plaques, microglial activation, astrogliosis, inflammation, oxidative stress, and synaptic loss [5-12]. Conversely, DS can be distinguished from AD based on the age of dementia onset, the higher degree of amyloid plaque burden and hippocampal neurofibrillary tangles, and the relatively decreased amount of neuronal loss. Cognitive impairment in DS has further been attributed to a combination of increased GABAergic neurotransmission and disrupted axonal function [5, 13]. DS is associated with degeneration of cholinergic neurons, and cholinesterases, namely donepezil and rivastigmine, have been studied in DS and found to be well-tolerated with a minimal, yet generally positive treatment effect in exploratory investigations [2, 14-17]. However, there are no current FDA-approved treatments for cognitive impairment associated with DS.

The AD brain is characterized by a severely impaired insulin-signaling pathway, including deficits of insulin, insulin-like growth factor insulin receptors, and central resistance to insulin action [18]. As a result of impaired insulin signaling, glucose uptake and utilization may be dramatically decreased in both patients with DS and AD. Fludeoxyglucose (FDG) PET scans of patients with AD show very little uptake and utilization of glucose within posterior temporo-parietal structures and similar processes involving posterior cingulate glucose utilization has been demonstrated in DS[10].

Originally thought to exist solely in the periphery, insulin has since been determined to be instrumental in the overall health and function of the CNS [19]. Central insulin and IRs have been established as differing from that of the systemically occurring counterparts that specifically regulate the utilization of glucose. Although central insulin does induce glucose uptake in the brain, it also functions in the modulation of various neurotransmitters and receptors involved in executive function and long-term potentiation of memories [20, 21]. For example, systems with impaired insulin signaling pathways have demonstrated inhibition of acetylcholine biosynthesis and subsequently have incurred debilitating effects on neuronal plasticity [22, 23]. Thus, cognitive function and memory falter as a result of decreased insulin-controlled regulation of, among others, acetylcholine, norepinephrine and activated NMDA receptors [23-25].

Consistent with evidence of insulin functioning as a neuromodulator in the facilitation memory is the high-density of IRs in the hippocampus and cerebral cortex, which are regions of the brain integral to the formation, retention and recall of information [19, 26]. Treatment of animal models with intracerebral ventricular insulin has benefited the model with improved memory in passive-avoidance tasks [27]. Further, intranasal insulin has been shown to reduce memory loss in aging diabetic animals. [28] In addition, intranasal insulin also ameliorates experimental diabetic neuropathy and prolongs lifespan when compared to systemic insulin treatment. [29]. Finally, intranasal insulin has been reported to ameliorate tau hyperphosphorylation in a rat model of type 2 diabetes [30].

Pre-clinical work has shown that insulin regulates the pathological hallmark proteins associated with both AD and DS, including NFTs and amyloid plaques (AP) [31-33]. Increasing central insulin concentration may decrease NFT formation through inhibition of tau phosphorylation by maintaining the phosphorylation equilibrium between kinase and phosphatase activity [34]. Insulin reduces amyloid plaque burden through the stimulation of insulin degrading enzyme (IDE) [35]. Finally, insulin receptor signaling increases synaptic density, which may counteract the characteristic loss of synapses occurring in AD and DS [36]. The numerous neuropathological similarities that exist in AD and DS characterize the insulin signaling pathway as a promising treatment approach in DS.

As a large, charged molecule, insulin does not readily cross the blood-brain barrier (BBB), and hence, intranasal (IN) delivery of offers a non-invasive route directly to the brain, while minimizing systemic exposure. Peptides, proteins, vaccines, drug treatments and charged molecules of various sizes are able to pass along the olfactory and trigeminal nerves and are deposited directly into the CNS without having to pass through the BBB that may degrade or limit the amount arriving at the target [37-44]. Intranasally-delivered insulin in healthy adults has been detected in the CSF as early as 10 minutes after IN administration without a significant decrease in systemic blood glucose or alteration in systemic blood insulin levels [32, 39, 45, 46]. or peripheral insulin levels

The majority of the work demonstrating the efficacy of insulin in the AD population has been performed by Dr. Suzanne Craft at the University of Washington, often in collaboration with Dr. William H. Frey II. A clinical trial consisting of 26 memory impaired subjects (13 with AD and 13 with mild cognitive impairment) and 35 normal controls showed that IN insulin 20 IU or 40 IU improved two declarative memory tasks compared to placebo within 15 minutes of drug administration [32]. IN insulin administered at 20 IU resulted in greater story recall whereas doses at 40 IU more favorably improved word list recall. Another study of 24 early AD/mild cognitive impairment subjects showed that 20 IU BID of intranasal insulin resulted in sustained benefit in over a 21 day period [46]. Furthermore, IN insulin resulted in favorable changes in the serum amyloid-beta 40/42 ratio while having no impact on systemic glucose or insulin levels. Most recently, Craft and colleagues have shown improved memory in AD patients following IN insulin treatment in a four month clinical trial [47].

Intranasal insulin's cognitive benefits extend beyond the MCI/AD population, having been demonstrated in healthy control subjects. A randomized double blind study of 38 normal controls treated with 40 IU IN insulin, 4 times/day over 8 weeks demonstrated benefits in

attention, immediate and delayed recall, and mood in the treatment group [48]. This same research center later performed a study in 38 normal subjects showing that rapid-acting insulin aspart resulted in greater declarative memory improvements than IN regular insulin over 8 weeks' time [49].

To test the hypothesis regarding the acute safety and efficacy of rapid acting (RA) insulin glulisine in AD, our group performed a double blinded, randomized, placebo-controlled, cross-over study in 9 mild-moderate ApoE4+ AD subjects. Glulisine is a rapidly absorbed insulin analogue lacking the zinc ingredient commonly found in insulin formulations, and may hypothetically be a safer, better tolerated insulin considering findings that zinc-containing compounds may be toxic to olfactory neurons [50, 51]. The study drug was administered intranasally using the LMA Mucosal Atomization Device (MAD). Treated subjects made fewer Trails B errors relative to controls. Otherwise, there were no significant difference between intranasal glulisine and placebo for cognitive tests of learning/memory, attention/executive function, language, or visuospatial function. Fingerstick glucose was not impacted by IN glulisine, but the drug resulted in a 19% decrease in insulin levels compared to baseline. The findings indicated that mild-moderate ApoE4+ AD patients were unresponsive to acute IN glulisine, but that the drug was safe and well-tolerated.

In the current study, we aim to demonstrate the safety of IN RA insulin glulisine in the DS population with a double blinded, randomized, placebo-controlled, cross-over study design. This investigation will enroll DS subjects with a high probability of elevated plaque burden, aged ≥ 35 , who may or may not be suffering from dementia. Recognizing the logistical limitations of positioning and administering study drug with the LMA MAD device, we will be using the Impel intranasal device (see description below), which would be expected to more efficiently deliver study drug to the target region.

2. SUMMARY OF DEVICE DESCRIPTION

2.1. Intranasal Pressurized Olfactory Delivery (POD) Device

The Pressurized Olfactory Delivery (POD) device is intended to be used with a variety of known and yet-to-be-known drugs. The POD device is designed specifically to deliver centrally acting drugs via the olfactory and trigeminal neural pathways in the roof of the nasal cavity and olfactory nasal epithelium.

Commercially available aerosol nasal devices such as the LMA MAD device are not specifically engineered to facilitate nose-brain delivery, and consequently deposit most of the drug within the lower nasal cavity, resulting in suboptimal CNS penetration. Other problems associated with traditional nasal sprays include variable aerosolized product, dependence on user position, and high frequency of device non-compliance/misuse. The POD device has specifically been developed to effectively and consistently deliver CNS therapeutics to the brain via the nasal/olfactory pathways. The device is not currently FDA approved, but numerous studies support its role in intranasal brain delivery of radiolabeled and therapeutic compounds.

3. OBJECTIVES AND ENDPOINTS

3.1. Primary Objectives

To demonstrate the safety of IN glulisine in DS.

To demonstrate the feasibility of performing a cognitive performance battery in DS.

3.2. Secondary Objectives

To estimate the effects of intranasal glulisine on cognition and memory in DS.

3.3. Primary Endpoint

Incidence of any or related adverse and/or serious events of intranasal glulisine versus placebo.

3.4. Secondary Endpoints

Change in performance of the Fuld Object-Memory Evaluation (FOME).

Change in performance on the Story Recall subtest of the Rivermead Behavioral Memory Test for Children (RBMT-C).

3.5. Safety

Frequency of change in clinically significant vital signs or physical exam.

4. STUDY DESIGN

This study is a single center, randomized, double-blind, placebo-controlled, cross-over pilot study designed to assess the safety of IN glulisine versus placebo in patients with DS.

After written informed consent has been obtained from the subject and their caregiver, subjects will be screened to assess study eligibility based on the study inclusion/exclusion criteria.

A total of twelve subjects (n=12) will be randomized in this cross-over study and within subject comparisons conducted between single treatment of IN glulisine and placebo. All subjects will receive a single test treatment of placebo prior to randomization to ensure adherence to study procedures. Twenty minutes after receiving IN treatment, DS subjects will undergo cognitive testing with Fuld Object Memory Evaluation and Story Recall subtest of the Rivermead Behavioral Memory Test for Children.

5. PATIENT SELECTION

5.1. Inclusion Criteria

- A subject will be included for consideration in this study only if all of the following criteria are met:
- Males or females aged 35-80 years with a Down syndrome diagnosis that is confirmed by karyotype.
- Vital signs must be within normal limits for their age. (Medically treated hypertension will be allowed).
- Must have an electrocardiogram free of clinically significant findings.
- Must have an authorized representative to provide written informed consent.
- Level of speech and comprehension of verbal commands are sufficient to understand and to answer simple requests.
- Must have a reliable caregiver or family member who agrees to accompany the subject to all visits, provide information about the subject as required by this protocol.
- Must be independent for activities of daily living.
- Must tolerate well the initial treatment of placebo and adhere to study procedures.

5.2. Exclusion Criteria

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

- Any current psychiatric or neurologic diagnosis other than Down syndrome or Down syndrome with dementia that is judged to impact cognition.
- Subjects who currently meet or have within the past five years met DSM-IV (Diagnostic and Statistical Manual) criteria for drug or alcohol abuse or dependence.
- Subjects residing in a skilled nursing facility or subjects who are anticipated to enter a nursing home within the next 6 months. (Subjects may reside in group homes, assisted living, or other residential settings where they do not require 24 hour skilled nursing.)
- Subjects receiving any experimental drug for Down syndrome within the past 30 days of screening visit.
- Subjects with significant allergies to or other significant intolerance insulin.
- Presence of active seizure disorder.
- Presence of significant aggression or agitation that may impact participation with testing and IN administration. All subjects must have NPI-C aggression and agitation subscore ≤ 4 (severity ≤ 2 ; frequency ≤ 2).
- Significant cerebrovascular disease with Modified Hachinski Score > 4 .
- Subjects who may not be able to comply with the protocol or perform the outcomes measures due to significant hearing or visual impairment or other issues judged relevant by the investigators.
- Subject has been diagnosed with any form of diabetes mellitus, actively takes insulin, or has HbA1c $\geq 6.1\%$ at screening.

6. STUDY ASSESSMENTS AND PROCEDURES

6.1. Neuropsychiatric Inventory

The Neuropsychiatric Inventory (NPI) was developed by Cummings et al. (1994) to specifically measure neuropsychiatric symptoms associated with both AD and non-AD dementias and has been shown to be reliable as well as valid [58]. The NPI examines 12 sub-domains of behavioral functioning: delusions, hallucinations, agitation/aggression, dysphoria, anxiety, euphoria, apathy, disinhibition, irritability/lability, aberrant motor activity, night-time behavioral disturbances and eating abnormalities.

6.1.1. Fuld Object-Memory Evaluation (FOME)

The FOME is a validated measurement of memory and learning in older adults. This test allows the examiner to evaluate memory and learning and eliminates disadvantages in relation to the effects of poor vision, hearing, language handicaps, cultural differences or inattention.

6.1.2. CAMDEX-DS: Cambridge Examination for Mental Disorders of Older People with Down syndrome and Others with Intellectual Disabilities

The CAMDEX-DS is a comprehensive assessment tool used for screening for cognitive impairment and diagnosing dementia in people with DS. This test is a modified version of the CAMCOG and the CAMDEX-R and was created specifically for the DS population. The measure includes questions assessing various domains of cognitive functioning including orientation, comprehension, expressive language, memory, attention/concentration, visuospatial skills, and executive functions.

6.1.3. Story Recall subtest of the Rivermead Behavioral Memory Test for Children (RBMT-C)

The RBMT-C provides an objective measure of everyday memory problems reported and observed in subjects with memory difficulties. The story recall subtest involves immediate free recall, cued recall, and delayed recall of short story material which is presented orally to subjects by the examiner. The RBMT-C is appealing for use in this population because the task is engaging, simple, and has been shown in other studies to be an effective measure of memory functions.

6.1.4. Vineland Adaptive Behavior Scale-II

This validated tool is utilized to measure and track adaptive behaviors (level of everyday functioning) in persons with intellectual and developmental disabilities and other disorders, such as autism, Asperger Syndrome, and developmental delays.

6.2. Procedures

6.2.1. Visit 1: Screening (Week -4)

- Obtain written informed consent from caregiver and subject (or subject's legally authorized representative) prior to any study related procedures.

- Review Inclusion/Exclusion Criteria.
- Review medical history, as it pertains to inclusion/exclusion criteria, such as research diagnosis, disease severity, and course of DS.
- Obtain demographic information
- Obtain details of medications taken over the course of the last 30 days.
- Complete physical exam, including neurological exam.
- Collect vital signs, height and weight.
- Scales for Hachinski and NPI-C.
- Collect laboratory samples for screening assessment.
- Perform a standard 12-lead ECG.
- CAMDEX-DS.
- Administer Vineland Adaptive Behavioral Scale-II to caregiver rater.

6.2.2. Visit 2: Treatment Visit

- Collect vital signs.
- Collect laboratory samples for screening assessment, for specific tests refer to Table 1.
- Review Inclusion/Exclusion Criteria.
- Review medical history, as it pertains to inclusion/exclusion criteria, such as research diagnosis, disease severity, and course of AD.
- Administer IN treatment
- Fingerstick glucose pre and 30 minutes post-treatment.
- Cognitive assessment 20 min post treatment with FOME and RBMT-C.
- Record AEs/SAEs.
- Visit 3 will be scheduled within 2 weeks (± 3 days).
- Follow-up phone call within 24 hours of dosing.

6.2.3. Visit 3: Treatment Visit

- Collect vital signs.
- Collect laboratory samples for screening assessment, for specific tests refer to Table 1.
- Review Inclusion/Exclusion Criteria.
- Review medical history, as it pertains to inclusion/exclusion criteria, such as research diagnosis, disease severity, and course of AD.
- Administer IN treatment
- Fingerstick glucose pre and 30 minutes post-treatment.
- Cognitive assessment 20 min post treatment with FOME and RBMT-C.
- Record AEs/SAEs.
- Visit 4 will be scheduled within 2 weeks (± 3 days).
- Follow-up phone call within 24 hours of dosing.

6.2.4. Visit 4: (Safety Visit)

- Collect laboratory samples for screening assessment, for specific tests refer to Table 1.

- Complete physical exam, including neurological exam. Collect vital signs, height and weight prior to ECG and blood draw.
- Perform a standard 12-lead ECG.
- Record AEs/SAEs.

6.3. Early Withdrawal

If subject withdraws from the study after the screening visit, no further evaluations are necessary. If subject withdraws from the study after visit 2, all safety assessments will be performed (see section 6.2.4.) and the investigators will be unblinded. For specific laboratory assessments refer to Table 1 Laboratory Assessments (see section 6.10.).

6.4. Safety

For all safety assessment described below, any clinically significant change will be recorded as an AE or SAE.

6.5. Physical Examination

Complete physical examination will be performed at visits 1 and 4 or if the subject withdraws or is withdrawn from the study early. Any abnormalities noted at Visit 1, will be documented as part of the subject's medical history.

6.6. Neurological Examination

Neurological examination will be performed at visits 1 and 4 or if the subject withdraws early. Any abnormalities noted at Visit 1, will be documented as part of the subject's medical history.

6.7. Vital Signs

Vital signs and O2 saturation will be recorded at visits 1, 2, 3, and 4. For within subject consistency, brachial artery pressure will be obtained in the routine fashion, the same arm will be used for all study measurements.

Blood pressure and heart rate to be measured after subject has been sitting quietly for a minimum of 5 minutes. Diastolic blood pressure will be measured at the disappearance of Korotkoff sounds. Vital sign and O2 saturation will be monitored by clinical staff during each visit of the study.

In addition, vital signs and O2 saturation will be measured pre and post study dose on Visits 2 and 3. The investigator will be notified for any baseline changes in blood pressure >20 mmHg systolic and >10mmHg diastolic or O2 saturation < 90%.

A baseline pre-dose fingerstick blood glucose will be measured followed by measurements post-study drug at 30 minutes. The investigator will be notified for any baseline changes. Percentage change from baseline glucose will be calculated as follows: $[(\text{pre-insulin serum glucose} - \text{post-insulin serum glucose}) / (\text{pre-insulin serum glucose})] \times 100\%$. Any change >10% will be considered clinically significant.

Vital signs will be taken prior to ECG and blood draw.

6.8. Weight

Body weight will be measured at all visits, without heavy outer clothing or footwear.

6.9. ECG

A standard 12-lead ECG will be performed on all subjects at baseline and visit 4.

6.10. Laboratory Samples

All subjects will be required to fast for a minimum of 12 hours prior to collection of each blood sampling. Any subject diagnosed with DS and lacking a karyotype-proven diagnosis will undergo blood draws for this test on visit 1.

During Visit 2 & 3 blood samples will be collected after study dose administration and prior to memory and cognitive testing.

7. INVESTIGATIONAL PRODUCTS

7.1. Description of Investigational Products

The Research Center will utilize the following investigation products:

- Insulin glulisine
- Placebo saline
- POD Device
- Syringe

7.2. Handling and Storage

The study drug must be handled and/or administered only by an authorized investigative staff member.

The study drug will be kept per label recommendations and institutional Standard Operational Policy, specifically, but not limited to temperature controlled secure area.

7.3. Treatment Assignment

Randomization will be stratified by gender *a priori* by the permuted block method. Sequences will be assigned for each gender by random selection of one of the twenty permutations of block of size 6 such that 3 of each gender are allocated to group 1, and 3 of each gender are allocated to group 2. The trial is a double blinded study so that neither the subject, the investigator, nor the trial coordinator will know to which sequence the subject has been randomized. To facilitate subject blinding, all subjects will undergo the same follow-up procedures. One clinician at the site will be unblinded to randomization and prepare the study dose for each subject. This person will not have responsibility for obtaining any study data. The investigators will be provided

with a sealed envelope containing the true sequence of glulisine and saline for each group if the blind needs to be broken due to unforeseen circumstances.

7.4. Packaging and Labeling

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

- IRB number
- Quantity statement
- Directions – To be administered only by investigative study staff
- Storage conditions per label
- “For Clinical Trial Use Only”

7.5. Occupational Safety

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

8. SUBJECT COMPLETION AND WITHDRAWAL

8.1. Subject Completion

Subjects completing all 4 study visits will be considered to have completed study.

8.2. 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 4 the safety procedures described in Section 6.2.4. and will be performed ideally within 14 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 final 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. Efforts will be made to recruit subjects to replace any withdrawals so as to maintain an n=12.

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

9. ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

9.1. 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. Interval development of illnesses or injuries will 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

9.2. Definition of SAE

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

- Death
- Life-threatening situation
- Hospitalization or prolongation of hospitalization
- Disability or incapacitation
- Other events determined by investigator to be medically significant in which subject's well-being is jeopardized (e.g. events that have high likelihood of escalating to the point of meeting criteria outlined above)

9.2.1. Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs and SAEs

Any new abnormal, vital, 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.

9.2.2. Time Period and Frequency of Detecting AEs and SAEs

Upon consenting, a subject is considered to be a participant 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.

10. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

10.1. Statistical Analysis

All primary and secondary endpoints will be summarized within strata defined by treatment and study period.

Treatment effects on both primary and secondary endpoints will be assessed using common statistical methodology for crossover trials. Continuous outcomes, such as the FOME and the RBMT-C, and count outcomes, such as adverse event incidence, will be analyzed using a mixed-effects linear model to account for period effects and patient characteristics such as age and sex. Plots of the observed effects will be constructed to aid with interpretation.

All statistical analyses will be completed using SAS® software. Visual representations of the data will be constructed using the package ‘ggplot2’ in R [63, 64].

10.2. Safety Outcomes

The primary endpoint is defined as the ‘incidence of any or related adverse and/or serious events of intranasal glulisine versus placebo.’ The difference in incidence of adverse events between subjects receiving glulisine and those receiving saline will be modeled in terms of rates using a mixed-effects Poisson regression model accounting for period and the treatment-period interaction as well as subject age and sex.

10.3. Cognitive Outcomes

The secondary endpoints consist of performance differences in the FOME and the RBMT-C between subjects receiving glulisine and those receiving saline. These differences will be assessed using normal mixed-effects regression accounting for period and the treatment-period interaction as well as subject age and sex.

10.4. Study Power

Power analysis for the primary endpoint (incidence of adverse events) is simplified to a comparison of paired means. The following table presents power estimates for a variety of assumptions. Assumptions common to all scenarios are a sample size of 12 and an alpha of 0.05. The difference column corresponds to the difference in number of adverse events between the treatment and the placebo. Although only half of these probable scenarios are adequately powered, such a result is acceptable for a pilot study such as this.

Difference	St. Dev.	Power
1.5	1.0	0.997
1.5	1.5	0.883
1.5	2.0	0.658
2.0	1.0	1.000
2.0	1.5	0.987
2.0	2.0	0.883

11. STUDY CONDUCT CONSIDERATIONS

11.1. Regulatory and Ethical Considerations

The study will be conducted in accordance with GCP guidelines. Subject privacy requirements will 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 all reporting requirements).

11.2. 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.

11.3. Study Closure

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

11.4. 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.

11.5. Provision of Study Results and Information to Investigators

Study results will be made available by the study statistician once analysis (Interim Analysis) is complete. Study Staff will not be unblinded in regards to individual subjects randomization status until after the database is locked.

11.6. 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, and password protected file).

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