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Description: Protocol for Intranasal Insulin for the Treatment of HAND as formatted for The Johns Hopkins University IRB [eForm A] including plan for statistical analysis. Version 3.1 was submitted to the Johns Hopkins IRB on 9/26/2018 and approved by the IRB on 10/16/2018 without changes. The protocol was current as of the last participants to consent and enroll.
23 pages (excluding cover)

JHM IRB - eForm A – Protocol

- **Use the section headings to write the JHM IRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.**
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1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

HIV-1-associated neurocognitive disorders (HAND) are characterized by disabling cognitive, behavioral, and motor dysfunction and can occur in up to 50% of HIV+ individuals even with combination antiretroviral therapy (ART) (1). The mechanisms for these residual impairments are not fully understood, but appear to involve poor penetrance of antiretroviral drugs into the central nervous system (CNS) (2), that creates a brain sanctuary for inadequately suppressed HIV infection with associated sustained inflammation. Adjunctive therapies with targeted neuroprotective agents are critically needed for the treatment of HAND. Insulin is involved in multiple CNS functions including food intake, metabolism, learning, and memory. Insulin has neuroprotective properties demonstrated in cell culture experiments and in vivo models, which provide strong evidence for its use as a therapeutic agent to treat HAND. Insulin modifying therapy (IMT) includes intranasal insulin administered via a novel nasal drug delivery device. IMT may play important roles in neuronal plasticity and survival by protecting hippocampal neurons against oxidative stress and apoptotic cell death induced by glutamate neurotoxicity. Previous studies support the proposed early phase trial of IMT as a novel therapeutic agent for HAND. Intranasal insulin and the drug delivery device will be purchased and distributed by the Johns Hopkins University Investigational Drug Pharmacy. The proposed trial uses several novel radiological and CSF surrogate markers to monitor the effects of IMT. Outcomes from these studies could have important implications for the design of future studies with IMT and other neuroprotective compounds for HAND.

2. Objectives (include all primary and secondary objectives)

The specific objectives of the trial are as follows:

Primary Objective: To examine the clinical effects of intranasal insulin in individuals with HIV associated neurocognitive disorder (HAND).

Primary Hypotheses:

1) Intranasal insulin will be safe and well-tolerated in HIV-infected patients.
2) Intranasal insulin will improve neuropsychological testing performance in patients with HAND.

Primary outcome measures:

1) Serious Adverse event frequency.
2) Cognitive performance as measured by the Global Dementia Scale (GDS)

Secondary Hypotheses:

1) Intranasal insulin will improve functional performance in patients with HAND.
2) Intranasal insulin levels will be detectable in the cerebrospinal fluid (CSF) as determined by pharmacokinetic analysis.
3) Fasting serum insulin levels will not be affected by the administration of intranasal insulin.

Secondary Objective: To examine the effect of intranasal insulin on markers associated with inflammation and CNS injury in individuals with HAND.

Hypotheses:

1) Intranasal insulin will improve neuroimaging markers associated with CNS damage and decrease markers associated with inflammation in HIV-infected patients.
2) Intranasal insulin will reduce markers of cell stress and injury while increasing energy substrate availability in CSF.
3) Intranasal insulin will increase levels of brain derived neurotrophic factor (BDNF) and amyloid-beta (Aβ) in CSF.

Secondary outcome measures:

1) Neuroimaging:
   i. Single voxel-magnetic resonance spectroscopy (SV-MRS) myoinositol, choline, and N-acetyl aspartate concentrations in frontal white matter and basal ganglia
   ii. Diffusion tensor imaging (DTI) whole brain fractional anisotropy, DTI whole brain mean diffusivity
   iii. Arterial spin labeling (ASL), a novel measure of cerebral blood flow
2) CSF biomarkers:
   i. Ceramide, Sphingomyelin, citrate, neurofilament protein.
   ii. BDNF, protein carbonyl, Aβ-42.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

SIGNIFICANCE/PUBLIC HEALTH CONTEXT OF THE STUDY

HAND continues to be an important neurological complication of HIV infection in the era of ART. HAND has a significant impact on cognitive and functional abilities (5) including employment status, driving ability, and medication adherence (6-11) that influence quality of life and longevity. The future treatment for HAND will likely need to include both ART and an effective adjunctive therapy to treat CNS-specific pathogenetic mechanisms. The urgency of this unmet need prompted us to search for an FDA approved drug that could be repurposed to treat HAND. Previous studies of the pathophysiology of insulin function in the CNS, the pathophysiology of insulin resistance in HAND, and our preliminary finding in tissue culture and rodent models of HAND strongly support the use of intranasal insulin as a potential therapeutic agent to treat HAND.

PRELIMINARY STUDIES FROM NON-CLINICAL AND CLINICAL STUDIES

Insulin resistance and HAND

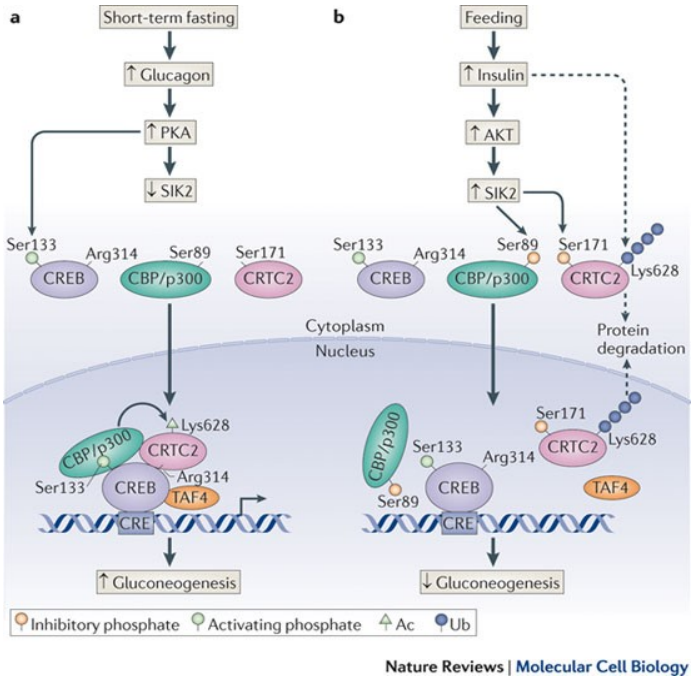
Previous studies have demonstrated that insulin resistance is associated with cognitive dysfunction in patients with HIV infection, especially in older patients ≥ age 50 years (3, 12). In the Hawaii Aging with HIV cohort, the mean serum insulin levels increased in HIV+ individuals with normal cognition (12.2), minor cognitive motor disorder (14.3), and dementia (17.1) [p=0.043, OR 1.036 (1.004-1.072) per unit of insulin], presumably as a compensatory mechanism for insulin resistance. When HIV+ individuals greater than age 50 years were examined, the strength of the association increased: mean insulin levels= 10.3, 15.04, and 19.0 for HIV+ individuals with normal cognition, minor cognitive motor disorder, and dementia, respectively [p= 0.003, OR 1.088 (1.030-1.149) per unit of insulin] (13). In a more recent study of 1036 women from the Women's Interagency HIV study (WIHS) cohort, higher insulin resistance as measured by the Homeostasis Model Assessment (HOMA) was associated with worse neurocognitive performance on the Stroop Color-Naming test, again suggesting that insulin resistance is associated with impaired cognition in HIV+ patients (3).

Neuroprotective effects of intranasal insulin

Our primary choice for the selection of intranasal insulin in the clinical trial of this project is intranasal insulin. Insulin is involved in the regulation of CNS functions such as food intake, metabolism, learning and memory, and neuronal survival. Receptors for insulin are widely distributed throughout the brain and are concentrated in the olfactory bulb, cerebral cortex, hypothalamus, hippocampus, cerebellum and choroid plexus. Disturbances in insulin sensitivity associated with diabetes mellitus impair cognitive function, and improving metabolic control in individuals with diabetes improves cognitive performance (14, 15). At the cellular level, insulin has been shown to regulate neuronal excitability, receptor expression, trafficking and function.

Exogenous administration of insulin protects neurons from a variety of insults including ischemia, oxidative stress, and glutamate induced excitotoxicity. This general neuroprotective effect of insulin involves the induction of CREB (cAMP response element binding protein a transcription factor that binds to CRE cAMP (response elements) and has an important role in learning and memory (16) (See Figure 1). Genes regulated by CREB include c-fos, BDNF, tyrosine hydroxylase, and a number of neuropeptides (somatostatin, enkephalin, VGF, and CRF). BDNF binds TrkB and p75 on neurons and promotes survival pathways. A signaling pathway that is activated by insulin and BDNF modulate learning and memory, neuroprotection and the regulation of food intake and glucose (17). The BDNF-activated pathway involves phosphatidylinositol 3-kinase and Akt kinase. Activation of this pathway has a crucial role in learning and memory, is neuroprotective in models of Alzheimer's, Parkinson's and Huntington's disease, and improves peripheral glucose metabolism by enhancing insulin sensitivity (18). Insulin also decreases levels of amyloid-beta (Aβ), the toxic protein associated with Alzheimer's disease (19), and protects against the deleterious effects of Aβ oligomers on synapses (20-22).

Figure1. Neuroprotective effect of insulin via induction of CREB



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These general neuroprotective and restorative effects of insulin suggest that insulin may also be protective against HAND. However, peripheral administration of insulin can be associated with hypoglycemia. To circumvent this potential problem, insulin has been delivered directly to the brain using an intranasal device. Intranasal administration of insulin provides rapid delivery of insulin to the CNS via bulk flow along olfactory and trigeminal perivascular channels, and slower delivery via olfactory bulb axonal transport (19). Intranasal insulin also has the advantage of not adversely affecting blood insulin or glucose levels as has been demonstrated in previous studies in humans (19). Intranasal insulin increases insulin levels in CSF to biologically relevant concentrations within 30-40 minutes and acutely enhances memory in human studies (23). In a recent study conducted by our collaborator/consultant Dr. Suzanne Craft, 104 individuals with amnestic mild cognitive impairment or Alzheimer's disease administered intranasal insulin showed improvement in neuropsychological testing, and general cognitive abilities as measured by the ADAS-cog score (a commonly used outcome measure in trials for Alzheimer's disease). Placebo-assigned patients in the study (19) also showed decreased fluorodeoxyglucose 18F uptake in parietotemporal, frontal, precuneus, and cuneus regions, whereas a matched group of insulin-treated patients showed no decline suggesting improvement in brain metabolism with insulin treatment. These positive changes in memory and function were also associated with changes in CSF Aβ42 levels. No significant adverse events were reported in this study.

Anti-inflammatory effects of intranasal insulin

Despite ART and suppression of plasma HIV replication, systemic and CNS inflammatory markers remain elevated in HIV infection. Intranasal insulin may have anti-inflammatory effects, which would be of benefit for the treatment of HAND. Anti-inflammatory effects have been observed with low doses of insulin in peripheral circulation (19, 24). Specifically, low dose insulin infusion in diabetic patients suppressed plasma concentration of chemokines including monocyte chemoattractant protein-1 (MCP-1), regulated on activation normal T-cell expressed and secreted (RANTES) and their receptors, chemokine receptor (CCR)-2 and CCR-5, in peripheral blood mononuclear cells (25). Insulin has also suppressed intranuclear nuclear factor Kβ (NFKβ), Egr-1 binding in peripheral blood mononuclear cells, and the plasma concentrations of adhesion molecules and cytokines, matrix metalloproteinases, tyrosine factor, plasminogen activator inhibitor-1, and vascular endothelial growth factor (25, 26). The effects of intranasal administration of insulin on CNS inflammation in HIV+ individuals have not been determined. Table 1 summarizes the outcomes reported in clinical studies using intranasal insulin with doses ranging from 10 to 160 IU.

Table 1. Summary of Intranasal insulin trials

REFERENCE	PATIENT POPULATION	DOSE OF INTRANASAL INSULIN TESTED	COGNITIVE ASSESSMENT TOOL
Benedict, 2004	Healthy	160 IU (long-term)	• Word list (immediate recall) • Word list (delayed recall)
Reger, 2006	Probable AD or MCI vs. healthy	20 or 40 IU (acute)	• Story recall (sum of immediate + delayed recall) • Word list (sum of immediate + delayed recall)
Benedict, 2007	Healthy men	20 IU Aspart* vs. 20 IU Regular (long term) **rapid acting*	• Word list (immediate recall) • Word list (delayed recall)
Benedict, 2008	Healthy, normal weight, with no medications	160 IU (acute)	• Digit span (immediate recall) • Object location (immediate recall) • Mirror tracing (immediate recall)
Hallschmid, 2008	Obese men	160 IU (long-term)	• Word list (delayed recall) • Word list (immediate recall) • Word list (delayed recall
Reger, 2008	AD or MCI	20 IU (long term)	• Memory saving score (immediate recall/20-min delayed recall ratio) • Voice onset time (immediate recall/20-min delayed recall ratio)
Reger, 2008	AD or MCI vs. healthy	10, 20, 40, 60 IU (acute)	• Story recall (immediate recall) • Word list learning (immediate recall) • Story recall (delayed recall) • Word list learning (delayed recall)
Krug, 2010	Healthy postmenopausal women	160 IU (acute)	• Digit span (immediate recall) • Object location(immediate recall)
Fan, 2011	schizophrenic	I40 IU (acute)	• Hopkins Verbal Learning Test (Immediate recall) • Hopkins Verbal Learning Test (Delayed)
Craft, 2012	AD or MCI	20 or 40 IU	• Story recall (delayed recall)
McIntyre, 2012	Euthymic with bipolar disorder	40 IU (long term)	• California Verbal Learning Test, second edition • Process Dissociation Task
Novak , 2013	Diabetic	40 IU (long term)	• Brief Visuospatial Memory Test-Revised • Verbal fluency measures
Fan, 2013	schizophrenic	40 IU (long term)	• Positive and Negative Syndrome Scale (PANSS) • Scale for Assessment of Negative Symptoms (SANS) • Cognitive battery.

Rationale for primary objective

We will conduct a 24 week double-blinded, placebo-controlled Phase I/II trial of intranasal insulin for the treatment of HAND in 40 individual subjects. The Johns Hopkins HIV Neurology group has participated in multiple clinical trials for the treatment of HAND including studies of abacavir, selegiline (ACTG 5090), minocycline (ACTG 5235, N. Sacktor PI), and paroxetine/fluconazole (NA_00037283). In the paroxetine/fluconazole study which required a lumbar puncture, 45 HIV+ individuals were enrolled over 48 months. Thus, the goal of recruiting 40 HIV+ individuals within a 36 month period as proposed in the current study is feasible. The gold standard for identifying patients with HAND remains neurological evaluation and neuropsychological (NP) test performance. Aim 1 will evaluate whether intranasal insulin for 24 weeks is safe and well tolerated and secondarily, improves cognitive impairment and functional performance in individuals with HAND. We anticipate that surrogate markers will further support and may extend the neuropsychological test results.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).
b. Study duration and number of study visits required of research participants.
c. Blinding, including justification for blinding or not blinding the trial, if applicable.
d. Justification of why participants will not receive routine care or will have current therapy stopped.
e. Justification for inclusion of a placebo or non-treatment group.
f. Definition of treatment failure or participant removal criteria.
g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Study Design

The study is designed as a 24 week double-blind, placebo-controlled Phase I/II clinical trial. Participants will be randomly assigned to one of two groups: 1) intranasal regular insulin or 2) placebo. The investigational product will be administered at the daily dose of 40 IU insulin (20 IU insulin twice a day, 30-60 minutes after a meal, i.e., after breakfast and dinner) or equal volume, twice daily placebo. Both will be administered with a nasal drug delivery device [POD, Impel Neuropharma Inc., Seattle, Washington]. The device works by releasing a metered dose of the investigational product into a chamber covering the participant's nose, which is then inhaled by breathing regularly for 2 minutes until the prescribed dose is delivered. The dose of intranasal insulin prescribed in this protocol is also currently being tested in a large multicenter study for the treatment of Alzheimer's disease because of its safety and efficacy as a neuroprotective strategy in prior trials for Alzheimer's disease.[Suzanne Craft – personal communication] The investigational product will be purchased by the JHU Investigational Drug Pharmacy, which will also perform randomization. A computer generated block randomization plan will ensure an approximately equal distribution of subjects (20 in each group) among the two treatment groups.

Prior to the baseline study visit, the protocol requires two visits during which screening assessments and procedures are performed. Randomization, start of study drug, and enrollment in the trial occur at the baseline visit. Once enrolled there are monthly follow-up visits focused on safety labs, monitoring adverse events, and assessing compliance with study drug administration. Outcome measures are collected at the baseline, week 12, and week 24 visits (details provided below). Those participants who agree to the optional pharmacokinetics portion of the protocol will have additional procedures at a week 1 and week 4 visit as well as the week 12 visit (see details for optional PK portion of the protocol below).

Use of placebo and blinding in this protocol is justified by the higher scientific validity of the double-blind, placebo-controlled trial design, given that there is currently no FDA approved alternative treatment for HAND and there is no evidence that the target population of this study is being denied care or at increased risk of harm if given placebo instead of the investigational product. Blinding will not put any undue burden on the participant or study team. The investigational product and placebo will look identical for all participants, and the integrity of blinding will be assessed by participant and researcher questionnaires. A Data Safety Monitoring Board (DSMB) will regularly review unblinded safety data. Participation in this study will not require a change in either routine care or current therapy.

Human Subject Selection

The study involves the recruitment of 40 individuals with HIV infection and cognitive impairment over three to four years. Study participants will include only adults (18 to 69 years old) and will be non-institutional (i.e., no prisoners). Study candidates who are too cognitively impaired to provide informed consent will not be enrolled because they are unlikely to be able to complete the protocol specified neurocognitive evaluations. Individuals over the age of 69 are also excluded from this study to avoid potential confounding effects of age-related decline in cognitive functioning.

Individuals with significant difficulty speaking, reading or writing English, or who have completed less than 7th grade education level will be excluded because they may provide inaccurate neuropsychological testing results or have difficulty completing the neuropsychological testing procedures. We currently do not have comparable neuropsychological measures and normative data for languages other than English.

Women who are pregnant or nursing will not be enrolled in the study due to, 1) the Category B status of insulin, and 2) the procedures and data collection needed to assess risk to fetus or nursing baby are outside the scope of this protocol. Participants who are able to become pregnant will be required to use appropriate methods of contraception for the trial duration, and all women able to become pregnant will be evaluated with a pregnancy test at screening and follow-up visits. Women 55 years of age and over who have not had a period for one year will be considered menopausal and will not need pregnancy testing or contraception. Women under age 55 will undergo pregnancy testing and will be required to use appropriate methods of contraception for the trial duration, unless there is a history of hysterectomy, bilateral oophorectomy, or medically-documented ovarian failure. Adequate methods of contraception include: implanted contraception, intrauterine device in place for at least 3 months, or barrier method in conjunction with spermicide. Women of childbearing potential must have a negative pregnancy test at screening and be non-lactating.

In the event of pregnancy, the study agent will be discontinued but the subject will remain on study through pregnancy outcome for safety monitoring; a report of the pregnancy will be made to the DSMB and IRB; and the participant will be advised to notify their obstetrician of exposure to the study agent.

It is justified to exclude children because of the low incidence of HIV infection among children, and because it would not be feasible to use these clinical tests (serial lumbar punctures) on children. In addition, age-appropriate neuropsychological tests would be needed and normative data for some of the neuropsychological tests are not available in children. Finally, imaging children would require sedation. For these reasons, we will NOT be studying children. The proposed cohort will be representative of the epidemic in Maryland. We estimate that we may need to screen up to 100 candidates in order to enroll 40 participants.

Clinical assessments

The screening visit and clinical assessments will include standard demographic information, medical history, antiretroviral medication history, alcohol and illicit substance use, and other medical illnesses. The neurological examination will include the ACTG macro-neurological examination (54). The schedule of events is detailed in Table 2.

Table 2

Evaluating	Screen	Pre-entry	Baseline	Wk. 1	Wk. 4	Wk. 8	Wk. 12	Wk 16	Wk 20	Wk. 24
HIV documentation; Incl./exclusion assessment	X	X	X							
Medical history	X		X(‡)	X(‡)	X(‡)	X	X(‡)	X	X	X
Concomitant medications	X		X(‡)	X(‡)	X(‡)	X	X(‡)	X	X	X
Neurological exam	X		X				X			X
Neuropsychological tests	X	X	X				X			X
Beck depression inventory	X		X				X			X
HAND Rating Scale	X		X				X			X
Functional, quality of life, fatigue assessments	X		X				X			X
Blood draw	X	X	X(‡)	(‡)	X(‡)	X	X(‡)	X	X	X
Urine tests	X	X	X		X	X	X	X	X	X
Pregnancy test	X		X		X		X			X
Optional Lumbar puncture/CSF tests		(X)(‡)					(‡)			(X)
MRI scan			X							X
Study drug compliance assessment				X(‡)	X(‡)	X	X(‡)	X	X	X
Smell function test			X							X
Blinding Questionnaire Investigator/Subject										X
(‡)Optional pharmacokinetics study – additional procedures/biosamples/assessments										

Neuropsychological (NP) testing is repeated on three occasions during the screening-baseline period to reduce changes due to practice effects during the 24 week period. During the trial, NP testing will be repeated at weeks 12 and 24. The NP test battery (Table 3) consists of 13 tests that cover 7 major cognitive domains, and it takes 60-75 minutes to complete. The tests have been shown to be sensitive to the earliest cognitive changes associated with HAND.(9) Z-scores will be calculated for each neuropsychological test using age and education adjusted norms to define cognitive impairment (see Section 5, inclusion criterion number 4). An optional baseline lumbar puncture (LP) will be performed during pre-entry on participants who are able and willing to undergo the procedure.

Week 4 evaluations include vital signs, blood tests, and, as needed, a targeted physical examination if the participant reports an AE requiring follow up. At weeks 12 and 24 evaluations will include the clinical assessments, neurological examination, safety evaluations/blood tests, NP testing, depression assessment, functional assessments and optional lumbar puncture (week 24/final visit). The optional pharmacokinetics procedures (see below) will include additional blood draws (baseline, week 1, week 4, week 12) and LP (week 12). A smell function test will be performed at baseline and week 24 visits to assess risk of changes to olfactory sensation. If a participant raises subjective symptoms suggesting altered or impaired smell during the trial, the study team will perform additional smell tests, as needed.

Routine safety blood tests will include fasting glucose and insulin levels (insulin resistance measured by HOMA), HIV viral load, CD4/T-cell count, electrolyte panels including liver function tests, and complete blood counts performed at screening, pre-entry, baseline, monthly after baseline, and, if deemed necessary by the investigator, *ad hoc* visits for safety evaluation. In addition there are non-routine blood tests performed prior to enrollment (refer to Table 1 in Appendix A, page 23, for complete schedule of lab tests). Urine toxicology screens will be performed at screening, pre-entry, baseline, and weeks 4, 8, 12, 18, 20 and 24. Routine safety labs will be performed in real time by the Johns Hopkins Hospital Laboratory Services (JHHLS). CSF specimens will be collected at the pre-entry visit and week 24 visit. CSF differential cell count, as well as protein and glucose levels will be performed by the JHHLS, while CSF samples for HIV RNA levels, fasting insulin levels, and outcome measures will be stored in a -80 degree C freezer for assays to be run at the conclusion of the study.

Table 3. Neuropsychological Test Battery

Cognitive Domain	Test/Subtests	Time (Mins)
Verbal memory	Hopkins Verbal Learning Test Delayed recall score Delayed recognition score	20
Visual Memory	Rey Complex Figure Delayed recall score	3
Visuo-construction	Rey Complex Figure Copy score	20
Psychomotor	Symbol Digit Modalities Test Trail Making Test A Computerized Reaction Time Test (CalCAP) Simple reaction time score Choice reaction time score	3 2 10
Motor Speed (fine)	Grooved Pegboard Dominant hand score Non-dominant hand score	5
Motor Speed (gross)	Timed Gait	2
Frontal/Executive	Trail Making Test B Verbal Fluency (F,A,S) Test Stroop Color Interference	3 5 5
Screening tests	International HIV Dementia Scale	3
Literacy (baseline only)	National Adult Reading Test	5

Unblinded data, including adverse events and toxicities, will be made available to a DSMB who will review semiannual closed reports (from PI) and open reports (from site pharmacist, the only unblinded member of the study team).

Participants will be removed: 1) if they develop a side effect, laboratory abnormality or medical condition that, in the opinion of the investigator, constitutes a significant safety risk for the subject; or 2) if a study participant is noncompliant with study medications as prescribed or unable to comply with study assessments. A study participant can stop participation in the study at any time or choose premature discontinuation of the investigational product (with continued follow up visits for intent to treat analysis). At the conclusion of the study, a participant's therapy will end and the participant will receive routine clinical care, including Department of Neurology consultation services if requested.

Therapy and Follow-up When the Study Ends or Participation Ends Prematurely

When the study ends, we will give participants the option of setting up a clinical appointment with the PI to assess options for follow up care.

In order to help evaluate safety, participants who stop their study drugs prematurely may be asked to return for their regularly scheduled visits. If a participant discontinues the investigational product due to an AE possibly or definitely attributed to the study drug, the study team will ask the participant to return for all remaining visits and procedures for intent-to-treat analyses. Follow-up for all other study drug discontinuations, either directed or undirected, will be evaluated based on: 1) reason for discontinuation, 2) the number of days following baseline when the study drug was stopped, and 3) the estimated total (cumulative) dose of study drug up to the point of discontinuation.

If the participant must altogether leave the study prematurely, we will ask the participant to come in for a final visit. At this final visit we will ask the participant to return all unused doses of the study drug, as well as the POD, and ask him/her to complete questionnaires, including the blinding questionnaire. The PI may also request certain tests or procedures from the study visits not yet completed. Returning to perform incomplete tests or procedures at the final visit will be requested at the PI's discretion after reviewing the reason for leaving the study and assessing how much of the study has been completed up until the time of leaving.

Neuroimaging protocol

The MRI study will be performed on the commercial Philips Trio 3.0 Tesla scanner, using the Philips phase-array head coil and the following protocols:

Standard MRI protocol: The MRI protocol includes a 3-plane localizer (TR/TE = 20/5 ms; 1 NEX; 128x256x1), a sagittal 3D MP-RAGE (TR/TE/TI = 2200/4.91/1000 ms; 1 NEX; 256x256x160), and an axial FLAIR sequence (TR/TE = 10,000/85 ms; 1 NEX; 205x320x28) to rule out any focal brain abnormalities. MRI scans will be reviewed by a site radiologist within one week for possible lesions (neoplasm, stroke, opportunistic infections); if clinically significant lesions are found (or incidental findings), we will immediately inform the participant and if serious his/her physician.

Quantitative 1H MRS protocol: Single-voxel 1H spectra will be acquired using a customized protocol [which includes water T2 measurement] that is based on the product version of the PRESS sequence. Voxels 6 cc in volume will be prescribed in 4 regions: midline frontal gray matter, right mid-frontal centrum semiovale (white matter), right basal ganglia (deep gray matter), and parietal cortex gray matter. Field homogeneity and water suppression will be adjusted using automated algorithms. Water suppressed spectra will be collected with TE/TR = 30/3000ms, bandwidth = 2000 Hz, 128 averages, 8-step phase cycling. The short TE yields excellent signal-to-noise ratio and allows observation of coupled spin systems, such as myoinositol (mI) and glutamate (Glu) plus glutamine (Gln). In addition, the customized protocol will automatically collect 7 single-scan, fully relaxed water FIDs from each voxel at variable echo times (TE=30, 45, 65,100, 200, 500 and 1500 ms; TR=10 ms) from which metabolite concentrations are calculated.

The total examination time per voxel is about 7 minutes. For quality control, a phantom spectrum will be obtained within 24 hours of each in vivo scan, using the identical MRS protocol as described above.

DTI protocol: A DTI series with whole-brain coverage will be acquired (spin-echo EPI, TR/TE = 3700/88 ms, b=[0,1000] s/mm2, 12 directions, 4mm slices with 0.5mm gap). Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps will be calculated and regions of interest (ROIs) will be manually drawn in a standardized fashion to assess mean diffusivity (MD) and FA, using DTI-Studio. The following regions will be evaluated in both right and left hemispheres: caudate, globus pallidus, thalamus, genu of corpus callosum, frontal white matter, and parietal white matter. Analysis of each individual ROI MD and FA as well as analysis of whole brain MD and FA will be performed.

ASL protocol: ASL will be performed using the 'FAIR' technique with a gradient-echo EPI readout, with a single axial slice at the level of the head of the caudate nucleus. Our collaborators have developed considerable experience in using ASL to measure cerebral blood flow (CBF) and now routinely acquire ASL images in the human brain at 3T. We performed a study of 12 cognitively normal HIV- subjects who were scanned four times (at 0, 3, 6, and 12 months) and no significant difference in CBF in the medial frontal gyrus was found over this period 55. Data obtained from the first two scans were analyzed by two readers, and showed high reliability [intraclass correlation coefficient (ICC) > 0.97] and reproducibility [within subject coefficient of variation (wsCV)
< 6%].

Measurement of markers of oxidative/nitrosative stress, energy metabolism, and neuronal/axonal injury

Measurement of lipid markers of oxidative stress in CSF and plasma will be conducted as described previously (46). The measurement of these lipid markers of oxidative stress (e.g., ceramide, sphingomyelin), and energy metabolism (e.g., citrate, acetate) will be performed in the laboratory of Dr. Norman Haughey. CSF concentrations of protein carbonyls will be measured by Western blot as described previously (45) and neurofilament protein (NFL) will be analyzed using an enzyme-linked immunoassay (ELISA) described previously, (49), in the laboratory of Dr. Avindra Nath at NINDS. BDNF will be measured by protein macroarray as described previously (51) in the laboratory of Dr. Norman Haughey. Insulin binding proteins 1-7 and Aβ42 will be measured using the multiplex XMAP Luminex platform (Luminex Corp, Austin, TX) in the laboratory of Dr. Carlos Pardo. Fasting AM CSF will be collected at the same time each day to prevent diurnal variation in Aβ levels. CSF will be collected using polypropylene tubes to prevent Aβ from sticking to polycarbonate tubes of standard LP kits. Blood and CSF will also be collected and stored for additional biomarkers to be determined at the conclusion of the study.

Sources of Data

Research data and materials include blood, urine, cerebrospinal fluid (CSF), neurological exam notes, neuropsychological test results, functional evaluations, interview data about medical history and drug use, and both imaging and quantification data from cranial MRI. Most outcome data for this study are not from clinically validated tests or procedures and therefore will be obtained for research purposes only. However, routine lab tests performed by the JHH core lab, clinical exam notes by the study team physicians, and clinically interpreted structural MRI reports will be part of the participant's electronic patient record and available for clinical use. All data obtained within this project will occur following written consent by each participant and will comply with HIPAA guidelines for data collection and disclosures. The confidentiality of the data is maintained through the use of a secure, double-lock office storage space and an encrypted, password-protected computer database.

Pharmacokinetics (PK) protocol (optional)

An optional PK protocol will be discussed with each participant during the consent process for the clinical trial. Target participation for the PK protocol is 10 subjects with complete evaluable data. During the screening period, the study team will ask each participant if he or she would be interested in the optional PK procedures. The study team will also assess whether the participant could practically, reliably, and safely complete the extra PK procedures (e.g., is phlebotomy or LP difficult for the participant?). For each participant who enrolls in the clinical trial and consents to consideration for the PK testing, selection will be made by order of enrollment until the target of 10 subjects is reached. Participants must be able and willing to undergo the optional baseline LP and must decide to participate in the PK protocol during pre-entry by the time of LP because it requires additional CSF to be collected at that time. Participants in the optional PK protocol can change their minds at any time.

The PK protocol has two components. The first will compare pre-intervention ARV levels in plasma and CSF with levels on intervention at Week 1 (plasma), Week 4 (plasma), and Week 12 (plasma and CSF). Blood and CSF will be collected in the morning before each participant takes his or her prescribed ARVs in order to measure pre-dose ARV C_{min} concentrations. Pre-dose blood samples will also be collected at Week 1 and Week 4. If the participant takes an ARV that can only be administered in the evening, the morning blood draw will be scheduled at the same time interval relative to dosing at each visit.

The second component of the PK protocol will occur during the regularly scheduled Week 12 visit, which will be extended to permit 8 blood draws by peripheral intravenous access line and an LP. The timing of the sample collection will follow the schema in Table 4. The morning dose of insulin will be administered in the clinic in a supervised manner, and sampling times calculated from the observed dosing. Blood samples for plasma insulin and ART concentrations will be obtained as indicated. The geometric mean of concentrations at the PK visits will indicate the plasma minimum concentration of insulin.

Table 4. Pharmacokinetic Study Schedule (Week 12 Visit)

Time following dose administration (minutes)	Blood draw	CSF collection	Neuropsychological testing
0	X		
10	X		
30		X	
45	X		X
90	X		
120	X		
180	X		
360	X		
480	X		

Once the PK protocol accrual target is met, the stored specimens will be transferred to the research laboratories responsible for testing. Insulin levels will be measured by ELISA (this is more sensitive than mass spectrometry) in the laboratory of Dr. Barbara Slusher. ART drug concentrations will be assayed in the Clinical Pharmacology Analytical Laboratory by Dr. Mark Marzinke using methods as described previously (68, 69). Common antiretroviral drugs including tenofovir, emtricitabine, efavirenz and several newer drugs including maraviroc and dapivirine will be measured.

Upon transfer of the specimens, the research pharmacist will provide Drs. Slusher and Marzinke with the treatment allocations associated with each set of specimens. A separate PK protocol specimen ID, (different from the clinical trial subject ID) will be used to identify each of the specimens. The treatment blinding will be lifted for those 10 subject specimens only and limited to Drs. Slusher and Marzinke. Communication between the research labs and the rest of the study team will be limited to only that which is necessary to transfer and carry out the procedures in order to avoid possible compromise to the treatment blinding. The research labs will not be able to link the PK protocol specimen IDs to the clinical trial subject IDs, clinical PHI, or other personal identifiers.

5. Inclusion/Exclusion Criteria

Inclusion Criteria:

1) HIV+ based on ELISA and confirmed by either Western blot or plasma HIV RNA,

2) Capable of providing informed consent,

3) Between the ages of 18-69 years,

4) Presence of neuropsychological testing impairment as defined by performance at least 1.0 standard deviation below age-matched and education-matched controls on three or more independent neuropsychological tests at the screening visit, or performance at least 2.0 standard deviations below age-matched and education-matched controls on one independent neuropsychological test and at least 1.0 standard deviation below age-matched and education-matched controls on a second independent neuropsychological test at the screening visit,

5) Stable HAART regimen for at least 6 months (180 days) prior to study entry, with no plans to change the antiretroviral regimen over the study period (confirmed by discussion with a patient's primary provider),

6) The following lab values within 2 weeks prior to entry: hemoglobin > 8.9 g/dl, absolute neutrophil count > 500 cells/mm3, platelet count > 50,000 cells/mm3, ALT < 2.5 X upper limit of normal, alkaline phosphatase < 3 X upper limit of normal, serum creatinine ≤ 2 X upper limit of normal,

7) Negative serum or urine β-HCG pregnancy test for all women of reproductive potential (have not reached menopause or undergone hysterectomy, oophorectomy, or tubal ligation),

8) Neurological examination by a physician to assess for space-occupying brain mass lesion, and if suspected, neuroimaging with CT or MRI must confirm the absence of a mass lesion prior to optional lumbar puncture and study entry,

9) Examination by physician revealing no contraindication or current impediment to using the intranasal device prior to starting the trial (e.g., traumatic obstruction to nasal passage, chronic sinus infections, significant and symptomatic seasonal allergies, etc.),

10) At least one plasma HIV RNA test within 12 months prior to the screening visit with results less than 400 copies/mL, as well as plasma HIV RNA tests at both the screening visit and a pre-entry visit (within 14 days prior to baseline/randomization) less than 400 copies/mL,

11) No clinical signs or evidence of intoxication during screening and pre-entry visits; evidence includes but is not limited to urine drug toxicology testing by the study team during each visit. Prior to enrollment participants must produce at least three different urine drug toxicology screen test results, per protocol, in which cocaine, methamphetamine, and heroin (or equivalent opiate/opioid metabolite) are negative; marijuana (THC) must be negative in the absence of taking prescribed medications known to produce a positive result for marijuana (THC), such as efavirenz or cannabinoid treatments, in which case clinical assessment of possible marijuana intoxication will be made at the discretion of the investigator; any test results positive for psychoactive prescription drugs included in the toxicology screen must be explained by documented prescription history, and clinical assessment of possible intoxication will be made at the discretion of the investigator.

Exclusion Criteria:

1) Current or past opportunistic CNS infection at study entry,

2) History or current clinical evidence of schizophrenia,

3) History of chronic neurological disorder such as multiple sclerosis or uncontrolled epilepsy,

4) Active symptomatic AIDS defining opportunistic infection within 30 days prior to study entry,

5) History of an uncontrolled medical illness or current severe affective disorder (e.g., depression with suicidal intention) which in the opinion of the investigators would constitute a safety risk for patients or interfere with the ability of a patient to complete the study,

6) Exclusion from the optional lumbar puncture procedure (but not the clinical trial) if the participant is currently treated with anticoagulants including coumadin, heparin, or low molecular weight heparin, or if upon screening exam, the investigator identifies any contraindications or undue risks to undergoing voluntary lumbar puncture,

7) History of diabetes or treatment with insulin or an oral hypoglycemic agent,

8) Amylase/lipase elevation (≥ 2X upper limit of normal) within 14 days prior to baseline/randomization,

9) Detectable plasma HIV RNA test ≥400 copies/mL within 6 months prior to baseline/randomization,

10) History of any endocrine related cancer including any thyroid tumor,

11) Current use of cocaine, heroin, or methamphetamine. *Current use* will be defined and determined by any evidence of such use within the two years (730 days) prior to study enrollment; evidence includes but is not limited to urine drug toxicology testing by the study team at screening and pre-entry visits,

12) HIV+ individuals with moderate or severe confounding illnesses. Specifically, the impact of comorbid conditions on neurocognitive performance will be examined using a comorbid condition scale developed by Dr. Robert Heaton at UCSD (9). The effects of comorbid conditions are rated as minimal, moderate, or severe in this scale. Examples of comorbid conditions included in the scale include mild learning disability, past substance use disorder, traumatic brain injury with loss of consciousness >30 minutes.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.
b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.
c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

Evidence shows that the dose of 40 IU intranasal insulin (20 IU of insulin twice a day) is safe and well-tolerated. This dose of intranasal insulin is currently being tested in a large multicenter study for the treatment of Alzheimer's disease because of its safety and efficacy as a neuroprotective strategy in prior trials for Alzheimer's disease.[Suzanne Craft – personal correspondence] This dose was also found to be efficacious in improving memory performance (water maze task) in the EcoHIV animal model for HAND. The intranasal drug delivery device we have chosen for the protocol [POD®, Impel NeuroPharma, Inc., Seattle, WA] is currently being used with regular insulin in other FDA approved clinical trials. For further clarification of the rationale and justification of this treatment and dose, see Section 3 of the eFormA (Table 1 lists the literature referenced for justification of drug and dose selection).

7. Study Statistics

a. Primary outcome variable.
b. Secondary outcome variables.
c. Statistical plan including sample size justification and interim data analysis.
d. Early stopping rules.

Safety and tolerability outcome measures/endpoints

To assess the safety and tolerability of intranasal insulin, we will maintain a record of adverse events using standardized ACTG clinical reporting forms and provide attribution to each of these events (e.g., study drug related). We will compute proportions of individuals reporting these adverse events in each group.

Safety and tolerability outcome analysis: Frequency of symptoms, signs, and toxicities by treatment will be examined with the Fisher's exact test. Time from treatment initiation to the development of a toxicity and/or symptom or sign will be compared across treatment arms using the log rank test. In addition, analyses will be performed for changes in laboratory test results, smell test results, vital signs and HIV markers for both this aim (e.g., HIV RNA levels) and aim 3 (e.g., surrogate measures). Variables that seriously violate the assumption of normality will be transformed using the natural logarithm for purposes of statistical analyses.

Neuropsychological testing and functional performance outcome measures/endpoints

This study also seeks to provide estimates for the effect of the two treatment groups (intranasal insulin and placebo) on cognitive impairment in individuals with HAND. Each individual will be characterized on the basis of the arithmetic difference between week 24 and baseline NP test battery performance. The primary outcome measure for NP performance will be the GDS (55). Secondary outcome measures will include the NPZ8, and subjective and performance based measures of functional performance.

The GDS score weighs the number and severity of deficits in an individual's neuropsychological test (NP) performance giving relatively less weight to performances within and above normal limits. The GDS is computed by converting demographically corrected standard scores (T scores) on individual NP measures (see Table 5) to deficit scores ranging from 0 (no impairment) to 5 (severe impairment). The deficit scores are then averaged to create the GDS measure (55)(56). The NPZ-8 is a global measure of cognitive functioning from tests outlined in the neuropsychological test battery for this study and closely correlates with HIV dementia stage (57, 58). The NPZ-8 is derived by taking the average of the age and education-adjusted Z scores for five tests (see Table 5) (59-66).

Other secondary endpoints of neuropsychological test performance will include the 24 week change in the individual neuropsychological tests, and the cognitive domain scores. Each cognitive domain will be derived as the average standardized Z score of the neuropsychological tests that corresponds to it. The cognitive domains that will be examined (and the neuropsychological tests involved) are: gross motor function (timed gait), fine motor function (average of Grooved Pegboard test for the dominant and non-dominant hands), psychomotor speed (Trail Making tests part A, Symbol Digit Modalities test, and the CalCAP choice and sequential reaction times), verbal memory (Hopkins Verbal Learning test-Revised, learning and delayed recall), visual memory (Rey complex figure delayed recall), visuo construction (Rey complex figure copy), and frontal/executive function (Stroop Color Interference Test, interference task, verbal fluency test, and Trail Making part B) (see Table 3). Depression symptomatology may be present in some patients, so the impact of depression symptomatology will be evaluated with the Beck Depression Inventory (67).

Table 5

Individual NP Testing Measures	Eligibility Assessment	NPZ-8	GDS
Hopkins Verbal Learning Test			
Trial 3			
Trials 1-3 total			X
Delayed recall	X		X
Delayed recognition	X		
Rey Complex Figure			
Copy score	X		X
Delayed recall score	X		X
Symbol Digit Modalities Test			
Total score (120 sec)	X	X	X
Trail Making Test			
Part A, time to complete	X	X	X
Part B, time to complete	X	X	X
Computerized (CalCAP) Reaction Time Test			
Simple reaction time, mean	X	X	X
Choice reaction time, mean	X	X	X
Grooved Pegboard			
Dominant hand, time	X	X	X
Non-dominant hand, time	X	X	X
Timed Gait			
Three trial mean, time		X	X
Verbal Fluency (F,A,S) Test			
Total score	X		X
Stroop Test (Kanalli Version)			
Trial 1, colors			
Trial 2, words			
Trial 3, color interference	X		X

Functional assessments will also be utilized to evaluate efficacy. These will include both subjective self-evaluation of performance and potentially more objective performance-based measures of function. Subjective measures will include 24 week change in the Karnofsky functional performance score and the Instrumental Activities of Daily Living (IADL) Questionnaire. Performance based measures of functional status will include the modified version of the medication management test which evaluates an individual's ability to manage medications (11) in a brief 5-10 minute assessment. Another performance based measure of driving capabilities, the Computerized Assessment of Mild Cognitive Impairment (CAMCI) Driving Simulation test will also be performed to evaluate the utility of this new functional outcome measure. Additional assessments of efficacy will include Quality of Life assessments.

Neuropsychological testing and functional performance outcome analysis: We have designed the study with the sample size of 20 per group for a total of 40 participants. We anticipate approximately 10% loss to follow-up with the longitudinal data. This would result in 36 evaluable participants (18 per group). Based on a previous study of GDS performance in patients with HAND, our study with 18 participants per aim will have 80% power with an alpha= 0.05 to detect a 0.45 SD improvement in GDS. We do NOT expect that the current study will provide conclusive evidence of efficacy. Rather, the current study will provide estimates of location (mean) and spread (standard deviation) of the distribution of change in NP test performance for each of the two treatment groups for future studies. Through randomization, it is expected that baseline demographic and clinical characteristics will be equalized between the two treatment arms (intranasal insulin and placebo). However, in the event that there are significant baseline differences between the two treatment arms with respect to demographic or clinical characteristics, our statistical plan can be expanded to incorporate the use of linear regression to model GDS as a function of treatment arm and controlling for significant covariates.

Blood and CSF biomarker outcome measures/endpoints

CSF biomarker measures include levels of ceramide, sphingomyelin, citrate, acetate, neurofilament protein, brain derived neurotrophic factor (BDNF), protein carbonyl, insulin binding proteins 1-7, and amyloid-beta (Aβ).

Blood and CSF biomarker outcome analysis: The change in the CSF surrogate markers will be examined for differences between the treatment arms using the Student's t-test in order to test for differences across the outcome measures. Using CSF protein carbonyl levels as an example of one of the markers which will be examined in this aim, in our previous data, the mean (SD) protein carbonyl level was 630.5 (141.0) among HIV+ individuals with normal cognition, and 859.0 (188.0) among HIV+ individuals with mild HIV dementia. If it is assumed that there would be no change in the protein carbonyl level from baseline to the 6 month follow-up visit among the HIV+ individuals with HAND in the placebo arm, and the change in protein carbonyl level among the HIV+ individuals with HAND in a intranasal insulin arm would be on the order of the difference observed between the HIV+ individuals with normal cognition and HIV+ individuals with HAND above, then using a Student t-test on the calculated change, if we were to enroll a total of 40 individuals and assume a 10% drop-out rate for 36 evaluable patients), we would have sufficient power (82%) to detect a true difference in change in protein carbonyl expression of 1.2 SD between the treatment arms. Based on the sample size, the proposed study will provide important preliminary data for a future larger study of efficacy using this marker.

Imaging outcome measures/endpoints

For the quantitative 1H MRS protocol, single voxels, 6 cc in volume, will be prescribed in 4 regions: midline frontal gray matter, right mid-frontal centrum semiovale (white matter), right basal ganglia (deep gray matter), and parietal cortex gray matter. Field homogeneity and water suppression will be adjusted using automated algorithms. Water suppressed spectra will be collected with TE/TR = 30/3000ms, bandwidth = 2000 Hz, 128 averages, 8-step phase cycling. The short TE yields excellent signal-to-noise ratio and allows observation of coupled spin systems, such as myoinositol (mI) and glutamate (Glu) plus glutamine (Gln). In addition, the customized protocol will automatically collect 7 single-scan, fully relaxed water FIDs from each voxel at variable echo times (TE=30, 45, 65,100, 200, 500 and 1500 ms; TR=10 ms) from which metabolite concentrations are calculated.

For the DTI protocol, apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps will be calculated and regions of interest (ROIs) will be manually drawn in a standardized fashion to assess mean diffusivity (MD) and FA, using DTI-Studio. The following regions will be evaluated in both right and left hemispheres: caudate, globus pallidus, thalamus, genu of corpus callosum, frontal white matter, and parietal white matter. Analysis of each individual ROI MD and FA as well as analysis of whole brain MD and FA will be performed.

The ASL protocol will be performed using the 'FAIR' technique with a gradient-echo EPI readout, with a single axial slice at the level of the head of the caudate nucleus.

Imaging outcome analysis: Changes in imaging measures will be based on a pre and post treatment design. Specifically, changes in MRS, DTI, and ASL measures from baseline to 24 weeks will be assessed through a Hotelling t-squared test to determine if there is an overall difference between the two treatment arms (intranasal insulin and placebo), using a Type 1 error level of 0.05. The Hotelling t-squared is a multivariable analog to the Student t-test to allow comparison of two groups on the vector of measures.

Based on MRS changes in another study evaluating insulin infusion in HIV- individuals in which those with high insulin sensitivity had an increase in NAA/Cr and a decrease in Cho/Cr compared to baseline, it is assumed that HIV+ individuals would behave similarly to those participants with high insulin sensitivity. The current study will enroll 40 participants (randomly assigned to intranasal insulin or placebo). We anticipate approximately 10% loss to follow-up, resulting in 36 participants (18 per group). We hypothesize to see a similar change in brain metabolites in the intranasal insulin group. We will examine this data using a Student t-test of change in measures between baseline and 24 weeks. The current design has adequate power to detect an increase in NAA/Cr (power=0.91) and a decrease in Cho/Cr (power =0.86) among HIV+ individuals in the intranasal insulin group relative to the placebo group. Power calculations were made using sampsi (Stata version 12, Stata Corp. College Station TX).

It is anticipated that randomization will ameliorate any baseline demographic and clinical characteristics between the two treatment arms. If this is not the case, our statistical plan can be expanded to incorporate the

use of linear regression to model brain metabolites as a function of treatment arm and controlling for significant covariates. MRS, DTI, and ASL markers, will be compared using both a nonparametric (Mann-Whitney U-test) and parametric (Students t-test) significance test. Bonferroni correction and also a false discovery rate (FDR) correction will be applied to control for multiple comparisons. Two-tailed tests will be used. Differences will be judged significant at $p < 0.05$ without correction, and at $p < 0.05/N$ with Bonferroni correction, where N is the number of regions compared. Our pilot study evaluating these neuroimaging markers after treatment with intranasal insulin will provide important preliminary data to evaluate the sample size needed for a larger study of efficacy.

Pharmacokinetics outcome measures/endpoints and analysis

PK protocol data will include ARV drug levels and insulin levels. Analysis of PK data will include the generation of concentration-time data for insulin and ART drugs using commercial software (PK solutions, Summit Research Services, Montrose, CO; WinNonlin, Pharsight, Cary, NC). Non-compartmental analysis will be used to estimate C_{max}, T_{max}, ALIC, CL/F, V/F, and half-life where possible given drug pharmacokinetics and 24 hour dosing intervals. Comparison of antiretroviral pharmacokinetics before and after the addition of insulin will be assessed using geometric mean ratio with 90% confidence intervals of pre-dose drug levels at baseline and those at both 1 week and 4 weeks after initiating insulin.

Toxicity management/assessment of safety and adverse events

The procedures used in Adult AIDS Clinical Trial Group (ACTG) HIV neurology studies will be used for management of study drug related toxicities. The study drug may be decreased to 50% of the daily dose twice a day or suspended to assess/treat clinical or laboratory adverse experiences. If after 1 week, the adverse experience leading to the temporary holding of the study drug improves sufficiently, the subject may be rechallenged at 50% of the daily dose regimen for 1 week (intranasal insulin 20 I U a day or matching placebo) or full dose study drug if the initial dose reduction was decreased to once daily. If the clinical or laboratory adverse experience does not occur again on the 50% of the daily dose regimen for 1 week, then the full daily dose regimen may begin. If the adverse experience recurs, the treatment may be suspended again for 7 or more days, and the subject may be re-challenged. If there is no improvement in the adverse experience with ≥ 31 day treatment suspension, the subject must be permanently discontinued from study treatment. The toxicity grading system of the ACTG will be used to characterize toxicity severity.

Data Safety and Monitoring Plan

The clinical trial will be monitored by a Data and Safety Monitoring Board (DSMB) led by Dr. David Clifford (Washington University-St. Louis). He will interact with the other 2 members of the board and review semiannual closed and open reports provided to him by the PI (blinded) and site pharmacist (unblinded). Unblinded data will not be made available to non-DSMB members. After discussion by the DSMB members, they will present their recommendations to project investigators, and the study team will forward the reports, as requested, to the IRB. Enrolled participants will be monitored throughout the study for untoward incidents (e.g., "Adverse Events") occurring during the study. The Principal Investigator (and other designated individuals, if necessary) will conduct regular monitoring for safety concerns and provide general oversight for human safety requirements. Where necessary these occurrences will be evaluated for severity, attribution, and possible trends. All adverse events that are anticipated or described in the informed consent form will be logged appropriately and reported to the IRB on at least an annual basis (e.g., at continuing review). Any Unanticipated Problems or unexpected "Serious Adverse Events" related to the study intervention and/or test article and affecting the risk/benefit profile of the study, will be reported to the IRB promptly, and, in all cases, within 10 business days of discovery. Unplanned and non-emergent deviations from the IRB approved protocol will be logged and reported to the IRB annually at continuing review; all planned deviations will be submitted as a Change in Research to the IRB for approval prior to implementation. FDA (IND) and NIH reporting requirements will be followed, and all required institutions will be notified of events/problems encountered in the study and/or changes in research, in accordance with all applicable regulations and guidelines.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.
b. Steps taken to minimize the risks.
c. Plan for reporting unanticipated problems or study deviations.
d. Legal risks such as the risks that would be associated with breach of confidentiality.
e. Financial risks to the participants.

Safety of Intranasal Insulin

Safety issues pertaining to intranasal insulin administration for the treatment of diabetes have been extensively explored for over two decades (93). The most common side effects associated with intranasal insulin from the recent study for Alzheimer's disease (19) were lightheadedness/dizziness (13.2%), runny nose (rhinitis) (7.0%), headache (5.3%), nosebleed (2.6%), upper respiratory tract infection (2.6%), falls (2.6%), and rash (2.6%) using the 40 IU/day dose of insulin proposed for the current study. There was no difference in the frequency of any of those specific adverse events in the insulin 40 IU group and the placebo group. In addition, there were no severe adverse events in the study (19). Anaphylaxis has not been seen with intranasal insulin, but has been observed rarely with other routes of administration. A recent safety study of intranasal insulin administration demonstrated no treatment induced changes in blood glucose levels, nasal airway patency, or transnasal pressure gradient (94).

Regarding the risk of hypoglycemia, at least five peer reviewed human studies (94-98) (submitted for publication) and four preliminary studies (19, 99-101) revealed no change in blood glucose levels following intranasal insulin administration with doses that included 40 IU 4 times daily for two months. There was one exception with the case of a single participant who experienced mild hypoglycemia (52 mg/dl) after skipping a meal and engaging in sustained vigorous exercise.

In addition, a recent safety study (94) examined intranasal insulin administration of 60 IU once a day for three weeks in 20 healthy adults. This randomized, double-blind, placebo-controlled crossover trial measured blood glucose levels six times a day during the first two and the last two days of treatment. Pre- and post-treatment blood laboratory tests and nasal examinations were performed. The nasal studies included rhinoscopy to detect local irritation, a saccharin particle test to analyze mucocilary clearance, and rhinomanometry to evaluate nasal airway patency and transnasal pressure gradient. Results indicated no change in blood glucose values with insulin, and no change in the frequency of glucose values above 3.0 mmol/L. The only symptomatic hypoglycemic value occurred during placebo treatment. Insulin treatment had no effect on other laboratory values (C-peptide, total cholesterol, HDL, LDL, triglycerides, creatinine, glutamyl transferase), blood pressure, or body weight. Nasal examinations revealed no adverse effects or functional disturbances following intranasal insulin administration. No serious adverse effects of treatment were observed in the preliminary studies (19, 99, 100, 100).

There are no known serious risks associated with intranasal insulin without absorption enhancers. One industry report raised the issue of rare but significant increases in lung cancer in smokers treated with inhaled insulin; six of 4740 patients taking inhaled insulin developed lung cancer compared with one of 4292 patients who received an active comparator (incidence per 100 patient year exposure, 0.13 vs 0.02). However, the inhaled insulin protocol used for diabetes treatment in this report included absorption enhancers to maximize delivery to lungs, whereas the nose-to-brain delivery device to be used in this study greatly minimizes lung delivery without using absorption enhancers.

There are fewer safety data for intranasal insulin use by patients with HIV infection and it is possible that long-term administration may induce CNS hyperinsulinemia or insulin resistance that may have deleterious effects. Such effects would presumably manifest as accelerated cognitive or functional decline. Thus an intensive safety-monitoring plan will be in place for this study that should detect such patterns and a conservative duration of treatment will be used that balances safety and scientific considerations.

Risks associated with use of the POD® device

Although not expected to be serious, there are risks that may occur from using the investigational intranasal drug administration device (POD®). It is important that participants carefully follow the instructions they are given about how to administer the study drug and how to clean the device. If they do not carefully follow the instructions, it is possible that they may not receive the correct dose or the device may become contaminated. Participants could experience some discomfort to their eyes or face if they do not hold the device to their nose as directed. There may also be risks associated with intentional misuse of the device to administer unapproved drugs or substances. The study team will train the participant on proper device operation prior to starting the clinical trial and monitor technique and device function throughout the trial.

Risks lumbar puncture

Risks to lumbar puncture include post-lumbar puncture headache, back pain, minor neurologic symptoms such as numbness or pain radiating down a leg, allergic reaction to the medication used for numbing the lower back (rare), bleeding into the spinal cord (rare), infection (rare), cerebral herniation (rare).

About 10% to 30% of people develop a headache after LP. These headaches typically affect the front or back of the head and happen within 24 to 48 hours of the LP. The pain is usually worse when standing or sitting upright and improved when lying down flat. For some people a post-LP headache may be bad enough to limit daily activity.

To lessen the risk of post-LP headache, we use a thin 22 gauge needle for the procedure (rather than the 20 gauge needle also commonly used) and ask participants to lie flat for at least 30 minutes immediately afterward. We also strongly advise the participants to continue to rest at home and avoid difficult physical activities for at least a day or two, as well as drink plenty of fluids following the procedure. In the event of a post-LP headache, we may prescribe anti-inflammatory medicine as needed, or recommend common over-the-counter anti-inflammatory medicine. If the post-LP headaches last for more than 2 to 3 days and are not helped by anti-inflammatory medications, we may recommend that the participant undergo an epidural blood patch procedure in which the participant's own blood is injected into the same area of the original LP. We will not perform this procedure as part of the study, but we can refer the participant to a doctor who can.

While uncommon, sometimes during the LP procedure people experience a brief, sharp pain (often described as "electricity") that radiates into the lower back, buttock, or leg on one side. This is a temporary sensation and does not indicate nerve damage. Nerve damage with permanent loss of muscle power, sensory function (ability to feel), or bowel or bladder function from LP is extremely rare. Most people have some mild back pain or soreness after the LP around the site of injection. This pain usually goes away within a day or two. Prolonged back pain greater than three days is uncommon. People with a history of back problems may have a greater risk of prolonged or severe pain after the LP.

A small percent of people are allergic to local anesthetics used to numb the skin during the LP. We typically use lidocaine (chemically similar to novocaine). An allergic reaction to this medicine could include itching, hives, swelling, shortness of breath, difficulty breathing, changes in blood pressure and heart rhythm, loss of consciousness, or in a rare case, death.

Serious bleeding that affects the spinal cord is an extremely rare but dangerous complication. It is primarily a risk for people who have very low blood platelet counts (thrombocytopenia) or other bleeding disorders, or for those who are taking anticoagulant therapy (for example coumadin or warfarin) prior to or immediately after the LP. We perform safety blood tests to make sure participants are not at significant risk of this complication. We will not perform the LP if the participant is shown to have a serious coagulation defect, a platelet count under 50,000/µL, or a blood INR greater than 1.4.

Infection during the LP is extremely rare. We perform the procedure using sterile techniques to avoid this complication. Another serious but extremely rare complication that can occur during the LP is cerebral herniation (brain swelling). This is primarily a concern for people in emergency situations who have increased intracranial pressure (ICP). Participants with any signs or symptoms of increased ICP will not receive an LP.

If abnormal findings are found as a result of the lumbar puncture analysis, we will refer the participant to their primary healthcare provider for further testing and treatment. If the findings are critically urgent, we will refer the participant to the Johns Hopkins Hospital Emergency Department.

Risks of Magnetic Resonance Imaging (MRI)

The effects of magnetic fields in an MRI scanner have been extensively studied, and there are no known significant risks with an MRI exam. Participants may be bothered by claustrophobia and by the noise made by the magnet during the procedure. Participants will wear earplugs or earphones while in the magnet. Participants will not receive an MRI if they have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices, history of brain surgery for a cerebral aneurysm, or history of shrapnel or other metal in the body (e.g. eye injury) without history of recent MRI or screening X-ray.

There are also risks associated with an incidental finding from the MRI exam. In general, the possibility or identification of an incidental finding may cause anxiety. Costs for any care that will be needed to diagnose or treat an incidental finding would not be paid for by this research study. These costs would be the participant's responsibility. The incidental finding will be part of the participant's medical record, and there is the potential risk that the participant could face greater difficulty in getting health or life insurance.

Risks of clinical and neurological procedures

The risks to participants from the neurological and neuropsychological evaluations and medical interview are minimal. Occasionally, neurological examination or neuropsychological test administration may induce anxiety, or blood tests and urine screening may provoke concern if they reveal a condition unexpected by the participant. Anxiety is usually controllable with appropriate counseling and explanation by the study team, and if appropriate, the study team may coordinate care with the participant's primary care provider to address medical concerns that he/she may have.

The risks of venipuncture include needle entry discomfort and bruising, and rarely infection. Possible complications from peripheral IV lines for blood draw include phlebitis, bruising, and hematoma formation. According to Up-to-Date: "thrombophlebitis occurs in up to 15 percent of those with peripheral venous catheters. This risk can be reduced by avoiding lower extremity IV placement, minimizing catheter movement, placing the smallest suitable catheter size, and removing the catheter as soon as possible." (Frank, RL. "Peripheral venous access in adults," Up-to-Date, Topic 13824 Version 12.0, accessed 8/3/2017)

Risks to subject's privacy

There is the risk of loss of privacy by joining this study. The investigators will do everything possible to minimize the risk. Any PHI collected for this study will be kept confidential through the use of a secure, double-lock office storage space and an encrypted, password-protected computer database. Biosamples will be stored in double-lock protected laboratory freezers; labels will include unique subject and specimen ID numbers and no personal names or identifiers.

Financial risks

The costs for the procedures, tests, drugs or devices that are part of this research will be paid for by the study. Participants are responsible for all other healthcare costs that may occur outside of the events and procedures we conduct in our protocol whether related to the study or not. If the participant has health insurance, he/she will be responsible for any co-pays or deductibles not covered by insurance.

If the participant cannot pay the costs that are his/her responsibility, the participant may request a financial hardship review. This would be done according to standard Johns Hopkins guidelines. Information about where and how to request a financial hardship review will be provided in the Insurance and Research Participant Financial Responsibility Information Sheet and in any bills received from Johns Hopkins. If the participant qualifies for financial hardship, the costs to the participant will be reduced. Taking part in this study may lead to added costs to the participant and his/her insurance company. In some cases, it is possible that the participant's insurance company will not pay for these costs because he/she is taking part in a research study.

9. Benefits

a. Description of the probable benefits for the participant and for society.

There is no expected direct benefit to volunteers in this study. The potential benefit to the participant and for society is that the study may provide preliminary data for an effective adjunctive therapy for the treatment of HIV associated neurocognitive disorder (HAND).

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Subjects will receive $50 for completion of the screening, week 12, and week 24 visits, and $100 for the lumbar puncture at the pre-entry visit and week 24 visit. Subjects will receive $20 for completion of the week 1, 4, 8, 16, and 20 visits. Subjects will also receive $50 for the MRI scan at baseline and week 24. Travel to and from the clinic by taxi will be arranged by the study team, or each participant will receive up to $20 per visit to cover transportation costs.

Participants who complete the optional PK protocol will receive $10 for the extra CSF drawn at pre-entry and $10 for each extra amount of blood drawn at the baseline, week 1 and week 4 visits. Participants will receive $100 at the week 12 visit for the sum total of serial blood samples drawn, and $100 for the additional LP at week 12.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

There will be no costs to the participants for the study procedures or study drug. Management of any adverse events resulting from study procedures will be performed through the participant's routine care and reimbursement services. (See Section 8, Financial Risks)

Appendix A

Table 1.

Schedule of Lab Tests	Intranasal Insulin for the treament of HAND (IRB00108564)												
Visit:	**Screen**	**Pre-Entry 1**	**Pre-Entry 2**	**D000 (Baseline)**	**D007**	**D028**	**D056**	**Midtrial**	**D112**	**D140**	**Final 1**	**Final 2**	**Ad Hoc**
Timeline (days):	-45 to -1	-21 to -1	-14 to -1	Day 0	7 ±3	28 ±3	56 ±3	84 ±3	112 ±3	140 ±3	161 to 175	161 to 182	-45 to 182
Blood Tests													
Complete Blood Count (CBC)	X			X		X	X	X	X	X	X		[X]
Comp Metabolic Panel (CMP), non-fasting	X		X			X	X	X	X	X	X		[X]
CMP, fasting for glucose													[X]
Glucose, fasting	[X]		[X]			[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]
Insulin, fasting	X		X			X	X	X	X	X	X	[X]	[X]
Amylase/Lipase	X		[X]								X		[X]
T-cell Subset	X			X		X	X	X	X	X	X		[X]
HIV RNA Viral Load	X		X			X	X	X	X	X	X		[X]
PT/aPTT	[X]						[X]			[X]			[X]
HIV ELISA (if needed)	[X]												
Thyroid Stimulating Hormone (TSH)	X												[X]
Syphilis/RPR	X												[X]
Vitamin B12	X												[X]
Hepatitis C testing	X												[X]
Repository, Blood		X	X	X	(X)	X	X	X	X	X	X	[X]	[X]
CSF Tests (LP Optional)													
Protein/Glucose, CSF		(X)						(X)			(X)		
Cell Count w/ Diff, CSF		(X)						(X)			(X)		
Repository, CSF		(X)						(X)			(X)		

Key:		
	X	Scheduled lab test at visit per protocol
	X	Single lab test required within range of visits
	X	One or the other lab test scheduled at visit
	[X]	Lab test/procedure to be performed as needed
	(X)	Lab test/procedure is optional

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