A Phase I Dose Escalation Study of Harmine in Healthy Subjects
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Icahn School of Medicine at Mount Sinai

Clinical Research Protocol

Phase 1 Dose Escalation Study of Harmine in Healthy Adults

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LIST OF ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
DAC	Depression and Anxiety Center
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISMMS	Icahn School of Medicine at Mount Sinai
MINI	Mini International Neuropsychiatric Interview
PI	Principal Investigator
PPHS	Program for the Protection of Human Subjects
SAE	Serious Adverse Event
SCID-5	Structured Clinical Interview for DSM-5 Disorders

PROTOCOL SYNOPSIS

TITLE	Phase 1 Dose Escalation of Harmine in Healthy Adults
FUNDING ORGANIZATION	National Institute of Diabetes and Digestive and Kidney Diseases/NIH/DHHS
NUMBER OF SITES	1
RATIONALE	The present study is an open label phase 1 dose escalation study in healthy volunteers. The primary goals of the trial are to determine the maximum tolerated dose of harmine as well as to characterize the psychoactive effects of the drug. A continual reassessment model with 7 doses of harmine at 100 mg, 200 mg, 300 mg, 500 mg, 700 mg, 900 mg, and 1200 mg will be utilized in order to monitor and determine the tolerability of the drug. The results of the study will inform future trials in regard to the safety and efficacy of harmine, which is thought to be an alternative medication for diabetes. The present study is a jump-off point for the next phases of treatment development. Regenerating insulin-producing beta-cells could potentially free millions of diabetes patients from daily doses of insulin, and thus, remains an important objective in developing treatments for diabetes. Loss of insulin-producing beta cells has long been recognized as a cause of type 1 diabetes and has recently been identified as an important contributor to type 2 diabetes as well. Though there have been efforts in producing a therapy to increase the number of functional beta cells in patients, the major obstacle has been identifying an agent that does so by a degree that is actually therapeutic. Recently, a harmala alkaloid called harmine has demonstrated promise to be an effective therapy for both type 1 and type 2 diabetes. In-vivo and in-vitro studies have shown harmine to robustly induce beta cell proliferation at rates that may have treatment implications. Further specificity studies identified dual specificity tyrosine-regulated kinase-1a (DYRK1A) as the likely target for harmine. Harmine occupied the ATP pocket of DYRK1A, leading to its inhibition and, ultimately, to beta cell expansion (Wang 2015). Given that harmine's therapeutic application for diabetes has never been studied in humans, we propose a phase 1 study of harmine to pioneer clinical research efforts of this potential new therapy. Harmine is consumed as part of Ayahuasca which is a hallucinogenic brew made from B. cappi bark and leaves of Psychotria viridis [which contain N,N-dimethyltryptamine (DMT)], and used recreationally by Amazonian tribes and has been studied recently for its potential psychotropic effects. Harmine in ayahuasca inhibits the breakdown of DMT and elevates its levels in systemic circulation in brain reversible inhibition of monoamine oxidase (MAO) A (MAO-A) enzyme. The hallucinogenic effects of ayahuasca are attributed to DMT, a 5-HT1A/2A/2C agonist. **Research Question:** This study is designed to investigate the safety of oral administration of harmine using an open label, two-stage, continual

	reassessment method with 7 doses of harmine at 100mg, 200mg, 300mg, 500mg, 700mg, 900mg and 1200mg in healthy volunteers. We anticipate that the proposed study will yield the maximum tolerated dose (MTD) for harmine, which will guide future research efforts toward novel diabetes treatments.
STUDY DESIGN	This is Phase 1 dose escalation study.
PRIMARY OBJECTIVE	The principal goal of our clinical trial is to establish the Maximum Tolerated Dose (MTD) of harmine in healthy patients that will inform the recommended phase II dose. *Hypothesis:* Orally administered harmine will be well-tolerated with minimal dose-limiting toxicities at our testing doses.

SECONDARY OBJECTIVES	Characterize psychoactive effects of harmine in healthy adults. *Hypothesis:* Orally administered harmine will be well-tolerated and will not induce burdensome psychoactive effects in healthy adults.
NUMBER OF SUBJECTS	N=40 patients dosed; N=54 anticipated screening
SUBJECT SELECTION CRITERIA	**Inclusion Criteria** • Male or female aged 18-55 years; • <u>Rationale:</u> *We wish to exclude children under the age of 18 and adults over the age of 55 in part due to concerns regarding neurodevelopmental and neurocognitive effects, respectively.* • Participants must have a level of understanding of the English language sufficient to agree to all tests and examinations required by the study and must be able to participate fully in the informed consent process; • Body Mass Index (BMI) between 19 and 30; • <u>Rationale:</u> *We wish to exclude individuals who are underweight as defined as a BMI <19, or who are obese as defined as >30 according to the Centers for Disease Control (CDC). These criteria are in line with the goals of a phase I dose finding and pharmacokinetic study.* • Women of childbearing potential and men must be using an acceptable method of contraception to avoid pregnancy throughout the study as judged by the investigator; • Women must not be breastfeeding; **Exclusion Criteria** • Presence of a significant medical illness i.e., delirium, metastatic cancer, decompensated cardiac, liver or kidney failure, major surgery, stroke or myocardial infarction during the three months prior to entry; • Presence of a significant neurological disease such as Parkinson's disease, primary or secondary seizure disorders, intracranial tumors, severe head trauma; neurodegenerative diseases; • Presence of neurocognitive or dementing disorders; • Presence or history of psychiatric disorder as diagnosed by Mini Neuropsychiatric Interview (MINI);

	• Urine toxicology positive for illicit drugs or dis-allowed concomitant medications as per study protocol;
	• Medications with primary central nervous system (CNS) effects are dis-allowed, including psychotropic medications, antidepressants, benzodiazepines, centrally acting hypnotic agents, and centrally acting anti-migraine therapies;
	• Medications with primary cardiovascular effects are dis-allowed, including beta-adrenergic antagonists, ACE inhibitors, calcium channel blockers, and diuretics;
	• Any OTC medications or herbal remedies that could interfere with the study drug, pose a risk to the subject, or contain high tyramine as outline in the Low Tyramine Diet attachment;
	• Any other medications that, in the opinion of the investigators, would pose a safety risk to the patient or that would interfere with the interpretation of study results;
	• Positive pregnancy test at screen or on the morning of the treatment day in women of childbearing potential;
	• Systolic blood pressure outside the range of 100 - 140 mmHg, diastolic blood pressure outside the range of 60 - 90 mmHg, and pulse rate at rest > 100 or < 60 bpm;
	• History of positive tests for hepatitis B surface antigen, hepatitis C antibodies;
	• History of HIV;
	• Significant ECG abnormalities as follows:
	  • Heart Rate < 60 and >100 bpm
	  • PR Interval <120 and > 220 ms
	  • QRS duration < 70 and >120 ms
	  • QTC Interval (Bazett) > 450 ms

TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Harmine will be administered orally at 100mg, 200mg, 300mg, 500mg, 700mg, 900mg and 1200mg. The drug will be manufactured at the University of Minnesota College of Pharmacy Institute for Therapeutics Discovery and Development.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	N/A
4 OF SUBJECT PARTICIPATION AND DURATION OF STUDY	We expect that for each subject, the study will last up to 8 weeks from recruitment to completion of all study procedures. Data analyses will be finished in up to 3 months after all the samples are collected.

CONCOMMITAN TMEDICATIONS	All of the following medications are prohibited: **Hypertension Drugs**: Ace inhibitors: enalapril (Vasotec, Epaned), lisinopril (Prinivil, Zestril, Qbrelis), and ramipril (Altace Angiotensin II receptor blockers (ARBs): valsartan (Diovan), losartan (Cozaar) Calcium channel blockers: amlodipine (Norvasc), diltiazem (Cardizem, Tiazac, others), nifedipine (Adalat CC, Procardia), and verapamil (Verelan, Calan)

EFFICACY EVALUATIONS	Efficacy will be the main focus of future Phase II and III studies. The current protocol is focused on safety, which will better inform future efficacy studies.
PRIMARY ENDPOINT	The primary endpoint of our clinical trial is to establish the Maximum Tolerated Dose (MTD) of harmine in healthy patients in a phase I dose escalation study for efficacy testing in phase II trials. See above for MTD and DLT definitions where safety is the primary endpoint.
SECONDARY ENDPOINTS	A secondary endpoint is to characterize psychiatric effects of orally administered harmine in healthy adults.
OTHER EVALUATIONS	N/A
SAFETY EVALUATIONS	• Psychosis / mental status changes: BPRS, other clinical scales • PRISE: nausea, vomiting, diarrhea and other general adverse events • Vitals: BP, HR, Temp, Respiration • Unanticipated effects: spontaneous reporting and structured assessment with the PRISE

STATISTICS **Primary Analysis Plan**	Human data on oral harmine administration are limited and of poor quality, as noted above. Briefly, there is only one study that employed oral doses in the range of 20 to 960 mg (Pennes et al., 1957). No subject had any adverse visual hallucinations. Some subjects (number uncertain) experienced "nausea, vomiting, slow coarse spontaneous tremor, 'waviness' of the environment, 'sinking' sensations of the body, subjective sense of body vibration, and subject numbness accompanied by reduced sensitivity to light touch and pinprick". These effects were apparently observed only in "some subjects" and only "occurred with oral doses higher than the threshold of 300.0-400.0 mg." In addition, the Callaway Ayahuasca study (Callaway et al., 1999) administered harmine in the Ayahuasca "tea" at the dose of 3.4 mg/kg (range 204-306 mg). Callaway et al. noted that the effects of this dose of harmine (along with other ingredients of Ayahuasca) was perceived to be mild. Thus, based on this limited data, doses below 200 mg are likely safe, and single oral doses as high as 1000 mg may also be safe and free of significant adverse effects For this reason, and an added layer of caution, we have elected to initiate dosing at 100 mg which is less than half of the lower dose range in Callaway study. The upper level of dose range (1200 mg) is 4-times that of the highest dose of Callaway study. In terms of selecting the number of doses tested between the established minimum (100 mg) and maximum (1200 mg) dose, the most important statistical consideration is whether the doses and dose range under investigation are likely to allow an accurate MTD estimate. In general, it is

recommended that phase I studies contain between 2 and 12 dosing levels (Penel and Kramar, 2013); more levels generally allow for a more accurate determination of the MTD. Based primarily on clinical data, together with the available pre-clinical safety data, and utilizing established methods for defining the incremental sequence of a range of doses (Le Tourneau et al., 2009), our study protocol specifies the following 7 oral harmine dose levels to be tested according to the dose escalation model described below: 100 mg, 200 mg, 300 mg, 500 mg, 700 mg, 900 mg and 1200 mg.

Our study will employ the continual reassessment method (CRM) to find the MTD of harmine (O'Quigley 1990). Generally, the CRM starts by selecting a target dose-limiting toxicity rate and a mathematical model assumed to govern the relationship between dose and toxicity; the prior dose-toxicity curve. After outcomes are observed (i.e., a patient experiences a DLT or not), the dose-toxicity curve is refit incorporating the latest outcome. The next dose assigned is that which is most likely to be associated with the target toxicity level (the MTD). At every step, the next patient is assigned the dose estimated to be nearest to the MTD. While the "3 + 3" design is the most commonly employed dose finding design, the CRM is generally accepted by statisticians as superior due to two main advantages: (1) the CRM provides a mechanism for stopping at a specified toxicity level, whereas the targeted toxicity rate for the MTD from the "3 + 3" design is necessarily in the range of 16%-33%, and (2) the CRM is more efficient; it can establish the MTD more accurately and precisely from fewer patients and it minimizes the number of patients treated at unsafe doses. The CRM is a model-based design while that of the 3+3 is a rule-based design.

Our implementation of the CRM is a Bayesian model-based method that will employ a logistic one-parameter prior for the model parameter α. We have chosen this model for two principal reasons; its simplicity and efficiency. Generally, the one-parameter model is more efficient than two parameter models. That is, it requires fewer patients to precisely estimate that aspect of the dose-toxicity curve that is allowed to vary (the slope or steepness parameter). This increased efficiency in parameter estimation may come at the cost of a slight decrease in the accuracy of depicting the entire dose-toxicity relationship. However, the CRM has proven to be robust in choosing an MTD, even if the functional form is moderately incorrect. In large part this robustness is due to the fact that the CRM is not concerned with accurately estimating all aspects of the dose-toxicity curve, but rather the area near the location of the MTD. The Inputs to specify for our model are: target toxicity pT as single-parameter (θ) dose-toxicity curve, K for number of doses, R for odds ratio (OR), and α for accuracy. If we use a one-parameter logistic model for the dose response curve, we would have a one-parameter dose response curve where d is the dose:

$$\varphi(d,\alpha) = \frac{e^{3+\alpha d}}{1+e^{3+\alpha d}}$$

We will assign a prior probability distribution to the model parameters, assigning a prior probability of DLT at each dose based on the available data

	(Legedza, 2001). In order to minimize risk, we will implement a common modification to the CRM such that the first patient treated in the study will be treated at the lowest available dose (e.g., 100 mg) regardless of the prior distribution (Goodman et al, 1995). The study will end when either the maximum number of allowable patients treated per protocol is reached (see below), or it will end early in the event that the probability that the next 10 patients to be dosed in the trial will be given the same dose level, regardless of DLT outcomes observed exceeds 90% (Zohar and Chevret, 2001).
Rationale for Number of Subjects	With an anticipated screen fail rate of 25%, 54 subjects will be enrolled in order to obtain a maximum of 40 eligible subjects. Phase I studies do not call for hypothesis testing per se. Sample size determination for phase I studies therefore are not driven by the usual statistical considerations related to type I error rate and estimated power for testing specific hypotheses. Planned sample sizes in phase I trials are generally dictated by practical constraints, such as the number of sites, projected recruitment rates, and number of dose levels. Samples sizes for model-based CRM studies typically range between 10 and 50, and the optimal sample size can be estimated based on considering model parameters that include the TTL (θ) and the number of dosing levels, K. Cheung (2013) has provided a sample size calculation package in R. We used his program to calculate sample size across a range of ORs along with our study parameters of K=7, θ=0.25 and α=0.5. For OR's as large as 3.0, N=10 provides an accurate estimate of the MTD, while N=48 would be needed for an OR of 1.5. Therefore our selected maximum sample size of N=40 will likely provide an accurate estimate of the MTD while minimizing the number of volunteers exposed to potential toxicities of the intervention.

1 BACKGROUND

1) Basic Characteristics

Harmine is a naturally occurring β-carboline alkaloid (in *Banisteriopsis caapi*). Use of harmine in humans is commonly used as part of Ayahuasca which is a hallucinogenic brew made from *B. cappi* bark and leaves of *Psychotria viridis* [which contain N,N-dimethyltryptamine (DMT)], is consumed recreationally by Amazonian tribes and has been studied recently for its potential psychotropic effects. Harmine in ayahuasca inhibits the breakdown of DMT and elevates its levels in systemic circulation in the brain. The hallucinogenic effects of ayahuasca are attributed to DMT, a 5-HT1A/2A/2C agonist, and harmine in the ayahuasca brew elevates levels of DMT in systemic circulation and brain[1].

Human studies of harmine have used three different routes: oral, subcutaneous, and intravenous. While most of the biological effects of harmine are attributed to its reversible inhibition of monoamine oxidase (MAO) A (MAO-A) enzyme, it is also a potent inhibitor of dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A)[2].

2) Relevance to diabetes

More recently, interest in harmine has emerged as a modulator of insulin release. Harmine has been

demonstrated to increase human beta cell proliferation to 1-1.5%, a range similar to that which humans experience during their first year of life[2]. Given that human beta cell proliferation strictly decreases 10-14 months after birth to levels well below this range[3] and that this decrease in beta cell proliferation contributes to type 1 and type 2 diabetes. Thus, harmine is a potentially revolutionary novel diabetes therapy that targets the source of insulin synthesis and is distinct from any currently available treatment.

3) Brief summary of previous rodent studies of Harmine

3.1 Preclinical Pharmacokinetics

In a pharmacokinetic study of harmine in rats, harmine was orally administered at 20 mg/kg[4]. The four parameters of pharmacokinetics – absorption, metabolism, distribution and excretion - were determined. Following intravenous injection of harmine, harmine was absorbed by the gastrointestinal tract with an absorption time of 3 minutes. Time to achieve maximum concentration of harmine after dosing (Tmax) was on average 9.7 minutes. Volume of distribution (Vd) was found to be 3.9 L/kg and harmine's bioavailability after oral administration was measured to be 3.1%. Lastly, systemic clearance was found to be 103.2 ml/kg/min. This rate of clearance is much higher than the rate of hepatic blood blow, suggesting that harmine may undergo extrahepatic metabolic processes in addition to hepatic clearance.

3.2 Preclinical Pharmacodynamics

Numerous studies have investigated harmine's therapeutic and behavioral effect in rodents. Together, these studies provide insight into harmine's safety profile and were used as support for the dosing regimen in this study. A specific rodent-to-human conversion factor was used to find the equivalent human dose (see "Rationale for Dose Determination" under section 5d for more detail).

One study investigating the antidepressant effects of harmine administered 10 doses of harmine at 20mg/kg over the course of 10 days to mice via injection[5]. Harmine exerted antidepressant-like effects by protecting against increased immobile time in the tail suspension test (TST) and fthe orced swimming test (FST). These effects were attenuated with administration of l-Alpha-Aminoadipic Acid (L-AAA), suggesting that harmine may induce anti-depressant effects via restoration of astrocytic function[5].
Another study investigating the anti-inflammatory effects of harmine administered 30mg/kg of harmine to mice via intraperitoneal injection[6]. Harmine inhibited NF-κB transactivity, resulting in reduced mRNA and protein levels of NF-κB downstream inflammatory cytokines. Harmine also prevented inflammatory damage of the lung, and decreased serum TNF-α, interleukin-1β (IL-1β) and IL-6 levels in an LPS-challenged mouse model. These results indicate that harmine may exert its anti-inflammatory effect by inhibition of the NF-κB signaling pathway[6].

In a study investigating the antioxidant properties of harmine, investigators observed both acute and chronic effects of harmine injected at either 5,10 or 15mg/kg in rats[7]. Acute treatment consisted of only one dose and chronic treatment consisted of once a day dosing for 14 days. Both acute and chronic treatment reduced lipid and protein oxidation in the prefrontal cortex and hippocampus and also increased superoxide dismutase ad catalase activity in the same regions[7].

Another study investigating the hypothermic effects of harmine in rats administered 2.5, 5, 10, 15 and 20mg/kg of harmine via intraperitoneal injection, showed harmine to produce hypothermic effects over the course of 1 hour[8]. Pretreatment with a 5-HT synthesis inhibitor attenuated the hypothermic effect, endogenous 5-HT stimulation of 5-HT1A receptor is responsible for hypothermic effects of harmine and other harmala alkaloids[8].

Lastly, another study explored therapeutic effects of harmine on memory deficits and administered 10, 20 and

30mg/kg of harmine intragastrically to cognitively dysfunctional mice for 7 days[9]. Harmine effectively ameliorated memory deficits in scopolamine-induced mice with the minimum effective dose being 20mg/kg.

3.2 Preclinical toxicology

In a review of pharmacological and therapeutic effects of harmine, the lethal dose in mice via intravenous route was found to be 38mg/kg and the median lethal dose in rats via subcutaneous route was found to be 200mg/kg. The review also summarized harmine's cardiovascular, neurological and antimicrobial effects as described below[10].

Cardiovascular Effects

Harmine has vasorelaxant activity which is attributed to its interaction with the alpha 1-adrenergic receptors in vascular smooth muscles and also more importantly to its increasing effect on nitric oxide (NO) release from the endothelial cells[11] . Harmine has been shown to have an ionotropic effect and also decrease heart rate in normal anesthetized dogs[12]. In addition, harmine demonstrated potent angiogenic inhibitory effects, significantly decreasing proliferation of vascular endothelial cells and reducing expression of different pro-angiogenic factors such as vascular endothelial growth factor, NO and pro-inflammatory cytokines[13]. Finally, there is some evidence that harmine may prevent platelet aggregation[14] .

Neurological Effects

In traditional medicine, *P. harmala,* the plant that contains a larger concentration of harmine, has been used among societies to treat some nervous system disorders such as Parkinson's disease[15] , in psychiatric conditions[16] such as nervosity[17] and to reduce nociception[18] .

As mentioned previously, harmine itself inhibits monoamine oxidase (MAO) enzyme[2]. Harmine has also been shown to increase Brain-derived neurotrophic factor (BDNF) protein[19].

Osteogenic Activity

Two different studies showed bone anabolic effects of harmine, *in vivo* and *in vitro*. It was revealed that administration of 10 mg/kg/day of harmine inhibits formation and differentiation of osteoclasts in mice[20]. Adversely, it enhances osteoblast differentiation, most likely via inducing the expression and activation of bone morphogenetic protein (BMP) and Runx2 pathways.

4) Previous clinical experience of Harmine

Radioisotope labeled harmine has been used in human Positron Emission Tomography (PET) studies in order to assess central occupancy of MAO-A. In one study, a dose of 370 MBq of intravenous [11C]harmine was

administered as a bolus for each PET scan. These studies indicated harmine as a suitable molecule for quantitative evaluation of MAO-A densities, suggesting it be used to study MAO-A dysregulation in several illnesses.

As mentioned previously, harmine has been administered via oral, subcutaneous, and intravenous route[21]. In one study of physically healthy patients with mental illness, mostly in age group of 18-35 years, 11 patients were given oral harmine in dose range 20-960 mg, 6 patients were given subcutaneous harmine between the

doses of 40-70 mg, and 11 patients were given intravenous harmine in between the doses of 100-300 mg[21]. Authors report that some patients got more than one dose of harmine (specifics are unclear). While authors report visual hallucinations with intravenous route, which was administered over 20-30 minutes, they reported that harmine was not hallucinogenic by oral or subcutaneous routine. In addition to visual hallucinations that occurred only when eyes were closed, authors reported the following adverse reactions with harmine: "nausea and vomiting, slow, coarse, spontaneous tremor of the extremities of an 'extrapyramidal' appearance; humming and buzzing noises; 'waviness' of the environment; 'sinking' sensations of the body; subjective sense of body vibration; and subject numbness that was accompanied by objective evidence of reduced sensitivity to light touch and pinprick." However, the authors reported, "These reactions, plus all the preceding, occurred in almost every patient with the intravenous route; and (except for hallucinations) some occurred with oral dosages higher than the threshold of 300-400 mg. The reactions were generally more intense by the former route".

There were no changes in heart rate or blood pressure reported for the group receiving oral administration[21]. However, bradycardia and hypotension occurred with all doses of intravenous harmine, limiting maximum dosage to 300 mg intravenously. With the intravenous administration, on average there was a decrease in pulse rate of 18 beats/minute and in blood pressure of 16mm mercury. Recovery occurred in about 30 minutes.

Another study administered 40mg of harmine subcutaneously to 40 subjects to explore anti-parkinsonian qualities in reserpine, chlorpromazine or encephalitis induced parkinsonism. Harmine showed to be an effective anti-parkinsonian agent in patients whose symptoms were induced by Reserpine but not in patients whose symptoms were induced by chlorpromazine. There was a wide variety of responses in this therapeutic effect of harmine, ranging from slight improvement to complete remission. Harmine also appeared to mildly improve the mood of patients. No severe adverse effects were reported in the paper and investigators concluded that harmine "is safe in man"[22].

Separately, harmine has been administered and studied as part of the ayahuasca brew[23]. DMT is the main hallucinogenic component of ayahuasca, whereas harmine is a MAO-A inhibitor that attenuates DMT breakdown, allowing for a longer lasting effect[1]. Controlled studies involving oral administration of single ayahuasca doses to healthy volunteers show that this botanical hallucinogen induces perceptual alterations, introspection, increases in autobiographical memories and positive mood[23]. These studies also suggest that ayahuasca has an acceptable tolerability, with nausea and vomiting as the most frequent adverse reactions.

4.1 Clinical Pharmacology

One study investigated the pharamacokinetics of Ayahuasca and its alkaloids in healthy volunteers[24]. Subjects ingested a brewed tea containing an average of 252 mg of harmine as well as 35.5 mg DMT, 158 mg THH and 29.7 mg harmaline. Pharmacokinetic values for harmine were determined for 14 volunteers. Results found a C_{max} of 114.8±61.7 ng/ml and a T_{max} of 102.0±58.3 min. $T_{1/2}$ was found to be 115.6 ± 60.1 min, k_{obs} was measured to be 0.016 ±0.027 min^{-1}, C1/F was 271.7±180.3 and V_{ss}/F was 49.6 ± 40.4 l/kg.

4.2 Clinical Toxicology

The clinical experience of the toxicology of harmine has been discussed above, (see section 2.4). Additionally, the toxicity of Ayahuasca, the brew that contains harmine as a significant component has also been investigated. In the pharmacokinetic study mentioned above, where subjects received an average of 253mg harmine, only 1 out of 15 healthy volunteers vomited[24]. In a systematic review of uncontrolled ayahuasca use, either ritual or recreational, only three case series and two case reports were identified where psychotic episodes were associated with ayahuasca intake[23]. It is important to note that several of the reports describe

subjects with a personal and possibly a family history of psychosis, nonpsychotic mania, or concomitant use of other drugs and also that DMT is believed to be the main hallucinogenic component of ayahuasca and not harmine. In conclusion, the controlled use of ayahuasca in experimental and ritual and recreational settings are not usually associated with psychotic episodes or intolerable toxicity.

2 STUDY RATIONALE

The present study is an open label phase 1 dose escalation study in healthy volunteers. The primary goals of the trial are to determine the maximum tolerated dose of harmine as well as to characterize the psychoactive effects of the drug. A continual reassessment model with 7 doses of harmine at 100 mg, 200 mg, 300 mg, 500 mg, 700 mg, 900 mg, and 1200 mg will be utilized in order to monitor and determine the tolerability of the drug. The results of the study will inform future trials in regard to the safety and efficacy of harmine, which is thought to be an alternative medication for diabetes. The present study is a jump-off point for the next phases of treatment development.

Regenerating insulin-producing beta-cells could potentially free millions of diabetes patients from daily doses of insulin, and thus, remains an important objective in developing treatments for diabetes. Loss of insulin-producing beta cells has long been recognized as a cause of type 1 diabetes and has recently been identified as an important contributor to type 2 diabetes as well. Though there have been efforts in producing a therapy to increase the number of functional beta cells in patients, the major obstacle has been identifying an agent that does so by a degree that is actually therapeutic.

Recently, a harmala alkaloid called harmine has demonstrated promise to be an effective therapy for both type 1 and type 2 diabetes. In-vivo and in-vitro studies have shown harmine to robustly induce beta cell proliferation at rates that may have treatment implications. Further specificity studies identified dual specificity tyrosine-regulated kinase-1a (DYRK1A) as the likely target for harmine. Harmine occupied the ATP pocket of DYRK1A, leading to its inhibition and, ultimately, to beta cell expansion (Wang 2015). Given that harmine's therapeutic application for diabetes has never been studied in humans, we propose a phase 1 study of harmine to pioneer clinical research efforts of this potential new therapy.

Harmine is consumed as part of Ayahuasca which is a hallucinogenic brew made from B. cappi bark and leaves of Psychotria viridis [which contain N,N-dimethyltryptamine (DMT)], and used recreationally by Amazonian tribes and has been studied recently for its potential psychotropic effects. Harmine in ayahuasca inhibits the breakdown of DMT and elevates its levels in systemic circulation in brain reversible inhibition of monoamine oxidase (MAO) A (MAO-A) enzyme. The hallucinogenic effects of ayahuasca are attributed to DMT, a 5-HT1A/2A/2C agonist.

This study is designed to investigate the safety of oral administration of harmine using an open label, two-stage, continual reassessment method with 7 doses of harmine at 100 mg, 200 mg, 300 mg, 500 mg, 700 mg, 900 mg and 1200 mg in healthy volunteers. We anticipate that the proposed study will yield the maximum tolerated dose (MTD) for harmine, which will guide future research efforts toward novel diabetes treatments.

2.1 Facilities and Resources

Facilities and Other Resources

The proposed research will take place at the Icahn School of Medicine at Mount Sinai. The research team will benefit from the facilities and resources available at the main campus that include specialized laboratories and highly trained personnel. Our research

team has a strong track of successful inter-Departmental collaborations and several ongoing collaborations with the facilities described below. We believe that the teamwork demonstrated by the study team and the facilities available at Icahn School of Medicine at Mount Sinai will allow for the successfully completion of the current proposal.

The Mount Sinai Health System

The Mount Sinai Health System is an integrated health system committed to providing distinguished care, conducting transformative research, and advancing biomedical education. Structured around seven members hospital campuses and a single medical school, the Health System has an extensive ambulatory network and a range of inpatient and outpatient services from community-based facilities to tertiary and quaternary care. The Health System's seven-member hospital campuses include Mount Sinai Beth Israel, Mount Sinai Beth Israel Brooklyn, The Mount Sinai Hospital, Mount Sinai Queens, Mount Sinai Roosevelt, Mount Sinai St. Luke's, and New York Eye and Ear Infirmary of Mount Sinai. These hospitals and the entire Mount Sinai network will benefit from synergies with the Icahn School of Medicine at Mount Sinai, one of the nation's leading medical schools, which is on the forefront of medical and scientific training, biomedical research, and patient care. The Health System includes approximately 6,600 primary and specialty care physicians, 12- minority-owned free-standing ambulatory surgery centers, over 45 ambulatory practices throughout the five boroughs of New York City, Westchester, and Long Island, as well as 31 affiliated community health centers.

Icahn School of Medicine at Mount Sinai (ISMMS)

Icahn School of Medicine at Mount Sinai is among the top twenty medical schools in the United States in both NIH funding and in the U.S. News and World Reports survey of America's Best Graduate Schools. Driven by a culture of innovation and discovery, ISMMS is guided by a $2.25 billion strategic plan that emphasizes translational science. The School's 14 multidisciplinary research institutes foster intensive collaboration along a continuum that runs from the laboratory to patient care. State-of-the-art laboratories support outstanding research, and abundant clinical venues offer superb patient care and clinical training opportunities. The 550,000 square foot Leon and Norma Hess Center for Science and Medicine, opened in December 2012, allows scientists and physicians to work in close proximity and stimulates collaboration that advances efforts to diagnose, treat and prevent human disease. Currently, over 1,100 students are enrolled in six degree-granting programs: MD; PhD in Biomedical Sciences or Neuroscience; Master of Biomedical Sciences; Master of Public Health; Master of Science in Genetic Counseling; and the Master of Science or PhD in Clinical Research. Approximately 55% of enrollees are pursuing a Doctor of Medicine (MD) degree, 25% are working toward a doctoral (PhD) degree and 20% are seeking a Master's (MS) degrees. Some students are pursuing dual degrees, primarily MD/PhD, MD/MSCR or MD/MPH. ISMMS also offers postgraduate research and clinical training opportunities. The School attracts outstanding students to its highly competitive programs within an invigorating academic environment. The student body is academically excellent and diverse. The Mount Sinai campus is located at the border of the Upper East Side, one of the wealthiest neighborhoods in America, and East Harlem, a vibrant and largely minority community where 39% of the residents live in poverty. East Harlem's rates of infant mortality, childhood asthma,

obesity, diabetes and hypertension are among the highest in New York City and the county. Mount Sinai is a major provider of medical care to both of these communities.

The Depression and Anxiety Center for Discovery and Treatment (DAC) at ISMMS

The Depression and Anxiety Center for Discovery and Treatment (DAC) at ISMMS, Directed by Project MPI James W. Murrough, M.D., Ph.D., is one of the major research and clinical programs of the Department of Psychiatry at ISMMS, with current research funding from NIH, non-profit foundations, and industry. Investigators at DAC have a long track record in successfully completing studies involving the assessment and treatment of individuals with major depressive disorder (MDD), posttraumatic stress disorder (PTSD), anxiety disorders and other stress-related disorders. Located on the main Manhattan campus of ISMMS, DAC currently includes two full-time psychiatrists, two full-time postdoctoral fellows (one in novel therapeutics and one in neuroimaging), two PhD-level clinical raters, seven full-time research coordinators, and a data manager. The PI and Director of DAC (Murrough JW), has a strong track record of overseeing the successful enrollment and completion of clinical research studies conducted in both healthy volunteers and in patients with MDD and PTSD. Within clinical research, our program has specific expertise in the conduct of prospective clinical trials, including phase I through phase IV studies. We have a robust workflow for recruiting and screening volunteers for research. In 2019, our clinical research program conducted over 400 telephone pre-screens and over 150 in-person new evaluations and diagnostic interviews.

DAC is located on 1399 Park Avenue on the main campus of ISMMS. Available space at DAC includes 4 private offices, 2 treatment rooms, one examination/phlebotomy room, 8 neuropsychology room, a conference room and 9 cubicles for research assistants and interns, as well as a comfortable waiting area, the clinic is an ideal setting for screening and interviewing potential study participants. The clinic has adequate office space for faculty members, research assistants, and post-doctoral fellows. All office space is fully equipped with necessary elements for the conduction of the research, including computers using a common network, printers, telephone, fax machine, photocopying machines, and supplies.

Psychiatry Infusion Suite

Harmine administration will take place at the Mount Sinai Psychiatry Infusion Suite, a research facility dedicated to supporting early phase and interventional study, located on the main campus of the medical school at 1425 Madison Avenue. This facility is equipped with continuous vital signs monitoring, infusion pumps and the necessarily medical equipment and supplies to support early phase pharmacological studies and pharmacokinetic studies. Nurses trained in research protocols provide clinical research support to the study physician. A crash cart is available in the infusion suite and a responsible study physician is located on site at all times during study procedures. Facilities and support for specimen collection and processing is also available.

The Clinical Research Unit (CRU)

Harmine visits may also occur at The Clinical Research Unit (CRU). The CRU is occupying 7500 square feet, is the hub for conducting clinical and translational research at Icahn School of Medicine at Mount Sinai. Resources include: private rooms and cubicles, telemetry, a core laboratory for specimen processing and short term storage. The staff

includes nurse practitioners, nurses, a research dietitian, and an information technologies specialist. Specimen processing and storage and database support are available. Nursing support may be provided for studies undertaken off-unit as well. A research subject advocate ensures the safe conduct of studies and to provide assistance with education concerning safe and ethical practices.

Mount Sinai Chemistry Clinical Services

Our Chemistry Laboratory combines modern automation with the personal skills of a dedicated team of pathologists, clinical chemists, and technologists. We provide a range of services, including routine testing, stat testing, point-of-care testing, and specialized tests, and report critical values to providers 24 hours a day, seven days a week.

Center for Biostatistics at Mount Sinai and Office of Research Services

The Center for Biostatistics at Mount Sinai at ISMMS (Director: Emilia Bagiella) is an academic clinical research center, whose mission is to support the design, conduct, and analysis of clinical research studies, including multi-site trials. The Office of Research Services (ORS) serves as a central resource for the Mount Sinai Health System (MSHS) research community. ORS assists the research community with how to navigate the internal research infrastructure and external research agencies; delivers research orientation for new faculty and staff; offers consulting services, ongoing training, education, and communication support; and provides a wide range of research tools. The lead statistician on the proposed project, Dr. Usha Govindarajulu, Ph.D., is senior faculty within the Center for Biostatistics and will work closely with PIs Dr. Stewart and Murrough to ensure that the proposed projects benefits from the outstanding resources and expertise of the Center. Dr. Govindarajulu will work with Dr. Murrough and the study database manager to finalize the trial design, the statistical analysis plan, and study monitoring and quality assurance plan, as well as the development of the database and the data capture instruments.

Scientific Computing

Mount Sinai's primary scientific computing resource, named Minerva after the Roman goddess of medicine and science, occupies a portion of the 30,000 square feet available in our computing facility. Minerva currently has 140 Teraflops peak speed and over 85 million CPU hours available each year, distributed over more than 300 nodes in the cluster with 256 GB of memory per node. These include 9 nodes with general-purpose graphics processing units (GPGPU) suitable for image-processing and deep-learning projects. Total storage will be expanded to 10 PB in 2018. An archival storage system encrypts and saves copies of data on tape to two geographically disparate locations. The software and programming environments are the best of breed, and include community standards such as Linux and MPI. Over 600 scientific software packages are available and regularly updated on Minerva. The workload on the Minerva is controlled by resource managers and schedulers that optimize utilization for maximum job throughput or time to solution based on the priorities of our scientific community. Minerva achieves well over 99% uptime, using scalable and reproducible configuration management techniques. Rigorous and industry-standard multi-factor authentication and other security policies ensure the integrity of the researcher's data and Mount Sinai's compute infrastructure.

Mount Sinai also employs several domain-specific PhD-level computational scientists with interdisciplinary expertise to assist researchers to make efficient and effective use of computing resources. These scientists lead basic and advanced training classes and accelerate the scientific discovery process by assisting with code development, optimization and troubleshooting. They also conduct annual surveys and actively solicit and respond to feedback. Additionally, Mount Sinai's robust computing and data cyber infrastructure has been designed for the rapid and accurate ingest of the sequencer output, and high performance post-processing and analysis by the computational and storage infrastructure. The cyber infrastructure resources have been tailored specifically to handle the computational and data workflow from the sequencer, including a high bandwidth network to the high performance computing and data facilities. Mount Sinai collaborates and partners with other facilities and vendors to continuously improve its state-of-the-art cyber infrastructure and services. The scientific computing staff track the communities' best practices and procedures to ensure that Mount Sinai's computing and data services are efficient and effective for its researchers.

Funding

Funds for this project are supplied through a two year R01 from the National

Institute of Diabetes and Digestive and Kidney Diseases/NIH/DHHS.

2.2 Risk / Benefit Assessment

Risks to Subjects:

1) Screening evaluation

The risks and discomforts of the screening and baseline evaluations are minimal. No discomfort is expected to be associated with the physical examination or the clinical interview. Venipuncture may result in pain and/or bruising. In addition, there is a slight possibility of infection.

2) Harmine Administration

Anticipated side effects include the following; nausea, vomiting, slow, coarse, spontaneous tremor of the extremities, humming and buzzing noises, ‘waviness’ of the environment, ‘sinking’ sensations of the body, subjective sense of body vibration, numbness and reduced sensitivity to light touch.

Blood pressure and heart rate are anticipated to decrease by an average of 16mm and 18 beats/minute, respectively. Recovery should be observed after 30 minutes.

The study team will closely monitor possible drug-related DLTs, which are defined as 1). Any serious adverse event with causality of at least “Possibly” or any moderate or severe adverse event with causality of at least “Possibly” based on FDA guidelines and MSSM Adverse Event tracking form, or 2). The event that the patient’s systolic or diastolic blood pressure (BP) or heart rate (HR) changes by greater than 20% relative to baseline values.

3) Questionnaires

Completing the questionnaires does not pose any risks. It might be a little tedious to repeat the same questionnaires over time. Participants may choose to decline to answer certain items and may stop testing at any point during the test session.

4) Intravenous catheter insertion

The insertion of the IV catheter may cause momentary discomfort. To minimize this, only experienced DAC personnel will perform the procedures involved.

5) Privacy risks

There always exists the potential risk for loss of private information. However, there are procedures in place to minimize the risk.

Potential Benefits to Subjects

No direct benefits may result from study participation. All participants will receive without cost an extensive psychiatric and medical evaluation. All participants will receive compensation for their participation in the study.

2.3 Recruitment Methods

The vast majority of potential participants for this study will be recruited from the greater NYC metropolitan area by media advertisements, including print and internet advertisements. All advertisements will be submitted to the IRB for approval prior to posting. Individuals who express interest will be given the telephone number of our research program and may call for information about the study. Trained research coordinators will briefly describe the study procedures to the potential participants, and if appropriate, will schedule the volunteer for the initial screening visit. Informed consent is obtained in person, prior to any diagnostic procedures. Interested participants will be screened and those that meet criteria will be offered the opportunity to participate in this study. Subjects will be compensated for the time and inconvenience associated with participation in this protocol.

Subjects may also be accepted as referrals from the MAP Screening Protocol (GCO 06-0945: A Screening Protocol for Adult Patients with Mood and Anxiety Disorders, Chronic Medical Conditions, and Healthy Volunteers; PI: Murrough, MD). The MAP Screening Protocol is not required for participation. The protocol is just one of a handful of ways for participants to find our program and therefore find this specific trial. It is not a mandatory part of this study and not all subjects will complete it. Part of the screening measures (SCID-5, self reports) may be completed under the MAP Screening Protocol as long as the assessments are completed within 6 weeks of the participant signing consent for the present protocol.

The MAP screening protocol is a protocol our program uses to screen every potential research participant. In other words, this protocol is the entry point into our program and is used to assess eligibility for any other research protocols that our program is running. A participant screened under this protocol may go on to participate in several other research protocols, or may only participate in the screening protocol if he/she is deemed ineligible for any other protocols. Because some of the assessments in the MAP screening protocol overlap with the assessments done in this proposed protocol (I.e. a diagnostic assessment, clinical self-report measures), we would not ask a participant to repeat these if he/she had completed the screening in the MAP Screening Protocol within the past 6 weeks and was interested in participating in the present study. For instance, a patient who had undergone a SCID-V assessment under the MAP Screening Protocol would not

be asked to undergo the MINI-7 as a part of screening for the present study as these assessments serve the same purpose as diagnostic tools.

The screening protocol specifically states that "data derived from [their] participation may be used… in a number of ways including: to make theories for future research, for any future DAC research protocols in which you participate…" It also asks participants to initial if they give DAC permission for future contact regarding additional study participation. A participant would only be re-contacted to participate in this proposed study if and only if they had given consent for future contact.

3 STUDY OBJECTIVES

3.1 Primary Objective

Aim 1: The principal goal of our clinical trial is to establish the Maximum Tolerated Dose (MTD) of harmine in healthy patients that will inform the recommended phase II dose.

Hypothesis 1.1: Orally administered harmine will be well-tolerated with minimal dose-limiting toxicities at our testing doses.

3.2 Secondary Objectives

Aim 2: Characterize psychoactive effects of harmine in healthy adults.

Hypothesis 2.1: Orally administered harmine will be well-tolerated and will not induce burdensome psychoactive effects in healthy adults.

4 STUDY DESIGN

4.1 Study Overview

Definition of Maximum Tolerated Dose (MTD)

In this modified Continual Reassessment Model (CRM) approach, the MTD is defined as the dose with a toxicity level closest to the target toxicity level of 25%. The CRM final MTD estimate is that dose that would have been given to the (N+1)*st* patient in the trial.

Definition of Dose Limiting Toxicity (DLT)

a) A serious adverse event with causality of at least “Possibly” related to the study drug or
b) A non-serious adverse event rated as at least moderate and “Possibly” related to the study drug
c) The event that the subject experiences any of the following psychiatric symptoms

   i) Visual hallucinations

   ii) Humming or buzzing noises

   iii) Sensations of sinking, body vibrations or waviness of the environment

d) The event that the subject develops any of the following changes in vital signs within 6 hours following administration of study drug:

i) Symptomatic hypotension, **or** > 20% decrease in systolic blood pressure (SBP) from pre-dosing **and** an absolute SBP < 90; or

ii) Symptomatic hypertension, **or** > 20% increase in SBP or diastolic blood pressure (DBP) from pre-dosing **and** absolute SBP > 170 or DBP > 95;

iii) New onset tachycardia (heart rate >100 bpm) and >20% increase from pre-dosing; or symptomatic bradycardia (heart rate <60 bpm) and > 20% decrease from pre-dosing.

Relationship	Attribution	Description
Unrelated to investigational drug	Unrelated	AE is clearly NOT related to intervention
	Unlikely	AE is doubtfully related to intervention
	Possible	AE may be related to intervention
Related to investigational drug	Probable	AE is likely related to intervention
	Definite	AE is clearly related to intervention

Table 1. Causal relationship between adverse event and investigational drug

Dose Escalation Rules

Generally, the CRM starts by selecting a target dose-limiting toxicity rate and a mathematical model assumed to govern the relationship between dose and toxicity; the prior dose-toxicity curve. After outcomes are observed (i.e., a patient experiences a DLT or not), the dose-toxicity curve is refit incorporating the latest outcome. The next dose assigned is that which is most likely to be associated with the target toxicity level (the MTD). At every step, the next patient is assigned the dose estimated to be nearest to the MTD.

While the "3 + 3" design is the most commonly employed dose finding design, the CRM is generally accepted by statisticians as superior due to two main advantages: (1) the CRM provides a mechanism for stopping at a specified toxicity level, whereas the targeted toxicity rate for the MTD from the "3 + 3" design is necessarily in the range of 16%-33%, and (2) the CRM is more efficient; it can establish the MTD more accurately and precisely from fewer patients and it minimizes the number of patients treated at unsafe doses. Initial criticisms of the CRM, largely that unsafe escalation could occur too quickly, have been addressed by modifications to the original approach. Our implementation of the CRM will include modifications that prohibit steep dose escalations and will include the standard safety considerations of the traditional designs.

We will implement a series of precedented safety modifications to the original CRM design suggested by O'Quigley et al. in order to ensure subject safety. The first modification will be to start dosing from the

lowest dose, far below the expected MTD, instead of starting at the prior MTD estimate (Wheeler 2019). Secondly, we will not skip untested dose levels when escalating doses in order to reduce the number of subjects exposed to potentially toxic doses. However, we can skip doses when de-escalating if the CRM recommends doing so (Wheeler 2019). Lastly, we will enforce coherent dose-escalation, where if the last patient has a DLT, the next patient will not receive a dose higher than that of the last patient, even if the model recommends it (Faries D 1994). These modifications are simple to implement and help to prevent overdosing subjects.

Dosing Design

We will employ the CRM with the following design parameters:

a. The target dose limiting toxicity rate is 25%.
b. 7 pre-defined dose levels: 100mg, 200mg, 300mg, 500mg, 700mg, 900mg and 1200mg with assumed prior toxicity rates of 2%, 5%, 10%, 20%, 30%, 50% and 65%.
c. Model for dose-toxicity:
    1. A maximum of 40 evaluable patients. We do not plan to implement any stopping criteria (other than for toxicity). In the absence of DLTs we plan to enroll all 40 patients in order to increase the precision of the estimated MTD.

Rationale for Dose Determination

The dose escalation for this Phase 1 study in healthy subjects described herein is based on the experience during the dose escalation study of harmine in medically healthy subjects with varying mental disorders[21] and supported by the preclinical rodent data described in section 2 above. Dose determination was guided by results of both orally and intravenously administered harmine. 11 subjects received oral harmine at doses between 20-960 mg and 11 subjects received IV harmine between 100-300 mg. All subjects were observed for 72 hours. It is important to note that 5 subjects out of the total 29 had auditory hallucinations or delusions before study enrollment and that the results section does not differentiate between the outcomes of these subjects and others. Hence, adverse event results may be confounded by subject susceptibility to experience psychiatric symptoms.

Oral Administration Results and Contribution to Dose Determination

Authors report that harmine was not hallucinogenic and did not change heart rate or blood pressure at any dose when given via oral route[21]. Harmine did, however, induce some (unclear on which) of the following mild to moderate adverse events when given above 300mg orally; nausea and vomiting, tremors, humming and buzzing noises, 'waviness' of the environment; 'sinking' sensations of the body, subjective sense of body vibration, numbness and reduced sensitivity to touch. Given that no serious or severe adverse events were reported for oral dosing up to the maximum administered dose of 960mg, we concluded that escalating from 100mg to at least 900mg using the modified Fibonacci sequence is warranted.

Intravenous Administration Results and Contribution to Dose Determination

In order to interpret results of the IV administration of harmine for the purpose of our oral administration study, we converted the IV dose to its equivalent oral dose. By definition, bioavailability of a drug given by IV is 100% because the drug is administered directly into the vascular space. Pharmacology studies indicate that harmine has relatively poor bioavailability via oral route, with results varying between 3-5.33% [25]. In an effort to remain conservative, we assumed double the upper limit of bioavailability and multiplied the IV dose by 10 in order to get the equivalent oral dose.

The threshold hallucinogenic dose through IV from the previously mentioned clinical study was between 150-200mg (equivalent to 1500-2000mg orally), with 5 out of 11 subjects reporting close-eye hallucination[21]. Bradycardia and hypotension limited the maximum IV dose to 300mg (oral equivalent = 3,000mg), almost double our highest proposed dose. Aside from closed-eye hallucinations, subjects receiving harmine via IV also experienced the mild to moderate adverse events mentioned above. Although bradycardia and hypotension can be serious adverse events, the limiting dose due to these parameters in this study is double what we are proposing to escalate to. Coupled with our conservative estimate of bioavailability, these results make our maximum potential dose of 1200mg a reasonable upper limit in our effort to identify harmine's maximum tolerated dose.

Preclinical Rodent Data and Contribution to Dose Determination

As described in the background section, numerous studies have been conducted administering harmine to rodents, some of which have included multiple administrations over the course of one to two weeks. We used rodent studies with extended administrations of harmine and lengthy observational periods to help inform dose determination.

In order to convert the animal dose to a human equivalent dose (HED), we utilized allometric scaling. Allometric scaling is an empirical approach where the exchange of drug dose is based on normalization of dose to body surface area. This approach assumes that there are some unique characteristics on anatomical, physiological, and biochemical process among species, and the possible difference in pharmacokinetics/physiological time is accounted by allometric scaling [26]. This method is frequently used to predict an approximate dose on the basis of data existing in other species.

In order to convert a mouse or rat dose in mg/kg to human equivalent dose, we divide the animal dose by 12.3 or 6.2, respectively[27]. Following this conversion, we found the HED for two of the described studies while taking into account route of administration and bioavailability. The HED from the study that administered 20mg/kg harmine to mice via injection for 10 days is 1.62mg/kg [5]. For an average American male, this correlates to 144mg of harmine as if it was administered via IV. Converting this to an oral dose equivalent using our conservative 10% estimate translates this to a 1440mg oral dose. The HED from the study where rats were given 15mg/kg harmine via injection once a day for 14 days is 2.4mg/kg [7]. The equivalent IV and oral dose in the average male are 215mg and 2,150mg, respectively.

The fact that the animal subjects were healthy enough to undergo behavioral testing and observation for 10 to 14 days suggests that the doses of harmine administered were not toxic. Converting these doses to their HED of 1440mg and 2150mg provides support for the dosing regimen outlined above.

Choice of model

Our study will employ the continual reassessment method (CRM) to find a MTD of harmine (O'quigley 1990). The model-based CRM approach has been shown to be more accurate in targeting the MTD and more efficient in terms of requiring fewer volunteers, compared to more traditional "3+3" rule-based approaches for phase I studies. The CRM uses a statistical model to estimate the relationship between dose and DLT risk, which then informs dose escalation decisions. Generally, the CRM starts by selecting a target DLT rate and a mathematical model assumed to govern the relationship between dose and toxicity: the prior dose-toxicity curve. After outcomes are observed (i.e., a patient experiences a DLT or not), the dose-toxicity curve is re-fit incorporating the latest outcome. The next dose assigned is that most likely to be associated with the target toxicity level (the MTD). At every step, the next patient is assigned the dose estimated to be nearest to the MTD. Our implementation of the CRM utilizes a one-parameter exponential (or empiric) model, the model originally proposed by O'Quigley et al. We will employ the model with a logistic one-parameter prior for the model parameter α. We have chosen this model for two principal reasons; its simplicity and efficiency.

Generally, the one-parameter model is more efficient than two parameter models. That is, it requires fewer patients to precisely estimate that aspect of the dose-toxicity curve that is allowed to vary (the slope or steepness parameter). This increased efficiency in parameter estimation may come at the cost of a slight decrease in the accuracy of depicting the entire dose-toxicity relationship. However, the CRM has proven to be robust in choosing an MTD, even if the functional form is moderately incorrect. In large part this robustness is due to the fact that the CRM is not concerned with accurately estimating all aspects of the dose-toxicity curve, but rather the area near the location of the MTD.

5 CRITERIA FOR EVALUATION

5.1 Primary Endpoint

The primary endpoint of our clinical trial is to establish the Maximum Tolerated Dose (MTD) of harmine in healthy patients in a phase I dose escalation study for efficacy testing in phase II trials. See above for MTD and DLT definitions where safety is the primary endpoint.

5.2 Secondary Endpoints

A secondary endpoint is to characterize psychiatric effects of orally administered harmine in healthy adults.

5.3 Safety Evaluations

We will be continuously assessing for AE/SAEs through the CRM model and through the questionnaires, we will administer as outlined in **Table 2**.

5.4 Other Evaluations

N/A

6 SUBJECT SELECTION

6.1 Study Population

Healthy adults ages 18-55.

6.2 Inclusion Criteria

Inclusion Criteria

- Male or female aged 18-55 years;
    - *Rationale: We wish to exclude children under the age of 18 and adults over the age of 55 in part due to concerns regarding neurodevelopmental and neurocognitive effects, respectively.*
- Participants must have a level of understanding of the English language sufficient to agree to all tests and examinations required by the study and must be able to participate fully in the informed consent process;
- Body Mass Index (BMI) between 19 and 30;
    - *Rationale: We wish to exclude individuals who are underweight as defined as a BMI <19, or who are obese as defined as >30 according to the Centers for Disease Control (CDC). These criteria are in line with the goals of a phase I dose finding and pharmacokinetic study.*
- Women of childbearing potential and men must be using an acceptable method of contraception to avoid pregnancy throughout the study as judged by the investigator;
- Women must not be breastfeeding;

6.3 Exclusion Criteria

Exclusion Criteria

- Presence of a significant medical illness i.e., delirium, metastatic cancer, decompensated cardiac, liver or kidney failure, major surgery, stroke or myocardial infarction during the three months prior to entry;
- Presence of a significant neurological disease such as Parkinson's disease, primary or secondary seizure disorders, intracranial tumors, severe head trauma; neurodegenerative diseases;
- Presence of neurocognitive or dementing disorders;
- Presence or history of psychiatric disorder as diagnosed by Mini Neuropsychiatric Interview (MINI);
- Urine toxicology positive for illicit drugs or dis-allowed concomitant medications as per study protocol;
- Medications with primary central nervous system (CNS) effects are dis-allowed, including psychotropic medications, antidepressants, benzodiazepines, centrally acting hypnotic agents, and centrally acting anti-migraine therapies;
- Medications with primary cardiovascular effects are dis-allowed, including beta-adrenergic antagonists, ACE inhibitors, calcium channel blockers, and diuretics;
- Any OTC medications or herbal remedies (see concomitant medications listed above) that could interfere with the study drug, pose a risk to the subject, or contain high tyramine as outlined in the Low Tyramine Diet attachment and above in the concomitant medication summary;
- Any other medications that, in the opinion of the investigators, would pose a safety risk to the patient or that would interfere with the interpretation of study results;
- Positive pregnancy test at screen or on the morning of the treatment day in women of childbearing potential;
- Systolic blood pressure outside the range of 100 - 140 mmHg, diastolic blood pressure outside the range of 60 - 90 mmHg, and pulse rate at rest > 100 or < 60 bpm;
- History of positive tests for hepatitis B surface antigen, hepatitis C antibodies;
- History of HIV;
- Significant ECG abnormalities as follows:
    - Heart Rate < 60 and >100 bpm
    - PR Interval <120 and > 220 ms
    - QRS duration < 70 and >120 ms
    - QTC Interval (Bazett) > 450 ms

Clinically significant abnormalities at screening or morning of visit

- Systolic blood pressure outside the range of 100 - 140 mmHg, diastolic blood pressure outside the range of 60 - 90 mmHg, and pulse rate at rest > 100 and < 60 bpm.
- History of positive tests for hepatitis B surface antigen, hepatitis C antibodies, and HIV.
- Positive urine drug test at screening and morning of dosing.
- Significant ECG abnormalities as follows:
    - Heart Rate < 60 and >100 bpm
    - PR Interval <120 and > 220 ms
    - QRS duration < 70 and >120 ms
    - QTC Interval (Bazett) > 450 ms

7 CONCURRENT MEDICATIONS

Concurrent medications are prohibited for this study. See the I/E criteria above for detail.

7.1 Allowed Medications and Treatments

See the I/E criteria above for details about concomitant medications.

7.2 Prohibited Medications and Treatments

See the I/E criteria above for details about prohibited medications.

8 STUDY PROCEDURES AND GUIDELINES

8.1 Clinical Assessments

A Schedule of Events representing the required testing procedures to be performed is diagrammedin Appendix 1 and described in more detail in **Table 2**.

Prior to conducting any study-related activities, written informed consent and the Health InsurancePortability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or the subject's legally authorized representative.

8.1.1 Diagnostic Assessment

A psychiatric diagnostic assessment will be performed to assess current and past psychiatric disorders. This may be assessed using the SCID-5, MINI, or any other standard clinical diagnosticinstrument.

8.1.2 Self-Report Questionnaires

Self-report scales, assessing symptoms of depression and anxiety and related sequelae and experiences, will be administered either by paper-and-pencil form or via secure online survey tool(REDCap).

8.1.3 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at screening. Dose, route, unit frequency of administration, and indication for administration and dates of medicationwill be recorded as available. Concomitant medications are largely prohibited as outlined in the I/E criteria above.

8.1.4 Demographics

Demographic information (date of birth, gender, race) will be recorded at screening.

8.1.5 Medical History

Relevant medical history, including history of current disease, other pertinent history, andinformation regarding underlying diseases will be recorded at screening.

8.1.6 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation tostudy participation will be recorded on the case report form (CRF).

8.1.7 Urine Toxicology Test (UTOX)

A rapid urine toxicology test (UTOX) may be performed at the DAC offices. Results will not be posted in the participant's medical record.

8.1.8 Pregnancy Test

A urine or serum pregnancy test may be obtained from female subjects who are of childbearing potential.

8.1.9 Urinalysis

Urine may be obtained and sent to the site's clinical laboratory for determination of aroutine urinalysis and drug of abuse screen.

9 EVALUATIONS BY VISIT

Psychiatric interview tools to be used

1. The Mini Neuropsychiatric Interview (MINI-7) is a diagnostic interview used to determine psychiatric diagnoses in research settings based on DSM-V criteria. The brief structured interview assesses the 17 psychiatric disorders that are thought to be most prevalent (Sheehan et al., 1998).

2. The Visual Analogue Scales (VAS) (Bond and Lader 1974) are used to assess subjective state changes. They are 100-mm horizontal lines marked proportionately to the perceived intensity of the subjective experience (0=not at all, to 10=extremely) for the following states: anxious, depressed, drowsy, high, hungry, nauseous, control/dominance, happiness/pleasure, excitement/arousal, and vividness of image.

3. The Perceived Stress Scale (PSS) (Cohen et al. 1983) is a 10-item scale that measures the perception of stress. Each item is rated on a 5-point scale ranging from never (0) to almost always (4). Positively worded items are reverse scored, and the ratings are summed, with higher scores indicating more perceived stress. PSS-10 scores are obtained by reversing the scores on the four positive items: For example, 0=4, 1=3, 2=2, etc. and then summing across all 10 items. Items 4, 5, 7, and 8 are the positively stated items.

4. The Columbia Suicide Severity Rating Scale (C-SSRS) (Posner et al. 2011) is a suicidal ideation and behavior rating scale used to evaluate suicide risk. The C-SSRS is made up of ten categories, all of which maintain binary responses (yes/no) to indicate a presence or absence of the behavior. The ten categories included in the C-SSRS are as follows: Category 1 – Wish to be Dead; Category 2 – Non-specific Active Suicidal Thoughts; Category 3 – Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act; Category 4 – Active Suicidal Ideation with Some Intent to Act, without Specific Plan; Category 5 – Active Suicidal Ideation with Specific Plan and Intent; Category 6 – Preparatory Acts or Behavior; Category 7 – Aborted Attempt; Category 8 – Interrupted Attempt; Category 9 – Actual Attempt (non-fatal); Category 10 – Completed Suicide. A yes/no binary response is also utilized in assessing self-injurious behavior without suicidal intent. The outcome of the C-SSRS is a numerical score obtained from the aforementioned categories.

5. The Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) is a clinician-administered assessment used to capture acute behavioral changes throughout treatment. The scale includes 16 items each aimed at assessing components of psychosis including 4 items assessing positive (+) symptoms of psychosis such as conceptual disorganization, hallucinatory behavior, suspiciousness, and unusual thought content. Another 3 items assess negative (-) symptoms of psychosis such as: blunted affect, emotional withdrawal, and motor retardation. The remaining items assess activation and hostility by capturing tension, mannerisms and posturing, uncooperativeness, and grandiosity. Items that assess guilt, anxiety, depressed mood, and somatic concerns are also present.
6. The Patient Rated Inventory of Side Effects (PRISE) is an adverse Event Visit Checklist is a clinician-administered questionnaire used to qualify side effects by identifying and evaluating the tolerability of each symptom.

7. The bipolar version of the Profile of Mood States (POMS-Bi) is a 72-item psychological rating scale used to assess transient, distinct mood states. Items are rated on a 4-point scale from 0, “much unlike this” to 3, “much like this”. It includes six bipolar scales; composed-anxious, agreeable-hostile, elated-depresses, confident-unsure, energetic-tired, and clearheaded-confused. It is also a useful instrument in identifying the effects of drug treatments (O’Halloran et al., 2004).

While paper-and-pencil versions of self-report scales will be available, should participants express apreference for them, standard protocol will be to administer these measures via REDCap, a secure, password-protected web application for the development and management of surveys and databases. While participants will be able to access their own databases (with research team help),they will not be able to access anyone else's data. All participants will enter a subject code rather than an individually identifying PHI for purposes of self-report measures. Participants will be given opportunity to fill out these measures via computers in our offices. REDCap has a save-and-continue feature, which provides a session- and subject-specific code for continuation should participants desire to complete assessments off-site, further reducing participant burden.

9.1 Screening Visit (V0)

After receiving complete disclosure about the research and being given the opportunity to fully review the consent form, potential participants will be encouraged to ask the investigators questions. If they choose to take part in the study, they will be asked to provide written informed consent.

All participants will also undergo a diagnostic assessment, using the SCID–V or the MINI, in order to determine diagnosis. Screening measures may be completed under the screening protocol "A Screening Protocol for Adult Patients with Mood and Anxiety Disorders, Chronic Medical Conditions, and Healthy Volunteers" (GCO: 06-0945; PI Dr Murrough), as long as the assessments are completed within 6 weeks of the participant signing consent for the present protocol.

At the screen all participants will receive:

1) Psychiatric Assessments
    a) MINI for psychiatric history (if a diagnostic assessment has not been completed already within the past 6 months under a DAC protocol.
    b) C-SSRS
    c) BPRS
    d) Concomitant medication log

2) Medical Assessments
    a) Medical history (Hx)
    b) Physical examination (PE)
    c) Vital signs (VS)
    d) Electrocardiography (ECG)
    e) Laboratory Tests: CBC, CMP, UTox, U/A, TSH
    f) Urine tests: Drug abuse screen, Urinalysis, Pregnancy test (if applicable)

The study investigators will review the psychiatric interviews and medical assessments and identify eligible participants. The screening visit may occur on 2-3 separate days. All screening procedures will be completed before the Treatment visit - visit V1, and within four weeks of the treatment visit. As long as the screening assessments are completed within four weeks of the signing of consent for this protocol, the screening measures will not be repeated. Those found eligible based on screening assessment will be scheduled for the treatment visit within 4 weeks upon completion review of all screening procedures. Upon scheduling a baseline visit, eligible participants will be instructed to begin the low tyramine diet discussed during screening 3 days before the scheduled treatment visit. Participants will be routinely reminded by a member of the study team 4 days before a given treatment visit to begin the low tyramine diet the following day and see if the participant has any questions. Participants will also be systematically reminded 24 hours before their treatment visit to avoid food and drink at least 8 hours before dosing is set to begin. Adherence to the low tyramine diet

and the 8-hour NPO will be confirmed and recorded by the study clinician at the beginning of the treatment visit.

9.2 Treatment Visit (V1)

Refer to Table 2 for Visit 1 procedures.

9.3 Follow-up/Study Exit (V2)

Post-Baseline: approximately 24 hours after the oral dose

a) Brief physical and interval medical history
b) Update AE and con-med log
c) Collect blood sample
   a. 10 mL plasma
   b. 3-5mL serum
d) Clinical Scales/Self-Reports
   a. C-SSRS
   b. BPRS
   c. POMS
   d. PRISE
   e. PSS
   f. VAS

Study exit: Physician assessment followed by decision for discharge using standardized assessment scale

Table 2. Treatment Visit (Visit 1) Procedures

Time	Procedures

-90 min	• Subject arrives at Infusion Suite or CRU
	• Name and number for escort home is collected
	• Vitals*
	• Urine tests: Drug abuse screen, Urinalysis, Pregnancy test (if applicable)
	• Study doctor confirms 8-hour NPO
	• Study doctor confirms adherence to low tyramine diet for past 72 hours
	• Clinical assessments
	  • C-SSRS
	  • BPRS
	  • POMS
	  • PRISE
	  • PSS
	  • VAS
	• Insertion of Hep-lock
	• Baseline plasma and Serum sample: 1 ETDA and 1 SST

-10 min	➢ Pre-treatment baseline of vital signs: vital signs will

	be taken following 5 minutes of rest, with the average of two taken 5 minutes apart.
0 min	➢ Oral Administration of Harmine
+30 min	➢ Vital signs ➢ PRISE ➢ VAS ➢ Plasma and Serum sample: 1 ETDA and 1 SST
+60 min	➢ Vital signs ➢ PRISE ➢ VAS ➢ Plasma and Serum sample: 1 ETDA and 1 SST
+90 min	➢ Vital signs ➢ PRISE ➢ VAS ➢ Plasma and Serum sample: 1 ETDA and 1 SST
+ 120 min	➢ Vital signs ➢ PRISE ➢ BPRS ➢ VAS ➢ POMS ➢ Plasma sample
+180 min	➢ Vital signs ➢ PRISE ➢ VAS ➢ Plasma and Serum sample: 1 ETDA and 1 SST
+ 240 min	➢ Vital signs ➢ PRISE ➢ VAS ➢ Plasma sample
+ 300 min	➢ Vital signs ➢ PRISE ➢ VAS ➢ Plasma sample

+360 min	➢ Vital signs ➢ C-SSRS ➢ BPRS ➢ POMS ➢ PRISE ➢ VAS ➢ PSS ➢ Plasma and Serum sample: 1 EDTA and 1 SST
+420 min	➢ Vital signs ➢ C-SSRS ➢ BPRS ➢ POMS ➢ PRISE ➢ VAS ➢ PSS ➢ Plasma sample
+480 min	➢ Vital signs ➢ C-SSRS ➢ BPRS ➢ POMS ➢ PRISE ➢ VAS ➢ PSS ➢ Plasma sample
Readily for discharge home	➢ Physician assessment followed by decision for discharge ➢ Participants picked up by pre-designated escort home

*Prior to each vital sign, measurement the subject will be resting quietly for a minimum of five minutes before the vital signs are measured. For each specified vital sign time point, two readings will be taken 5 minutes apart and the average of the two readings will be used.

a) Safety parameters for monitoring

- Psychosis / mental status changes: BPRS, other clinical scales
- PRISE: nausea, vomiting, diarrhea and other general adverse events
- Vitals: BP, HR, Temp, Respiration
- Unanticipated effects: spontaneous reporting and structured assessment with the PRISE

b). Plasma and Serum sampling

At screening, blood will be drawn for clinical screening labs including chemistry and hematology. For this purpose up to 16mL of blood will be drawn. On a separate treatment day, blood will be collected at eight timepoints over approximately eight hours for pharmacokinetic analysis. At each of these timepoints, 4mL of blood will be drawn. A 10ml of blood will be collected at timepoints -90, +60, +90, +180, +360 minutes and +24 hours for immune cell characterization in addition to pk analysis. At the same timepoints 3-5mL of serum will be drawn for analysis of c-peptides, insulin, and glucose.

c). Dosage of Harmine

The doses of harmine for oral administration are 100mg, 200mg, 300mg, 500mg, 700mg, 900mg and 1200mg. The rationale for using these doses is based on the modified Fibonacci sequence (i.e. 1, 2, 3.3, 5, 7, 9, 12, 16…) that is widely used in Phase I clinical trials (Le Tourneau, Lee et al. 2009).

d) Drug source, storage and preparation

a) Source: Harmine will be provided and compounded by The University of Minnesota College of Pharmacy ITDD.
b) Harmine will be stored by the Investigational Drug Service (IDS) of the Mount Sinai Pharmacy
c) The manufacturing process of harmine consists of purification from the plant followed by salt formation to form harmine HCL under GMP conditions.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 Adverse Events

An AE is any untoward medical occurrence in a study subject administered an investigational product and that does not necessarily have a causal relationship with atreatment.

An AE therefore can be any unfavorable and unintended sign (including laboratory finding), symptom or disease temporally associated with participation in an investigational study, whether or not considered drug-related. In addition to new events, any increase in the severity or frequency of a pre-existing condition that occurs after the subject signs a consent form for participation is considered an AE. This includesany side effect, injury, toxicity, or sensitivity reaction.

Any condition, laboratory abnormality, or physical finding with an onset date prior to thesubject signing consent for study participation is considered to be pre-existing in nature and part of the subject's medical history.

AE Severity

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.

Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

1). Attribution of Adverse Events:
No: Adverse events are clearly not related to the investigational agents
Unlikely: Adverse events are doubtfully related to investigational agents
Possibly: Adverse events may be related to investigational agents
Probably: Adverse events are likely related investigational agents
Definitely: Adverse events are clearly related to the investigational agents

2). Plan for grading adverse events:
The FDA's definition of serious adverse events (21 CRF 312) include any untoward medical occurrence that at any time results in death or the immediate risk of death, hospitalization or the prolonging of an existing hospitalization, persistent or significant disability/incapacity or congenital anomaly/birth defect (NOH Guide, 6/11/99).

Grades of Risk:
1 = Non-Serious Adverse Event
2 = Serious Adverse Event

The PI will report all serious adverse events, verbally and in writing, to the Mount Sinai IRB within 48 hours.

10.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life threatening experience defined as any adverse experience that places the subject, in the view of the treating physician, at immediate risk of death at the time of occurrence; ie, it does not include a reaction that, had it occurred in a more severeform, might have caused death.
- Requires inpatient hospitalization or prolongation of an existing hospitalization (except scheduled hospitalizations for non-acute, unrelated cause such as an electivesurgery)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in the offspring of an exposed subject
- Important medical events that may not result in death, be life threatening, orrequire hospitalization, may be considered an SAE when, based upon appropriate medical judgment, it jeopardizes the subject and may require medical or surgical interventionto prevent one of the outcomes listed in this definition.
- Any death occurring within 30 days of the subject receiving study drug, regardless ofthe

subject having discontinued from the protocol, must be reported as an SAE.

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention toprevent one of the outcomes listed.

AE/SAE Reporting Procedure

To Mount Sinai PPHS (IRB)

AEs are reportable to the IRB within 5 business days (SAEs within 24 hours of knowledgeof the event) *when they meet the following definition*:

> Any 'harm' experienced by a subject or other individual that in the opinion of the investigator is *unexpected* AND *at least probably related* to the research

All AE/SAEs with an onset date after the subject signs consent for study participationmust be reported to the IRB *at the time of annual renewal*. Details of the event must include severity, relationship to study drug, duration, action taken, and outcome.

All AE/SAEs that are considered related to study drug must be followed to resolution or stabilization if improvement is not expected. AE/SAEs that completely resolve and then recur should be recorded as a new AE/SAE. AE/SAEs continuing at 30 days post-last dose should have acomment in the source documents by the PI that the event has stabilized or is not expected to improve.

11 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

Participants will be exited from the study once a screening determination is made (eligible/ineligible) for further DAC studies or once they become lost to follow-up. Participants may re-enter the study for re-screening if necessary.

All subjects are free to withdraw from participation at any time, for any reason, specifiedor unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

Subjects who withdraw from the study will not be replaced.

12 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator,or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specifiedor unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint

criteria. Protocol violations for this study include, but are not limitedto, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Positive urine toxicology screening

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Principal Investigator (PI) will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 DATA SAFETY MONITORING

Safety and data information will be reviewed in an ongoing matter by the study PI at monthly project meetings. The clinical trial team, led by PI Dr. Murrough, will monitor for the occurrence of DLTs in real time throughout the study. The occurrence (Yes/No) of a DLT for every newly enrolled participant will be formally reviewed at each of the weekly research meetings and at the bi-weekly project meetings that are attended by the larger investigational team, to include Drs. Stewart, DeVita, Smith, and Govindarajulu. Please see the related **Data Safety and Monitoring Plan** and **Protection of Human Subjects** documents for details regarding dose escalation procedures for the study.

The following individuals will lead the efforts of the data safety and monitoring committee:

MSSM Principal Monitor:
Principal Investigator
Last Name: Murrough
First Name: James
Academic Title: MD, Ph.D.
Department: Psychiatry
Mailing Address: One Gustave L. Levy Place Department of Psychiatry, Box 1230 New York, New York 10029
Phone: 212-585-4640
Fax: 212-241-3354
E-mail: james.murrough@mssm.edu

MSSM Additional Monitor:
Team Member
Last Name: Ables
First Name: Jessica
Academic Title: Assistant Professor
Department: Psychiatry and Neuroscience
Mailing Address: One Gustave L. Levy Place Department of Psychiatry, Box 1230 New York, New York 10029
Phone: 212-241-3354
Fax: 212-241-3354
E-mail: : jessica.ables@mssm.edu

Safety Monitor:
Independent:

Last Name: Levy
First Name: Carol
Academic Title: Professor
Department: Endocrinology, Medicine, Diabetes and Bone Disease, Obstetrics, Gynecology and Reproductive Science
Mailing Address: 1190 Fifth Avenue, 1st Floor, New York, NY 10029
Phone: (212) 241-3422
Fax: 212-241-3354
E-mail: carol.levy@mssm.edu

Dr. Murrough is the director of the Depression and Anxiety Center (DAC) and intimately knowledgeable of this study. He will be involved in the clinical care of the subjects and will provide oversight of the entire study. He has been involved in many studies in DAC.

Dr. Ables is a co-director of DAC and has extensive experience in clinical trials and psychopharmacology in mood disorders.

Dr. Levy is a clinical endocrinologist and certified diabetes educator at Icahn School of Medicine at Mount Sinai, and has volunteered to join us as a Safety Monitor. Dr. Levy is the director of the Mount Sinai Diabetes Center and knows T1D very well clinically and in terms of clinical research. In the event of a suspected DLT, Dr. Levy along with Drs. Murrough and Ables will review the event and make the formal determination of the occurrence of a DLT, which will subsequently inform the CRM and the dose level of the next patients treated in the study.

The DSMC will focus on the following items:

- Study-related adverse events
    - We would like to note that we are also monitoring for adverse events as per our DLT definition (see **Definition of Dose Limiting Toxicity** above).
- Cardiovascular effects: Any of the following that occur after administration of study drug will be considered a cardiovascular effect if temporally related to administration of the investigational intervention and considered at least possibly related to the investigational intervention:
    - Symptomatic hypotension, **or** > 20% decrease in systolic blood pressure (SBP) from pre-dosing **and** an absolute SBP < 90; or
    - Symptomatic hypertension, **or** > 20% increase in SBP or diastolic blood pressure (DBP) from pre-dosing **and** absolute SBP > 170 or DBP > 95;
    - New onset tachycardia (heart rate >100 bpm) and >20% increase from pre-dosing; or symptomatic bradycardia (heart rate <60 bpm) and > 20% decrease from pre-dosing.
    - Signs and symptoms of cardiac ischemia, defined by acute ischemic changes on ECG with or without concomitant symptoms
- Protocol compliance
- Dropouts
- Confidentiality of participants' information
- Other study-specific safety information

15 STATISTICAL METHODS AND CONSIDERATIONS

Human data on oral harmine administration are limited and of poor quality, as noted above. Briefly, there is only one study that employed oral doses in the range of 20 to 960 mg (Pennes et al., 1957). No subject had any adverse visual hallucinations. Some subjects (number uncertain) experienced "nausea, vomiting, slow coarse spontaneous tremor, 'waviness' of the environment, 'sinking' sensations of the body, subjective sense of body vibration, and subject numbness accompanied by reduced sensitivity to light touch and pinprick". These effects were apparently observed only in "some subjects" and only "occurred with oral doses higher than the threshold of 300.0-400.0 mg." In addition, the Callaway Ayahuasca study (Callaway et al., 1999) administered harmine in the Ayahuasca "tea" at the dose of 3.4 mg/kg (range 204-306 mg). Callaway et al. noted that the effects of this dose of harmine (along with other ingredients of Ayahuasca) was perceived to be mild. Thus, based on this limited data, doses below 200 mg are likely safe, and single oral doses as high as 1000 mg may also be safe and free of significant adverse effects For this reason, and an added layer of caution, we have elected to initiate dosing at 100 mg which is less than half of the lower dose range in Callaway study. The upper level of dose range (1200 mg) is 4-times that of the highest dose of Callaway study.

In terms of selecting the number of doses tested between the established minimum (100 mg) and maximum (1200 mg) dose, the most important statistical consideration is whether the doses and dose range under investigation are likely to allow an accurate MTD estimate. In general, it is recommended that phase I studies contain between 2 and 12 dosing levels (Penel and Kramar, 2013); more levels generally allow for a more accurate determination of the MTD. Based primarily on clinical data, together with the available pre-clinical safety data, and utilizing established methods for defining the incremental sequence of a range of doses (Le Tourneau et al., 2009), our study protocol specifies the following 7 oral harmine dose levels to be tested according to the dose escalation model described below: 100 mg, 200 mg, 300 mg, 500 mg, 700 mg, 900 mg and 1200 mg.

Our study will employ the continual reassessment method (CRM) to find the MTD of harmine (O'Quigley 1990). Generally, the CRM starts by selecting a target dose-limiting toxicity rate and a mathematical model assumed to govern the relationship between dose and toxicity; the prior dose-toxicity curve. After outcomes are observed (i.e., a patient experiences a DLT or not), the dose-toxicity curve is refit incorporating the latest outcome. The next dose assigned is that which is most likely to be associated with the target toxicity level (the MTD). At every step, the next patient is assigned the dose estimated to be nearest to the MTD. While the "3 + 3" design is the most commonly employed dose finding design, the CRM is generally accepted by statisticians as superior due to two main advantages: (1) the CRM provides a mechanism for stopping at a specified toxicity level, whereas the targeted toxicity rate for the MTD from the "3 + 3" design is necessarily in the range of 16%-33%, and (2) the CRM is more efficient; it can establish the MTD more accurately and precisely from fewer patients and it minimizes the number of patients treated at unsafe doses. The CRM is a model-based design while that of the 3+3 is a rule-based design.
Our implementation of the CRM is a Bayesian model-based method that will employ a logistic one-parameter prior for the model parameter α. We have chosen this model for two principal reasons; its simplicity and efficiency. Generally, the one-parameter model is more efficient than two parameter models. That is, it requires fewer patients to precisely estimate that aspect of the dose-toxicity curve that is allowed to vary (the slope or steepness parameter). This increased efficiency in parameter estimation may come at the cost of a slight decrease in the accuracy of depicting the entire dose-toxicity relationship. However, the CRM has proven to be robust in choosing an MTD, even if the functional form is moderately incorrect. In large part this robustness is due to the fact that the CRM is not concerned with accurately estimating all aspects of the dose-toxicity curve, but rather the area near the location of the MTD. The Inputs to specify for our model are: target toxicity pT as single-parameter (θ) dose-toxicity curve, K for number of doses, R for odds ratio (OR), and α for accuracy. If we use a one-parameter logistic model for the dose response curve, we would have a one-parameter dose response curve where d is the dose:

$$\varphi(d,\alpha) = \frac{e^{3+\alpha d}}{1+e^{3+\alpha d}}$$

We will assign a prior probability distribution to the model parameters, assigning a prior probability of DLT at each dose based on the available data (Legedza, 2001). In order to minimize risk, we will implement a common modification to the CRM such that the first patient treated in the study will be treated at the lowest available dose (e.g., 100 mg) regardless of the prior distribution (Goodman et al, 1995). The study will end when either the maximum number of allowable patients treated per protocol is reached (see below), or it will end early in the event that the probability that the next 10 patients to be dosed in the trial will be given the same dose level, regardless of DLT outcomes observed exceeds 90% (Zohar and Chevret, 2001).

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents and paper Case Report Forms(CRF) corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available. Subjects will not be identifiedby name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a unique ID number and initials.

For eCRF data, recorded via REDCap, a date and time stamp tracks data entry and updates to entered data and creates an electronic audit trail. For paper CRFs, if corrections are needed the study staff will line through the incorrect information, write the correct data, and initial and datethe change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness andaccuracy by the Investigator.

16.2 Data Management Procedures

The data will be entered into a validated database, stored on REDCap.

All data collected will be kept confidential and used for research purposes only. Each participant will be assigned a coded identifier that will be used to associate stored data with each participant. Diagnostic interviewers will only enter coded identifiers on their notes and forms. The list associating participants' names with coded identifiers will be maintained separately from the data and in a locked file. No participant's identifying data will be published. All electronic records will be kept confidential to the extent permitted by law, stored in a file (including the linking file) on an electronically secure database in the Department of Psychiatry at ISMMS maintained by Sinai's IT department.

This database is password protected and only study personnel will be given the password. A backup of the database will be conducted each day and stored on the secure database. Only study personnel will have access to the database and backup. The study data will be stored in the database for 2 years after the study is completed, allowing for any follow-up analyses. The study personnel will be responsible for the receipt or transmission of the data.

The data from the pre-screener is stored in the secure, encrypted online database, Redcap. The database has been designed such that no one has access to the subjects' answers. The study team, including Dr. Murrough

who is also the PI, only has access to the subject's contact information along with an indication as to whether or not the participant appears initially eligible based on the parameters set up in the pre-screener.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the electronic database, REDCap, directly. The study database willbe updated in accordance with the resolved queries. All changes to the study database will be documented.

16.4 Archiving of Data

The database is safeguarded against unauthorized access by established security procedures;appropriate backup copies of the database and related software files will be maintained.
Databases are backed up by the database administrator in conjunction with any updates orchanges to the database.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representativesof the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A filefor each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. TheInvestigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study FileNotebook, etc.) will be kept secured in keeping with Mount Sinai institutional policies.

16.6 Subject Confidentiality

Confidentiality will be protected through the use of participant study ID numbers, generated basedon the order in which individuals are screened, rather than any identifying information (e.g. names, date of birth). Questionnaires, forms and cognitive test results will contain only the subject's ID number; no identifying information will be included on the data. Diagnostic interviewers will only enter coded identifiers and initials on their notes and forms. All health protected information will be kept in locked files or on a secure MSSM server that is maintained byIT personnel and designed to house confidential information. The only forms that will contain the participants' names and identifying information will be the consent forms, which will be stored in alocked file in the principal investigator's locked office. Only the PI and other approved members of the DAC research team will have access to the code list which links that participant study ID number to health-protected information. The list associating participants' names with coded identifiers will be maintained separately from the data and in a locked file and will be destroyed after study completion.

Participants have the right to withdraw consent at any time by contacting the study principle investigator. At that time, samples that have not already been used for research will be promptlywithdrawn from storage and destroyed by trained laboratory personnel.

Results will be published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientificreports, or publications, in order to maintain anonymity.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of HumanVolunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (Health Insurance Portability and AccountabilityAct of 1996).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Principal Investigator (PI). Protocol amendments can not be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs arenotified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reportedto the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment,payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB unconditional approval statement will be transmitted by the Investigator to ISMMS or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without priorwritten IRB approval except when necessary to eliminate immediate hazards to the patients orwhen the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; seriousand/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re- approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA), and localregulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. Theconsent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will store an IRB -approved copy of the Informed Consent Form at thelocal study site.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legally authorized representative) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the subject willalso be obtained. Alternatively, Participants will also be given the option to complete the consent remotely, using a telehealth platform (VSee, HIPAA-compliant Zoom) or over the phone (using Doximity) and the electronic consent process via REDCap survey that will be sent to individuals. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or the subject's legally authorized representative and the original will be maintained with the subject's records.

17.4 Publications

The publication or presentation of any study results shall comply with all applicable privacy laws,including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes afternotifying the IRB, except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB reviewand approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Ensure that all associates, colleagues and employees assisting in the conduct of thestudy are informed about their obligations in meeting the above commitments.
5. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and tomake those records available for inspection.
6. Ensure that an IRB that complies with the requirements of §21 CFR part 56 willbe responsible for initial and continuing review and approval of the clinical study.
7. Promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and safety reports).
8. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
9. Comply with all other requirements regarding the obligations of clinical investigators and allother pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS

	Screening Visit	Dosing Visit	Follow-Up/Exit Visit
Informed Consent	x		
Pregnancy Test (Urine or Serum)	x	x	x
Urinalysis	x	x	x
Medical History and Clearance	x		
Urine Toxicology Screening (UTOX)	x	x	x
Self-Report Scales	x	x	x
Concomitant Medication Review	x		
Adverse Experiences Review	x	x	x
Harmine Administration		x	
Blood Sampling		x	x