

Clinical Trial Protocol

1 INFORMATION ON CLINICAL TRIAL PROTOCOL

This protocol has been designed for a clinical trial subject to the European Communities (Clinical trials on Medicinal Products for Human Use) Regulations, 2004 (S.I. No 190 of 2004), as amended.

2 STUDY TITLE

Ketamine for relapse prevention IN recurrent DepRessive Disorder: a randomised, controlled pilot trial with blood biomarker evaluation: The KINDRED Trial

3 STUDY SPONSOR

St Patrick's Mental Health Services as represented by the Medical Director, Prof. James Lucey

4 APPLICATION DETAILS

4.1 Study title

Ketamine for relapse prevention in recurrent depressive disorder: a randomised, controlled pilot trial

4.2 Reference numbers

Protocol identification (code or reference number): SPMHS Ref: 20/15

EudraCT number: 2015-002020-37

Date and version number: Version 3.0, as approved by the Research Ethics Committee of the Mater Misericordiae Hospitals on 15.9.16

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Funder : Medical Research Charities Group - Health Research Board Joint Funding Scheme 2016 "HRB-MRCG Joint Funding Scheme 2016 Contracts MRCG-2016-23"

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5 CONFIDENTIALITY STATEMENT

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigative team, regulatory authorities, and members of the Research Ethics Committee.

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7 DOCUMENT HISTORY

Document	Date of Issue	Summary of Change
Original protocol	19.5.15	Not applicable
Version 2.0	18.7.15	Addition of information regarding traffic law
Version 2.1	24.8.15	Addition of Childhood Trauma Questionnaire
Version 3.0	Approved by REC on 15.9.16	Change in eligibility criterion: "Meet DSM-V criteria for recurrent depressive disorder (RDD): ≥ 2 previous depressive episodes with at least 2-months(consecutive) subthreshold or no symptoms in between PLUS must also have experienced ≥ 3 major depressive episodes (including index episode) within the previous 2 years." to "Meet DSM-V criteria for recurrent depressive disorder (RDD): ≥ 2 previous depressive episodes with at least 2-months (consecutive) subthreshold or no symptoms in between."

8 SYNOPSIS

Title of study	Ketamine for relapse prevention in recurrent depressive disorder: a randomised, controlled pilot trial
Name of sponsor/company	St Patrick's Mental Health Services as represented by the Medical Director, Prof. James Lucey
Phase of development	Feasibility study for Phase II Trial
Objectives	The overall aim is to assess ketamine for depression relapse prevention. Objective 1: To conduct a randomised controlled patient- and rater-blinded pilot trial of two-weekly ketamine vs. midazolam over eight-weeks for reducing relapse during the six-months following successful treatment of depression in people with RDD. Objective 2: To assess safety and tolerability of repeated (x4) infusions of ketamine vs. midazolam in this RDD population.

	<p>Objective 3: To explore the role of ketamine-induced changes in peripheral blood neuroplasticity molecules for: (i) monitoring biological response to ketamine during the first infusion and (ii) for evaluating this biological response in predicting lower relapse rates over six-months.</p> <p>Objective 4: To investigate epigenetic modulation of depression/stress-related genes in patients with recurrent depression receiving inpatient treatment</p>
Trial design	<p>Randomised, controlled, parallel-group, pilot clinical trial of ketamine vs. midazolam for depression relapse prevention in persons at high risk. The main purpose of the pilot study is to assess trial processes to help inform a future definitive trial.</p> <p>Participants will be recruited at admission to St Patrick's University Hospital for treatment of DSM-IV-diagnosed recurrent unipolar depression and followed-up weekly to assess recovery according to standard criteria. Blood samples for epigenetic studies will be taken at baseline. Treatment-as-usual will continue throughout the entire trial. Participants who meet standardised response criteria will then be invited to be randomised to course of four two-weekly ketamine or midazolam (active comparator) infusions. Block randomisation will be independently performed. Physical, psychotomimetic and cognitive outcomes will be monitored before, during and after infusions. Blood samples will be taken at four time-points in the first infusion session and before the final infusion for neuroplasticity biomarker studies.</p> <p>Trial Interventions: participants will receive four two-weekly infusions of either ketamine at 0.05mg/Kg or midazolam at 0.045mg/Kg. All infusions will be administered by a consultant anaesthetist. Repeated infusions of ketamine have been shown to be safe and well-tolerated by patients with mental illness. Minor haemodynamic changes and psychotomimetic side-effects can occur and will be assessed regularly during infusions and for 200 minutes afterwards.</p> <p>Participants will be followed up over six months to assess for relapse according to standardised criteria. This is the highest-risk period for relapse and I hypothesise that ketamine will provide additional neurotrophic support (assessed by the laboratory biomarker project) which will result in lower relapse rates when compared to midazolam.</p>
Key inclusion criteria	<ul style="list-style-type: none"> • ≥18 years old • HRSD-24 score of ≥21 • Voluntary admission for treatment of acute depressive episode • Meet DSM-V criteria for recurrent depressive disorder (RDD): ≥2 previous depressive episodes with at least 2-months(consecutive) subthreshold or no symptoms in between <p>For the randomised pilot trial, RDD patients must have:</p> <ul style="list-style-type: none"> • received antidepressant treatment for the acute depressive episode(pharmacological, psychotherapeutic or multidisciplinary) • ≥60% decrease from baseline HRSD-24 score and score ≤16 • Mini-Mental State Examination (MMSE) score of ≥24 • (v) able to provide informed consent

Key exclusion criteria	<ul style="list-style-type: none"> • Current involuntary admission • Medical condition rendering unfit for ketamine/midazolam • Active suicidal intention • Dementia • History of Axis 1 diagnosis other than RDD • ECT for treatment of index depressive episode • Alcohol/substance abuse in previous six-months • Pregnancy or inability to confirm use of adequate contraception during the trial
Number of subjects	N=98 to be recruited upon admission, expecting that at least n=51 will meet response criteria and that n=40 will consent to be randomised. This provides a sample size of n=20 per group
Test product, dose and mode of administration	<p>Investigational Medicinal Product: Ketamine: Ketalar 10mg/ml Solution for Injection/Infusion, Pfizer Ireland</p> <p>Active comparator: Midazolam: Hypnovel 10mg/5ml solution for injection, Roche Pharmaceuticals Ireland</p> <p>Both made up as 50 ml colourless saline solutions and administered over 40-minutes using a syringe driver pump, in an eight-week course of four infusions</p>
Duration of treatment	Eight weeks
Statistical methods	Data analyses will be performed blinded to allocation by Prof Daly in the Centre for Support and Training in Analysis and Research (CSTAR). Pilot trial data will be analysed on an intention-to-treat basis for all patients who completed at least one infusion. Descriptive statistics will be used to report process outcomes. Relapse-free survival times will be compared between groups using Kaplan-Meier survival curves and log-rank test. Cox proportional hazard regression analysis will provide a 95% confidence interval for a hazard ratio for ketamine versus midazolam groups. These data will inform a future definitive trial
Sample size	We wish to recruit 20 patients per group, a total of 40, an acceptable number for the purposes of a pilot trial. A formal sample size calculation is not appropriate as per the Medical Research Council Guidelines. Response rates to inpatient depression treatment are approximately 80%. However, as this is a heterogeneous sample with RDD, we expect response rates to be lower at 50-60%. Allowing for a 15% drop-out rate, We seek to recruit 98 patients upon admission, expecting that at least n=51 will meet response criteria and that n=40 will consent to be randomised. Prof McLoughlin's team recently finished recruiting severely depressed inpatients (n=140) to an RCT of ECT at St Patrick's University Hospital (see http://www.controlled-trials.com/isrctn/pf/23577151). 60 patients were recruited in the first 16 months. As the proposed study is less intensive, with the attraction of an additional therapy and a wider cohort to recruit from, I expect the recruitment rate to be higher and to be able to recruit 98 participants to the pilot trial within 16 months.

9 ABBREVIATIONS

ACE-R	Addenbrooke's Cognitive Examination, Revised
AE	Adverse event
Akt	Ak-Thyoma/Protein Kinase B
AR	Adverse reaction
BDNF	Brain Derived Neurotrophic Factor
BPRS	Brief Psychiatric Rating Scale
CA	Competent authority
CADSS	Clinician-Administered Dissociative States Scale
CRA	Clinical research associate
CRF	Case report form
CRO	Contract research organisation
CSTAR	Centre for Support and Training in Analysis and Research, University College Dublin
CT	Clinical trial
CTA	Clinical trial authorisation
CXR	Chest x-ray
DNA	Deoxyribonucleic Acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
ECG	Electrocardiogram
ECT	Electroconvulsive therapy
eEF-2	Eukaryotic elongation factor 2
EHQ	Edinburgh Handedness Questionnaire
EU	European Union
e-CRF	Electronic case report form
FBDS	Forward and Backward Digit Spans
GCP	Good Clinical Practice
GP	General Practitioner
GSK-3	Glycogen Synthase Kinase 3
HRSD-24	Hamilton Depression Rating Scale, 24-item version
HPRA	Health Products Regulatory Authority
HSE	Health Service Executive
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonisation
IMP	Investigational medicinal products
IMPD	Investigational medicinal product dossier
LMP	(Date of) Last Menstrual Period
MDD	Major depressive disorder
MMSE	Mini Mental State Exam
MSTRD	Maudsley Staging for Treatment-Resistant Depression
mTOR	Mammalian target of Rapamycin
NART	National Adult Reading Test
NMDA	N-methyl-D-aspartate
NOK	Next of kin
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PI	Principal Investigator (Refers to Prof. Declan McLoughlin)
PIL	Patient/subject information leaflet
PRISE	Patient-Rated Inventory of Side Effects

QIDS-SR	16 Quick Inventory of Depressive Symptoms, Self-Report, 16-item
RA	Nominated Responsible Adult
RDD	Recurrent Depressive Disorder
REC	Research ethics committee
ROI	Republic of Ireland
SAE	Serious adverse event
SAR	Serious adverse reaction
SCID	Structured Clinical Interview for DSM-IV Axis I Disorders
SmPC	Summary of product characteristics
SOP	Standard operating procedure
SPUH	St. Patrick's University Hospital
SUSAR	Suspected unexpected serious adverse reaction
TCIN	Trinity College Institute of Neuroscience
YMRS	Young Mania Rating Scale

10 INTRODUCTION

10.1 Background information

DEPRESSION AND RELAPSE

Depression is projected to become the second greatest cause of disability worldwide by 2020. According to the Irish mental health charity Aware, over 300,000 people in Ireland experience depression at any time (<http://www.aware.ie/help/information/information-on-depression>). It is the most costly brain disorder in Europe, accounting for 1% (€118 billion annually) of the total European economy². Indeed, depression is currently the second largest cause globally for years lived with disability¹. It is thus a public health priority, additionally so in Ireland with worryingly high suicide rates (see <http://nsrf.ie/wp-content/uploads/reports/SSISReport2013.pdf>). One of the major reasons for the social and economic costs of depression is that it can be a chronic disorder. Over 50% of people who experience one depressive episode will have further episodes, with on average 5-9 episodes in their lifetime³. Even following successful antidepressant therapy for an acute episode, relapse rates are high, ranging 40-70%⁴. The first six months after remission represent the highest-risk period, with an average time to relapse of 3.5 months³. Much of the burden of depression is therefore contributed to by its relapsing nature.

RELAPSE PREVENTION

It is well established that continuation treatment with antidepressants reduces the odds of relapse by 70%, independent of the underlying risk of relapse.⁵ Nonetheless, relapse rates within six months are still too high at 40-70%.⁶ A major challenge now is how best to prevent relapse after successful treatment of depression. However, remarkably, the evidence base for relapse prevention is small. For example, the National Institute for Health and care Excellence (NICE) in the UK have identified that evidence on relapse prevention in depression is limited and recommended research in this area⁸ (and <https://www.nice.org.uk/guidance/cg90/chapter/4-research-recommendations>). To date, the reported randomised controlled trials for relapse prevention following successful antidepressant therapy have focused on the effect of 12 months' tricyclic or SSRI antidepressant therapy, showing consistent but limited reduction in relapse rates as above.⁷

Ongoing registered trials of relapse prevention in depression (<http://www.controlled-trials.com/isrctn> and <http://clinicaltrials.gov>) focus on psychological or internet-based

interventions. Interestingly, there are no currently registered trials of pharmacological treatments for relapse prevention. This possibly reflects a lack of industrial interest in this important, but difficult, area. There is clearly a need for other, potentially better, methods for relapse prevention. One such possibility is the anaesthetic/analgesic ketamine.

KETAMINE AND DEPRESSION

Ketamine is a competitive glutamate N-methyl-D-aspartate receptor (NMDAR) antagonist with a half-life of 2-3 hours. Ketamine has a remarkably rapid antidepressant effect,^{9,10} targeting core symptoms in treatment-resistant depression when given as single sub-anaesthetic doses (usually a 40 minute 0.5 mg/kg intravenous infusion).¹¹ It is psychotomimetic (with abuse potential) but at low dosage it is safe.^{9;10;11;12} Only 1.45% of sub-anaesthetic infusions in patients and healthy controls have been reported to cause mild dissociative and psychotic symptoms that resolve soon after finishing infusions.^{13,14} To control for these effects, and also avoid “carry-over” effects in crossover studies while improving blinding, midazolam is now being used as an active control in parallel-group design trials rather than inactive placebo saline.¹¹ Thereafter robust antidepressant effects (average four-hour response rate of 77%)⁹ occur and can persist for a few days, i.e. beyond immediate NMDAR blockade. Chronic, mostly recreational, high-dose ketamine use can cause uropathy and dependency¹⁵. However, repeated (e.g. 2-3/week for two weeks) infusions of sub-anaesthetic ketamine are safe with more sustained antidepressant effects in both antidepressant-free and ongoing-treated patients.⁹

Together, these findings have led to the most exciting development in treating and understanding depression in over 50 years and represent a paradigm shift away from conventional slow-acting monoaminergic antidepressants.¹⁰ Preclinical studies have shown that within just two hours ketamine increases synaptogenesis and spine formation in rodent prefrontal cortex and rapidly reverses chronic stress-induced depressive behaviours and prefrontal neuronal atrophy.¹⁶ These effects are mediated, at least in part, via Akt/GSK-3/mammalian target of rapamycin (mTOR) signalling and increased dendritic translation of synaptic proteins¹⁷ as well as deactivation of eukaryotic elongation factor 2 (eEF2) kinase, resulting in de-suppression of brain-derived neurotrophic factor (BDNF) translation.¹⁸ BDNF mediates synaptic plasticity and is implicated in mechanisms of antidepressants.^{18,19}

Interestingly, a lesser response to ketamine was found in low-activity Met BDNF Val66Met polymorphism carriers while increased plasma BDNF was detectable in responders compared to non-responders four hours post-infusion¹⁸ though this hasn't been found in all studies. Changes in blood mononuclear cell levels of phosphorylated mTOR, eEF2 and GSK-3 β have also been associated with response to ketamine^{20,21} suggesting potential as biomarkers for response.

The PI and team has started a HRB-funded pilot trial of ketamine for depression relapse prevention following electroconvulsive therapy (EudraCT number 2014-004262-14; and NCT02414932 at www.clinicaltrials.gov). However, no other trials are currently registered or reported using ketamine as an adjunctive treatment to reduce relapse rates following successful depression treatment - an important potential use of ketamine that this proposal addresses.

KETAMINE-ASSOCIATED BIOMARKERS

Ketamine causes acute neurotrophic effects which may result in more sustained positive clinical effects. Potential biomarkers to investigate ketamine in the novel role of relapse prevention are therefore molecules that have been associated with both neuroplasticity and ketamine's antidepressant effects. Based upon the above findings, the most appropriate biomarkers for initial evaluation include neuroplasticity markers, i.e. BDNF as well as phosphorylated species of mTOR, eEF2 and GSK-3 β . Additionally, several meta-analyses have established that both serum

and plasma BDNF are reduced in major depression and rise to normal levels with effective antidepressant treatment.

EPIGENETICS AND DEPRESSION

Epigenetics refers to control mechanisms that work alongside DNA sequences, altering their activity without changing the sequence itself. Recent research shows that depressed patients have a different epigenetic profile compared with control subjects.^{39,40} We will examine epigenetic modulation of depression/stress-related genes (e.g. BDNF, FKBP5) in our sample of inpatients with recurrent depressive disorder. Epigenetic modifications that will be examined may include DNA methylation, which involves the addition of a methyl group at the promoter region of a gene's DNA sequence. This modification usually suppresses gene expression and may be measured by pyrosequencing⁴¹ a DNA sequencing technique that identifies methylated DNA nucleotide bases. A second type of epigenetic modification that may be measured is chromatin activation status. To allow the storage of condensed information within the nucleus, DNA is stored in the form of chromatin. Active, open chromatin promotes gene expression while chromatin in its closed state suppresses gene expression. Chromatin activation status can be analysed using a technique called chromatin immunoprecipitation⁴² whereby genomic DNA associated with chromatin in its open or closed state can be precipitated with targeted antibodies and analysed by quantitative PCR. Small non-protein coding RNA molecules known as microRNAs (miRNA) that also act as epigenetic modulators of gene expression, by suppressing messenger RNA translation to protein, may be measured using reverse transcription PCR. The downstream changes modulated by these epigenetic changes can be examined by measuring messenger RNA (mRNA) and protein products of the genes involved. Examining such epigenetic modulations may provide insight into the pathogenesis of depression.

OUTCOMES, OUTPUTS AND TIMESCALE

The purpose of this research programme is to conduct a randomised pilot trial of ketamine for relapse prevention in depression and refine molecular biomarkers for ketamine response. Outcomes of the proposed study will include: knowledge gained from the pilot trial to inform a future randomised controlled trial; safety and tolerability data on ketamine in this novel treatment group; and the utility of neuroplasticity biomarkers in the relapse-prevention response to ketamine. This proposal addresses the Irish Government's 2012 Research Prioritisation exercise regarding the Platform Science and Technology Area "Basic Biomedical Science", with relevance to the "Diagnostics" Priority Area (http://www.forfas.ie/media/ffs20120301-Research_Prioritisation_Exercise_Report.pdf). Additionally, the proposal fits in well with Trinity College Dublin's major research theme of "Neuroscience" as identified in its most recent Strategic Plan 2014-2019 (<https://www.tcd.ie/strategy/>).

This study has the potential to directly impact on clinical practice by testing a novel therapeutic strategy in a unique population with a major unmet clinical need. Developing a truly new therapy for depression relapse prevention will improve quality of life for patients and their families, inform health services' treatment guidelines, raise public awareness of mental health, and could have a positive socioeconomic impact in Ireland and abroad.

10.2 Rationale for the study

INVESTIGATIONAL MEDICINAL PRODUCTS

Ketamine (ketamine hydrochloride 0.5 mg/kg; Pfizer Healthcare Ireland) and midazolam (0.045 mg/kg; Roche Products Ireland Ltd) will be made up as 50 ml colourless saline solutions and administered over 40-minutes using a syringe driver pump, in an eight-week course of four infusions. The optimal dosing regimen for a study such as this has not been established, however it is known that repeated sub-anaesthetic doses of ketamine are well-tolerated by a psychiatric population. The dosing schedule of four two-weekly infusions over a total of eight weeks was chosen as there is evidence that ketamine's effects last up to 14 days. It is hypothesised that a course of ketamine infusions over this schedule may provide additional neurotrophic support which would reduce relapse rates when compared with the active comparator, midazolam. Midazolam was chosen as an active comparator based on other studies as it results in similar acute effects as ketamine at sub anaesthetic doses^{27,28}.

RISKS AND BENEFITS

Physical:

(i) Phlebotomy-Related Risks and Hazards:

Serious adverse effects of phlebotomy are rare, but may include loss of consciousness with seizures. Injury to adjacent anatomical structures is rare and vasovagal attacks may occur occasionally. Bruising and haematoma may occur in up to 12.3% of subjects (WHO, 2010). In this study, phlebotomy will only be performed by experienced doctors and St Patrick's University Hospital protocol on phlebotomy will be strictly adhered to. All efforts will be made to reduce the likelihood of any adverse events due to phlebotomy; however, discomfort during the procedure is common.

Classification: Transient to serious.

Probability: Serious adverse effect=Rare, Transient adverse effect=Common.

(ii) Risks and Hazards associated with Ketamine: for more detail please see Appendix: investigator's brochure. Ketamine is psychotomimetic (with abuse potential) but is safe at low dosage, with patients and healthy controls experiencing mild dissociative and psychotic symptoms (which can be unpleasant) that resolve soon after finishing infusions. In sub anaesthetic doses, ketamine is a safe drug but can cause transient rises in pulse and blood pressure during infusion and for up to 80 minutes afterward. Thus monitoring procedures will be followed as per "interventions" in this trial protocol. A recent review of ketamine in depression concluded that outside recreational usage, there have been no reports of persistent adverse effects with sub anaesthetic uses of ketamine.

(iv) Risks and Hazards associated with Midazolam: for more detail please see Appendix: investigator's brochure. Midazolam has recently been used as an active comparator to ketamine in parallel-group design trials as it mimics some of the effects of ketamine and may improve blinding over inactive placebo saline. At sub anaesthetic doses there have been no reported serious adverse events. However transient physical symptoms can occur during infusions, including minor lowering of blood pressure. The monitoring procedures detailed in "interventions" have been put in place to ensure possible harm is minimised. Infusions will be administered by an experienced consultant anaesthetist with assistance from researchers in a facility equipped for general anaesthesia and resuscitation.

Classification: Transient to serious.

Probability: Serious adverse effect=Rare. Non-serious adverse effect=Unlikely.

Transient adverse effect=Common.

(v) Risks and hazards associated with peripheral venous cannulation and intravenous administration of investigative medicinal products:

Complications that can arise following the procedure of cannulation include infiltration, extravasation, venous spasm, phlebitis, thrombophlebitis, haematoma, nerve injury, arterial puncture, embolism and needle stick injury (HSE, 2013). In this study, peripheral venous cannulation will be performed by a consultant anaesthetist using aseptic technique and in accordance with the local venous cannulation policy. Cannulae will be used for a 40-minute infusion and removed 30 minutes prior to discharge from the infusion clinic. The cannulation site will be monitored during the infusion and regularly for 200 minutes thereafter. Discomfort is common during the insertion of a peripheral venous cannula, however every effort will be made to minimise pain or discomfort, including the use of topical anaesthetics where indicated.

Classification: Transient to serious.

Probability: Serious adverse effect=Rare, Transient adverse effect=Common.

2. Psychological:

(i) Distress: Some participants may find questionnaires distressing or anxiety-provoking, or the experience may change the way they view or manage their illness. This is difficult to predict, however researchers will be vigilant for possible negative psychological effects and seek to minimise these wherever possible. In the case of patients, where distress is noted, this will be brought to the attention of the treating team.

Classification: Transient. Probability: Unknown

3. Psychosocial:

(i) Inconvenience: Attending for assessments or interventions may cause inconvenience to participants. We will seek to minimise lifestyle inconvenience by keeping assessments concise, accommodating participants on return visits to the hospital for non-trial-related appointments where possible, or telephone/home assessments as necessary and reimbursing participants for travel and meal expenses where applicable.

Classification: Transient. Probability: Common.

11 STUDY OBJECTIVE

The overall aim is to assess the feasibility of this study of ketamine for depression relapse prevention, to inform a future definitive trial.

11.1 Primary objective

Objective 1: To conduct a randomised controlled patient- and rater-blinded pilot trial of two-weekly ketamine vs. midazolam over eight-weeks for reducing relapse during the six-months following successful treatment of depression in people with RDD.

11.2 Secondary objective

Objective 2: To assess safety and tolerability of repeated (x4) infusions of ketamine vs. midazolam in this RDD population.

11.3 Exploratory objectives

Objective 3: To explore the role of ketamine-induced changes in peripheral blood neuroplasticity molecules for: (i) monitoring biological response to ketamine during the first infusion and (ii) for evaluating this biological response in predicting lower relapse rates over six-months.

Objective 4: To investigate epigenetic modulation of depression/stress-related genes in patients with recurrent depression receiving inpatient treatment

11.4 Primary and secondary outcome measures

The primary outcomes for this pilot trial are process outcomes to inform a future definitive trial²², e.g. completion of assessments; success of blinding. Although some attrition can be expected during the six-month follow-up period, information collected on rates and reasons for drop-out will form a valuable feasibility outcome. Non-compliance is not expected due to intravenous administration of agents.

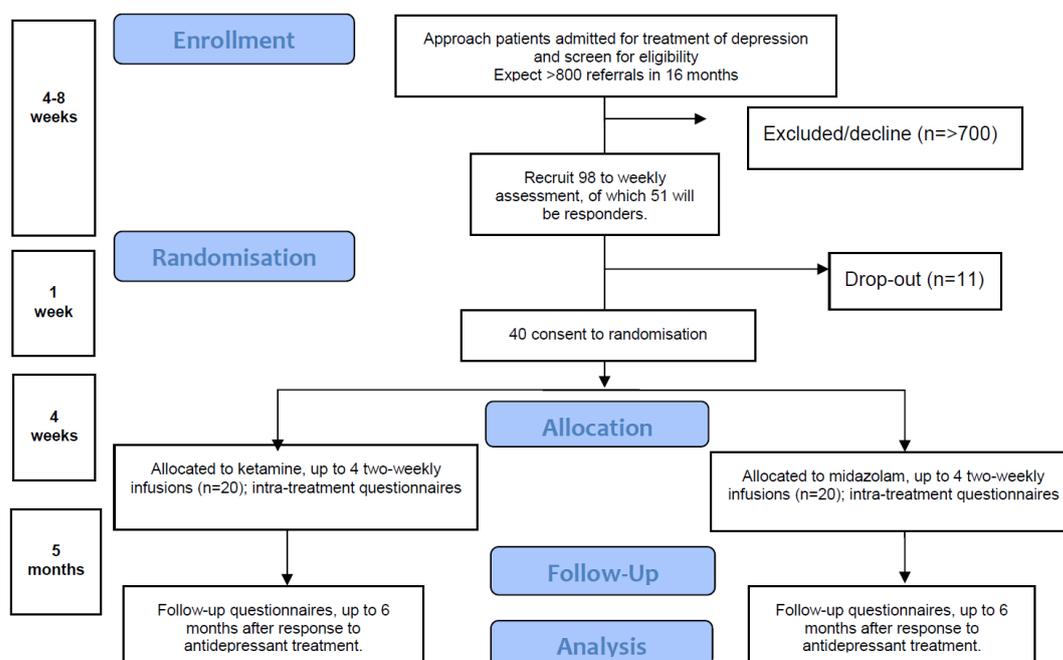
Clinical outcomes are secondary. The primary clinical outcome is relapse rate at six months, measured using the objectively-rated 24-item Hamilton Rating Scale for Depression (HRSD-24)²⁵. Standard criteria for depression severity, treatment response, remission and relapse will be used (please see definitions in "assessments") in a six-month follow-up schedule which involves the HRSD-24 and other instruments at weeks 12, 20 and 26 post-treatment-response. Safety and tolerability outcomes consist of psychotomimetic, dissociative, cognitive and physical health effects of repeated ketamine infusions, measured before, during and after infusions using a range of validated instruments. Outcomes in the biomarker study are changes in blood levels of molecules including total Shank3 and phosphorylated and total BDNF, pMTOR, pGSK3 β and peEF2 in response to the first ketamine/midazolam infusion following immunoassays performed on blood samples collected at time points -60, +40, +120 and +240 minutes, and a final sample before the fourth infusion. Outcomes in the epigenetics study are e.g. DNA methylation and chromatin activation status.

12 TRIAL DESIGN

12.1 General considerations

This single-site, randomised, controlled, parallel-group pilot trial²³ will take place at St Patrick's University Hospital.

Figure 1: CONSORT Trial Flow Diagram, Pilot Clinical Trial



12.2 Selection of study population

12.2.1 Overall description of trial subjects

Trial participants will be people who have been admitted to St Patrick's University Hospital for treatment of depression and who have a history of recurrent depressive disorder.

12.2.2 Inclusion criteria

- ≥ 18 years old
- HRSD-24 score of ≥ 21
- Voluntary admission for treatment of acute depressive episode
- Meet DSM-V criteria for recurrent depressive disorder (RDD): ≥ 2 previous depressive episodes with at least 2-months(consecutive) subthreshold or no symptoms in between

For the randomised pilot trial, RDD patients must have:

- received antidepressant treatment for the acute depressive episode(pharmacological, psychotherapeutic or multidisciplinary)
- $\geq 60\%$ decrease from baseline HRSD-24 score and score ≤ 16
- Mini-Mental State Examination (MMSE) score of ≥ 24
- able to provide informed consent

To be eligible for inclusion, each subject must meet each of the following criteria at Screening and must continue to fulfil these criteria at Baseline.

12.2.3 Exclusion criteria

All candidates meeting any of the exclusion criteria at screening/ baseline will be excluded from study participation.

Subjects are excluded from the study if any of the following criteria are met at Screening or at Baseline:

- (i) Current involuntary admission
- (ii) Medical condition rendering unfit for ketamine/midazolam
- (iii) Active suicidal intention
- (iv) Dementia
- (v) History of Axis 1 diagnosis other than RDD
- (vi) ECT for treatment of index depressive episode
- (vii) Alcohol/substance abuse in previous six-months
- (viii) Pregnancy or inability to confirm use of adequate contraception during the trial

12.3 Study assessments and procedures

Table 1: Schedule of assessments

Assessment	Patients admitted for treatment of depression			Antidepressant treatment responders randomised to ketamine or midazolam			
	Baseline (admission)	Weekly during admission	On response to depression treatment	Pre-infusions	Infusions #1-4; weeks 1-8	Weeks 6, 8, 12, 20	Final follow-up week 26
DIAGNOSIS AND TREATMENT							
Background, SCID, NART	✓						
Treatment review	✓			✓	✓ (1-4)	✓ (6-20)	✓
CLINICAL OUTCOMES							
HDRS-24	✓	✓	✓		✓✓✓✓ (1-4)	✓ (6-20)	✓
QIDS-SR	✓	✓	✓		✓✓✓✓ (1-4)	✓ (6-20)	✓
COGNITIVE OUTCOMES							
ACE-III			✓		✓✓ 1st + 4th		✓
Digit spans			✓		✓✓ 1st + 4th		✓
Trails A + B			✓		✓✓ 1st + 4th		✓
FCSRT			✓		✓✓ 1st + 4th		✓
KETAMINE EFFECTS							
CADSS					✓✓✓✓ (1-4)		
BPRS					✓✓✓✓ (1-4)		
YMRS					✓✓✓✓ (1-4)		
PHYSICAL EFFECTS							
PRISE					✓✓✓✓ (1-4)		
Regular HR, BP, O ₂ , ECG					✓✓✓✓ (1-4)		
BLOOD SAMPLES							
Phlebotomy					✓✓✓✓ 1 st and 4 th		
CONSENT							
Signed Consent	✓			✓			
Verbal Assent	✓	✓	✓		✓✓✓✓ (1-4)	✓ (6-20)	✓
ELIGIBILITY							
Eligibility check	✓	✓	✓	✓	✓✓✓✓ (1-4)	✓ (6-20)	✓
RANDOMISATION							
Allocation				✓			

SCID: Structured Clinical Interview for DSM Disorders
 NART: National Adult Reading Test
 HDRS-24: 24-item Hamilton Rating Scale for Depression
 QIDS-SR: Quick Inventory of Depressive Symptoms, Self-rated
 ACE-III: Addenbrooke' Cognitive Examination, Version III
 FCSRT: Full and Cued Selective Reminding Test
 CADSS: Clinician-administered Dissociative States Scale
 BPRS: Brief Psychiatric Rating Scale
 YMRS: Young Mania Rating Scale
 PRISE: Patient-rated Inventory of Side-effects

The following windows constitute a **protocol violation**:

- (i) Recruitment more than four days after admission
- (ii) First infusion clinic more than two weeks after discharge
- (iii) Any more than four infusions
- (iv) Infusions taking place less than two weeks apart
- (v) Any more than five follow-up assessments
- (vi) Final follow-up assessment taking place more than seven months after discharge

Informed consent will be obtained prior to any study-related procedures being undertaken .

12.3.1 Description of Study Assessments

Medical and Surgical History

Details of current and previous diagnoses and treatments will be recorded.

Demographics

The date of birth, gender and race will be recorded.

Vital Signs

Vital signs will not be recorded for the purposes of the trial outside of infusion clinics. During infusion clinics participants' heart rate, pulse oximetry and blood pressure will be monitored before and during infusions and for a further 200 minutes.

ECG Test

A 12-lead ECG will be examined by the investigator at screening. ECG monitoring will be performed for all participants at infusion sessions, before and after administration of the IMP. Abnormal findings will be noted for clinical significance, and the report will be signed by the investigator.

Clinical Laboratory Tests

Clinical laboratory tests will not be performed as part of this trial. All participants will have had recent laboratory investigations (FBC, UE, LFT, TFT) at admission, and these will be examined by a researcher at screening and also by the anaesthetist prior to the first infusion clinic.

Pregnancy Tests

Women of child-bearing potential who participate in the study are requested to inform researchers if there is any possibility they may be pregnant. At this point, urine pregnancy test may be performed with consent. Date of last menstrual period (LMP) will be documented at the first infusion clinic. Information relating to the importance of contraception during the trial is provided in the Participant Information Sheet.

Concomitant Medication and other therapies

All over-the-counter or prescription medication, vitamins, and/or herbal supplements will be recorded on CRFs. The indication for treatments will be recorded. Contraindicated medications are listed in this Protocol. The Maudsley Staging Method for Treatment-Resistant Depression (MSTRD) will be used as a measure of treatment resistance in participants at admission baseline and prior to the first infusion clinic, and repeated at each infusion clinic and follow-up session.

Cognitive Assessments

Cognitive measures including Addenbrooke's Cognitive Examination- Revised (ACE-R)³⁴; Digit Spans³⁵; Trails A and B³⁶ will be performed at baseline and end-of-inpatient-treatment, and at baseline and final infusion session, as well as at final follow-up at week 26. Parallel versions will be used where available³⁷. These validated cognitive measures will be performed and scored by trained researchers, and have been chosen as each assesses an important aspect of cognition.

Psychiatric Assessments

Structured Clinical Interview for DSM-IV Axis I Disorders (SCID)²⁴ will be performed once by trained researchers at baseline assessment. SCID is a structured interview which is diagnostic for disorders in DSM-IV and will be used in this study to confirm a diagnosis of recurrent depressive disorder.

Childhood Trauma Questionnaire

Childhood Trauma Questionnaire (CTQ) is a validated measure to assess for adverse early life events and will be completed once at baseline assessment.

Hamilton Depression Rating Scale, 24-item (HRSD-24)^{25,26}

A validated depression rating measure which will be performed on a repeated basis by trained researchers at various points throughout the trial as a measure of depressive symptomatology and response to treatment.

Quick Inventory of Depressive Symptoms, self-report version (QIDS-SR)³⁹

A validated self-report measure of depressive symptoms. It is best practice to include both self- and clinician-rated measures of depressive symptoms to assess treatment response in depression.

National Adult Reading Test³³

National Adult Reading Test (NART) is a measure of premorbid intelligence relative to expected intelligence (i.e. NART assesses IQ) and will be performed once at baseline assessment.

Side Effect Measures

During infusion sessions, adverse or psychotomimetic effects of either ketamine or midazolam will be monitored using validated scales comprising: Clinician-Administered Dissociative States Scale (CADSS)³⁰, Brief Psychiatric Rating Scale (BPRS)³¹, Young Mania Rating Scale³², and Patient-Rated Inventory of Side Effects (PRISE), administered before, during and after infusions in order to capture the range of possible subjective and objective side effects of either agent

Neuroplasticity Biomarkers

At the first infusion session, changes in blood levels of markers including BDNF, pMTOR, pGSK3beta and peEF2 in response to the ketamine/midazolam will be compared at time points -60, +40, +120 and +240 minutes. A final sample will be taken prior to the fourth and final infusion clinics to assess for the possibility of a sustained response over the course of repeated infusions. Blood samples will be collected using EDTA vacutainer tubes and centrifuged to generate plasma that will be aliquoted and stored at -80°C. BDNF levels will be determined using ELISA (ChemiKine, USA) following the manufacturer's instructions. PBMCs will be collected, pelleted and stored at -80°C as described above. Semi-quantitative immunoblotting of PBMC lysates will be used to measure changes in levels of the other ketamine-induced proteins using antibodies against activated mTOR phosphorylated at serine residue 2448 (AbCam, USA), inhibitory serine-9 phosphorylated GSK-3beta (Cell Signalling Technology, USA), and phospho-

eEF2 (Thr56; Cell Signalling Technology, USA) with appropriate secondary antibodies and controlling respectively for total levels of mTOR, GSK-3beta and eEF2 measured using relevant antibodies. Protein bands will be identified using standard chemiluminescent techniques (Millipore) visualised in a darkbox imager (LAS 3000, Fujifilm) and analysed using ImageJ (Image Processing and Analysis in Java) software. One blood sample in a PAXgene tube will also be collected at time points -60 & +120 minutes for extraction of total RNA to examine RNA species such as mRNA or miRNA using reverse transcription PCR.

Epigenetics

Blood samples will be collected using one PAXgene tube and one EDTA tube at baseline to analyse epigenetic modulation of depression/stress-related gene expression. Epigenetic modifications that will be examined may include DNA methylation, measured by pyrosequencing, a DNA sequencing technique that identifies methylated DNA nucleotide bases. A second type of epigenetic modification that may be measured is chromatin activation status, using chromatin immunoprecipitation whereby genomic DNA associated with chromatin in its open or closed state can be precipitated with targeted antibodies and analysed by quantitative PCR. Small non-protein coding RNA molecules known as microRNAs that also act as epigenetic modulators of gene expression, by suppressing messenger RNA translation to protein, may be measured using reverse transcription PCR.

12.3.2 Endpoints assessments

Efficacy Assessment

This pilot trial is not designed to assess efficacy. Rather, a 95% confidence interval for the difference between relapse rates between ketamine and midazolam groups will be obtained, to inform a future definitive trial. This will be based on the following clinical outcomes.

The following will be used to obtain baseline, intra-treatment, and end-of-treatment and follow-up data at timepoints indicated in Table 1.

(i) *Diagnosis and treatment history*: Diagnosis of recurrent depressive disorder will be confirmed using the mood episodes module of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) at admission baseline.

Additional demographic data obtained at admission baseline from patient interview and case-note review will include: age, gender, weight, height, occupation, educational attainment, duration of index depressive episode, number of previous depressive episodes, history of medical illness and surgical treatments, personal and family history of alcohol/substance dependency, presence of psychotic symptoms (detected by SCID), current medications and other therapies. Changes in medications will be documented at follow-up interviews.

(ii) *Depression outcomes*: The primary clinical outcome measure is the relapse rate at six months as measured using the objectively-rated 24-item Hamilton Rating Scale for Depression (HRSD-24). To enter the study patients must score ≥ 21 at baseline.

- Response to antidepressant treatment is defined as achieving $\geq 60\%$ decrease from baseline HRSD-24 and score ≤ 16
- Remission criteria are $\geq 60\%$ decrease in HRSD from baseline and score ≤ 10
- Criteria for relapse are ≥ 10 point increase in HRSD-24 compared to responder baseline score plus HRSD ≥ 16 ; in addition, increase in the HRSD should be maintained one week later (if indicated, additional follow-ups will be arranged).

During the infusion sessions in the pilot trial HRSD-24 scores will be obtained 60 minutes before the infusion begins and at +120 and +240 minutes afterwards. Baseline scores on sleep and appetite items will be maintained for repeated measures within one day. The +240 HRSD-24 scores will serve as the weekly HRSD scores up to follow-up-week 4. Depression measures will be repeated at weeks 6, 8, 12, 20 and 26 during the six-month follow-up. Subjective mood ratings will be measured at the above timepoints using the Quick Inventory of Depressive Symptoms, self-report version (QIDS-SR16).⁴

Safety Assessment

Psychotomimetic and dissociative symptoms

Acute psychotomimetic effects of ketamine are usually short-lived and restricted to the infusion period, resolving within one hour.⁶ An extensive review of safety in healthy volunteers showed adverse mental status events in only 1.45% of infusions. In line with previous ketamine trials we will use the following instruments before, during (+35-40 mins) and after (+240 mins) ketamine infusions (WP2.1):

- Dissociative effects: Clinician-Administered Dissociative States Scale (CADSS).
- Psychotomimetic effects: positive symptoms subscale of the Brief Psychiatric Rating Scale (BPRS). The 4-item positive symptoms subscale measures suspiciousness, hallucinations, unusual thought content, and conceptual disorganisation.
- Mood elevation: Young Mania Rating Scale (YMRS; mood item).

Cognitive Effects

Our population of responders to antidepressant treatment are at high risk of relapse but are not depressed at the time of ketamine administration. Cognitive assessment is an important aspect of investigating potential effects of ketamine in the novel role of relapse prevention. Ketamine in healthy volunteers can cause transient impairment in working and episodic memory, procedural and semantic memory, executive function, verbal fluency, and verbal memory, resolving shortly after the infusion. Procognitive effects of repeated doses of ketamine have been suggested in some studies. Baseline and acute neurocognitive performance are associated with likelihood of antidepressant response to ketamine in depressed patients, however it is not known whether this is the case in recovered depressed patients.

The following validated instruments will be used to assess cognitive outcomes at responder baseline (prior to randomisation) and during the pilot trial: one day after the first and fourth infusions and at six-months (Table 1). Parallel versions will be used to reduce practice effects. The National Adult Reading Test (NART) will measure premorbid ability at responder baseline.

- Addenbrooke's Cognitive Examination III (ACE-3): global cognition, also generates verbal fluency scores (letter and category). The ACE-3 provides a total score (maximum=100) plus subscale scores for different aspects of cognition including attention and orientation, and has been used to study cognition in depression.
- Forward and Backward Digit Spans: immediate short-term memory, attention and working memory
- Trail Making Test (Part A): motor and psychomotor speed

- Trail Making Test (Part B) plus letter and category verbal fluencies: frontal executive function

Physical Side Effects

In a recent review, the most common adverse events of single ketamine infusions were drowsiness, dizziness, incoordination, blurred vision, and dissociative symptoms, with only 1.95% of 205 infusions discontinued. Approximately 33% of participants experienced mild haemodynamic changes (systolic or diastolic blood pressure (BP) > 180/100 or >20% increase above pre-infusion reading or tachycardia > 110 beats/min). The following monitoring will take place to assess physical side-effects

- Heart rate, blood pressure, pulse oximetry, and ECG before and during infusions and for a further 200 minutes, to measure haemodynamic changes
- The Patient-Rated Inventory of Side Effects (PRISE) will be used to document other general adverse events by patients before, during (+35-40 mins) and after (+240 mins) infusions

All adverse medical, psychotomimetic and general events will be reported to Trial Steering and Data Monitoring and Ethics Committees.

12.3.3 Screening procedure

All patients admitted for treatment of acute depression will be screened by means of a chart review to ascertain whether they have a history of recurrent depressive disorder or any exclusion criteria are present. This chart review will be conducted by a researcher within three days of admission. Laboratory and ECG results will also be noted – these are routinely performed on all patients at admission. Date of screening, subject age, gender and reason for ineligibility (if subject is not eligible) will be recorded. The results of the screening evaluation must meet the inclusion/exclusion criteria for the subject to continue in the study.

Following screening, eligible patients will be approached on the ward by a researcher and provided with information about the study. If agreeable to participate, informed consent will be obtained and the initial HRSD-24 and SCID assessment performed to ensure further eligibility criteria are met.

12.3.4 Baseline assessments

The following pre-treatment Baseline assessments will be performed at recruitment:

- confirmation of eligibility (review inclusion/exclusion criteria)
- recording of demographics, medical history and concomitant medications
- Structured Clinical Interview for DSM IV Disorders
- National Adult Reading Test
- Hamilton Rating Scale for Depression, 24-item
- Quick Inventory of Depressive Symptoms – Self-Rated (QIDS-SR)
- Childhood Trauma Questionnaire
- blood collection for biomarkers as detailed above

12.3.5 Subsequent study visits and procedures

Participants will undergo usual inpatient care as prescribed by their treating team during the admission for treatment of the index acute depressive episode. Following the baseline assessment above, participants will be assessed weekly during their ongoing inpatient treatment, using the HRSD-24 and QIDS-SR.

Those who are identified as responders to inpatients treatment for the acute episode of depression and continue at weekly assessment to meet eligibility criteria, will be invited to be randomised. Participants must score ≥ 21 at baseline to be eligible for the study and achieve at least response to antidepressant treatment to participate, i.e. $\geq 60\%$ decrease from baseline HRSD-24 and score ≤ 16 . Following randomisation and prior to the commencement of the randomised treatment phase, cognitive outcomes will be assessed (ACE-III; Trails A+B; Digit Spans). Treatment review will take place at each subsequent study visit to monitor changes in medications. A detailed description of each of the assessments is provided in section 12.3.1. Randomisation will take place as detailed below. Participants will attend the hospital for four infusion clinics at which time they will receive an infusion of either ketamine or midazolam. Participants will be asked to fast for 8 hours prior to infusion clinics and to have a nominated adult who can drive them home and stay with them for 24 hours after infusions. Pre-infusion monitoring will be performed and the infusion will take place over 40 minutes as detailed below. Ongoing monitoring of safety and tolerability outcomes will continue for 200 minutes after completion of the infusion. A researcher will contact each participant 24 hours after each infusion to check for potential adverse effects. Travel expenses incurred by participants will be reimbursed and breakfast and lunch provided for infusion sessions.

Ketamine (ketamine hydrochloride 0.5 mg/kg; Pfizer Healthcare Ireland) and midazolam (0.045 mg/kg; Roche Products Ireland Ltd) will be made up as 50 ml colourless saline solutions and administered as slow infusions over 40 minutes using a syringe driver pump, as per previous similar studies. The drugs will be securely stored in the Hospital pharmacy and made up for use by the Consultant Anaesthetist (Dr Enda Shanahan) on the mornings when infusions will be given, using the St Patrick's University Hospital Electroconvulsive therapy clinic as the treatment facility. Like ketamine at 0.5 mg/kg, midazolam at 0.045 mg/kg has anaesthetic effects and causes some sedation and disorientation with a similar time course and adverse effect profile. In subanaesthetic doses, ketamine is a safe drug but can cause transient rises in pulse and blood pressure during infusion and for up to 80 minutes afterward. All patients will therefore be monitored for heart rate, blood pressure, pulse oximetry, and ECG before and during infusions and for a further 200 minutes. Infusions will be discontinued by the Anaesthetist if there are persisting haemodynamic changes (i.e. heart rate >110 /minute or systolic/diastolic blood pressure $>180/100$ or $>20\%$ increase above pre-infusion BP for more than 15 minutes) that do not respond to beta-blocker therapy. Assessments including cognitive and mood assessment will be performed as outlined in section 12.3.1, before, during and after infusions.

Patients will be withdrawn from the trial if: (i) an infusion is discontinued for the above haemodynamic reasons or other serious medical contra-indications, e.g. over-sedation, hypoxia, intolerable adverse physical reactions; (ii) the patient develops mania or psychosis; (iii) the patient becomes severely depressed and/or suicidal. To ensure patient safety, the first infusion session will take place while an in-patient within one week of meeting response criteria. If there are no major adverse effects, subsequent sessions can happen as out-patients. Participants will

be advised not to drive or operate heavy machinery for 24 hours post-commencement of infusions, and provided with information on recent changes to the Road Traffic Act 2014, which includes provisions for roadside intoxication testing. Participants will be asked to ensure they have a nominated adult who can stay with them for 24 hours on outpatient treatment days, and will be contacted by a researcher 24 hours after each session to enquire about side-effects.

Participant will be followed up over six months to assess for relapse. Cognitive assessment will be repeated at week 26 and this assessment will take place in person, either at the site or via home visit by two researchers and according to the Home Visit Protocol developed by the Research Department at St Patrick's University Hospital. HRSD-24 and QIDS will be repeated at weeks 6,8,12, 20 and 26. Assessments other than the final follow up at week 26 can take place over the telephone. Reasonable meal and travel expenses incurred by participants attending for follow-up appointments will be reimbursed.

12.3.6 Method of assigning Subjects to treatment groups

Participants will be recruited at admission for treatment of a depressive episode and assessed weekly. Those identified as responders according to the criteria defined above, will be invited to be randomly assigned to one of two treatment groups in a 1:1 ratio.

Randomisation

Computerised random allocation, using randomly permuted blocks will be done independently by the Centre for Support and Training in Analysis and Research (CSTAR, University College Dublin, www.cstar.ie). To ensure allocation concealment, allocation information will be provided in a randomisation list available only to the anaesthetist. This will be stored in a locked cabinet to which only the anaesthetist has the key. A matching set of opaque randomisation envelopes will also be provided by CSTAR to be accessed by clinical staff in the event of emergency unblinding. This system has been successfully piloted in an existing trial at St Patrick's University Hospital (NCT02414932).

Blinding

Study treatment assignment will be blinded for both the raters and the participants. To ensure patient safety during infusions and in the post-infusion period, the anaesthetist administering the ketamine/midazolam infusions will not be blinded but he will not be involved in assessments or data analysis. Infusions will be prepared by the anaesthetist in a location separate to the infusion area and labelled as "trial infusion" prior to transfer to the infusion area. Success of blinding for patients and raters will be assessed after the first and final treatments and at the end of the six-month follow up.

The matching set of envelopes containing allocation information will remain unopened but may be used where emergency unblinding is indicated. Unblinding for one or all participants will take place if it is in the best interests of the participants. In the case of an emergency, when knowledge of the treatment assignment is essential for the clinical management of the subject, any investigator may unblind a single subject. Please see further details in section 13.7.2.

Circumstances in which unblinding for multiple or all subjects may take place include – multiple SAEs or SUSARs, new information regarding safety of the investigative medicinal products, and unsatisfactory progression of the trial.

Any breaking of the blind, whether intentional or unintentional, will be recorded and reported to

the sponsor as soon as possible. Unblinding for multiple or all subjects will be discussed by the trial steering committee at the next meeting. Unblinding will be recorded and justified in the final report.

12.4 Definition of end-of-trial

End-of-trial is defined as the final follow-up visit/ home visit/telephone assessment of the last participant. End-of-trial will be reported to the REC, TSC, DMC and HPRA within 90 days, or 15 days if the study is terminated prematurely. The investigators will inform subjects and ensure that the appropriate follow-up is arranged for all involved. A summary report of the study will be provided to the REC, Sponsor and HPRA within 1 year of end-of-trial.

The end-of-study visit form will include:

- Assessment of endpoints i.e. clinical (HRSD-24, QIDS-SR) and cognitive (ACE-R, digit spans, Trails A and B) outcomes
- Assessment of safety - check for any adverse effects
- Recording of concomitant medication

The Sponsors and/or the trial steering committee (TSC) have the right at any time to terminate the study for clinical or administrative reasons.

12.4.1 Premature termination of the study

The Sponsor and/or the TSC have the right at any time to terminate the study for clinical or administrative reasons. The DMC may request that the trial be prematurely terminated and this request will be discussed in a timely manner by the TSC.

Premature termination of the trial may take place in the event of the following:

- (i) New information regarding safety of investigative medicinal products
- (ii) Multiple SAEs or SUSARs
- (iii) Unsatisfactory progression of the trial
- (iv) Major breach of data confidentiality
- (v) Any situation in which premature termination of the trial is judged by the investigators and/or Sponsor to be in the best interests of trial participants.

Premature termination of the trial will be reported to the REC, TSC, DMC, HPRA, and Sponsor and justified in the final report.

12.5 Discontinuation/withdrawal of subjects from study protocol

Subjects have the right to voluntarily discontinue study treatment or withdraw from the study at any time for any reason without any consequences. The investigator has the right to discontinue a subject from study treatment or withdraw a subject from the study at any time if it is in the best interest of the subject.

Subjects must discontinue the investigational medicinal product(s) and be withdrawn from the study for any of the following reasons:

- withdrawal of consent by the subject

- any medical condition that the investigator or sponsor determines may jeopardize the subject's safety if she or he continues receiving the study treatment
- pregnancy
- ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- an adverse event which requires discontinuation of the study medication
- treatment failure and disease progression
- Lack of compliance with the study and/or study procedures (e.g., dosing instructions, study visits).
- Lost to follow-up – at least three documented attempts must be made to contact any subject lost to follow-up.

Ideally all subjects who discontinue should comply with specified follow-up procedures as detailed in this protocol, i.e. assessment via interview comprising clinical and cognitive measures. The only exception to this requirement is when a subject withdraws consent for all study procedures. There is no mandatory physical health monitoring to be performed in the event of a subject withdrawing consent after a complete infusion session including 200 minutes post-infusion monitoring, or between infusion sessions. However, in the event that consent is withdrawn during an infusion session, monitoring of vital signs and mental health must be performed for 200 minutes following the end of the infusion.

If a subject is withdrawn before completing the study, the reason for withdrawal must be entered on the appropriate case report form (CRF) page. If a subject is withdrawn due to an adverse event, the investigator will arrange for follow-up visits until the adverse event has resolved or stabilised.

13 TREATMENT OF TRIAL SUBJECTS

13.1 Description of study treatments

Investigative Medicinal Product - Ketamine Hydrochloride 10 mg/ml infusion at 0.5mg/kg (Pfizer Healthcare Ireland) made up as 50ml colourless saline solution and administered intravenously over 40 minutes via syringe driver pump.

Active Comparator - Midazolam Hydrochloride (Hypnovel) 10mg/5ml solution at 0.045mg/kg (Roche Products Ireland Ltd) made up as 50ml colourless saline solution and administered intravenously over 40 minutes via syringe driver pump.

13.2 Formulation, packaging and handling

Commercial labels will remain on both study treatments. These detail the component, date of expiry and manufacturer. Further labels will be attached to each unit of product indicating:

- Use of the treatment in this trial
- Name of sponsor, and principal investigator;
- Trial reference code allowing identification of the trial site, investigator and trial subject.

These labels will be added by pharmacy staff to each vial on receipt of delivery of the treatment to St Patrick's University Hospital Pharmacy, in accordance with Annex 13 (EU Guidelines to Good Manufacturing Practice, Investigational Medicinal Products).

Suppliers:

Ketamine: Pfizer Healthcare Ireland, 9 Riverwalk, Citywest Business Campus, Dublin 24.

Midazolam: Roche Products (Ireland) Ltd., 3004 Lake Drive, Citywest, Naas Rd., Dublin 24.

Pharmacy performing additional packaging:

St Patrick's University Hospital Pharmacy, James' St, Dublin 8.

13.3 Storage and disposition of study treatments

The treatments will be stored securely in a clean dry area of the pharmacy department at St Patrick's University Hospital. Ampoules will be stored in the outer carton (labelled as above) in order to protect from light. Products will be prepared into infusions in the clinic room of the ECT department by the consultant anaesthetist who will administer them. The anaesthetist will be unblinded throughout and patients and raters will remain in a separate area for infusions and assessments. Once made up as identical colourless solutions, the underlying labels will be obscured by bags and infusions will begin within one hour of preparation. Any unused product will be returned to pharmacy and disposed of according to the protocols specified by the pharmacy for destruction of unused pharmaceutical products.

The study treatment will be stored at St Patrick's University Hospital Pharmacy Dept. under the responsibility of Ms. Amanda Fitzpatrick, Chief Pharmacist, St. Patrick's University Hospital. Temperatures in the storage area of the pharmacy are monitored constantly by electronic thermostat and a printed record is available. An alarm process is instigated if the temperature varies from the specified room temperature. The study treatment will be stored locked in a secure area until dispensed for use or returned to the sponsor. The IMP ketamine is for investigational use only and is only to be used within the context of this study.

13.4 Accountability of the study treatments

The study medication will be supplied to the pharmacy by Pfizer Healthcare Ireland (ketamine) and Roche Products (Ireland) Ltd (midazolam). Standard shipment arrangements will continue. Upon delivery, receipt of the products will be recorded by pharmacy staff and labels applied as described here, with products then transferred to the secure storage area. Unopened products which are unused by end-of-trial will be returned to the manufacturer. Opened unused products will be destroyed in the pharmacy following the protocol for destruction of pharmaceutical products at the end of every infusion session.

The investigator is responsible for the control of the treatments under investigation. Adequate records for the receipt and disposition of the IMP will be maintained.

The investigator will use a standard prescription form and the investigator/research nurse will collect the medication from the pharmacy no more than three hours before dosing.

Accountability and subject compliance with study treatments will be assessed by maintaining dispensing and return records. Discrepancies in these return records will be dealt with initially by re-checking and communication with pharmacy. Should a discrepancy arise which cannot be accounted for, this will be recorded and discussed by the TSC at their next four-monthly meeting.

13.5 Assessment of compliance

In this study, interventions will be administered intravenously by the research team and thus there is no opportunity for non-compliance. The investigator is responsible for ensuring that the study treatment is administered in compliance with the protocol. Subject compliance will be assessed by maintaining dispensing records.

13.6 Overdose of study treatment

Given the safeguards in place (consultant anaesthetist to prepare and administer infusions, assisted by members of the research team), it is deemed unlikely that an overdose of study treatment could occur. In the improbable event of an overdose, the study subject will be monitored for any change in neurological status or vital signs by the consultant anaesthetist and research team members. If there are signs of change, e.g. drowsiness, change in blood pressure/heart rate and a known overdose has occurred, the subject will be counselled and accompanied by a member of the research team to the local emergency department (St. James' Hospital) for medical investigation. A letter detailing treatment and doses administered will be provided. Reasonable efforts will be made to contact the NOK/RA if the subject consents. Should the subject require medical investigation and/or treatment due to an overdose of study treatment, cost will be covered by the SPUH indemnity policy, unless due to negligence or malpractice.

13.7 Prior and concomitant therapy

Any medication, other than the study medication taken during the study will be recorded in the CRF. Medications will be documented at the point of consent, at Baseline, and changes will be noted at every assessment or intervention appointment thereafter.

13.7.1 Permitted medications/non- investigational medicinal products

All medications aside from those listed in section 13.7.2 are permitted. Treatment-as-usual will continue for all participants during this study. No non-investigational medicinal products will be used outside authorisation for the purposes of this trial.

13.7.2 Prohibited medications

The following medications are contraindicated during the randomised treatment period as they may alter the pharmacokinetics of ketamine. Additionally, the medication theophylline is contraindicated as concomitant use of ketamine and theophylline may significantly reduce the seizure threshold with reports of unpredictable extensor-type seizures.

Contraindicated medications:

Ketoconazole	Clarithromycin	Diltiazem
Voriconazole	Saquinavir	Fluconazole
Itraconazole	Nefazodone	Verapamil
Telithromycin	Erythromycin	Theophylline

Participants taking any of these medications on randomisation will be excluded from the trial. Medication history will be checked at Baseline and each subsequent assessment, and participants who have been prescribed these medications during the trial will not receive further

interventions (i.e. infusions of ketamine or midazolam), however will be followed up according to the framework presented here. Data collected will be included in intention-to-treat analyses if one infusion and one follow-up assessment have been completed. It is not permitted for subjects to participate in investigational treatment studies while participating in this study.

14 SAFETY REPORTING

14.1 Definitions

14.1.1 Adverse event (AE)

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product

14.1.2 Adverse reaction (AR)

All untoward and unintended responses to a medicinal product related to any dose.

The phrase 'responses to a medicinal product' means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

14.1.3 Serious adverse event

Any untoward medical occurrence or affect that at any dose:

- results in death,
- is life-threatening*,
- requires hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect
- important medical events**

*Regarding a life-threatening event, this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

**Some medical events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events (hereinafter referred to as 'important medical events') should also be considered as 'serious' in accordance with the definition

14.1.4 Severe adverse events

The term 'severity' is used here to describe the intensity of a specific event. This has to be distinguished from the term 'serious'.

14.1.5 Suspected unexpected serious adverse reactions

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational medicinal product or summary of product characteristics for an authorised medicinal product).

14.2 Evaluation of AEs and SAEs

14.2.1 Assessment of seriousness

The investigator should make an assessment of seriousness as defined in section 12.1.4.

14.2.2 Assessment of causality

All adverse events judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to an investigational medicinal product qualify as adverse reactions.

The causality assessment given by the investigator should not be downgraded by the sponsor. The investigator/sponsor must make an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions:

Unrelated

Where an event is not considered to be related to the study medication.

Possibly

Although a relationship to the study medication cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.

Probably

The temporal relationship and absence of a more likely explanation suggest the event could be related to the study medication.

All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g. possibly, probably) to the study medication will be considered as ARs/SARs.

All AEs/SAEs judged as being related (e.g. possibly, probably) to an interaction between the study medication and another medication will also be considered to be ARs/SAR.

Alternative causes such as natural history of the underlying disease, concomitant therapy, other risk factors and the temporal relationship of the event to the treatment should be considered.

14.2.3 Assessment of severity

The investigator will make an assessment of severity for each AE/SAE and record this on the CRF according to one of the following categories:

Mild

An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with every day activities.

Moderate

An event that is sufficiently discomforting to interfere with normal everyday activities.

Severe

An event that prevents normal everyday activities.

Note: the term 'severe', should not be confused with 'serious' which is a regulatory definition

based on subject/event outcome or action criteria

14.2.4 Assessment of expectedness

The expectedness of an adverse reaction will be determined by the sponsor according to the reference document e.g. the investigator's brochure for a non-authorized investigational medicinal product, or the summary of product characteristics for an authorized medicinal product which is used according to the terms and conditions of the marketing authorisation.

14.2.5 Emergency unblinding procedures

Emergency unblinding can be performed by any investigator by opening one or all of the set of envelopes containing allocation information. These will be securely stored in the ECT department where infusions are to be administered. Instructions for emergency unblinding will be included on each CRF/ e-CRF, and will also be prominently displayed in the ECT department. A successful trial run of this system with the clinical staff group involved in out-of-hours procedures has taken place.

14.3 Reporting procedures for all adverse events

All AEs occurring during the study observed by the investigator or reported by the subject, whether or not attributed to the study medication, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to the study medication, other suspect medication or device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the study medication as judged by an investigator or the sponsor will be followed until resolution or until the event is considered stable. All related AEs that result in a subject's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the subject's removal from treatment. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the subject must undergo an end-of-study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

The severity of events will be assessed on the following scale: mild, moderate, severe.

The relationship of AEs to the study medication will be assessed by the investigator.

Any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed-up for congenital abnormality or birth defect.

14.4 Reporting procedures for serious adverse events

The investigator will report all serious adverse events immediately to the sponsor except for those that the protocol or investigator's brochure identifies as not requiring immediate

reporting. The immediate report will be followed by detailed, written reports. The immediate and follow-up reports will identify subjects by unique code numbers assigned to the latter.

The immediate report will be made by the investigator within a very short period of time and under no circumstances should this exceed **24 hours** following knowledge of the serious adverse event.

All SAE information must be recorded on an SAE forms and sent expeditiously to the sponsor. Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form and sent expeditiously to the sponsor.

The sponsor will keep detailed records of all adverse events which are reported to him by the investigator or investigators.

In cases where reporting is not required immediately the investigator will report within the appropriate time frame, taking account of the specificities of the trial and of the serious adverse event, as well as possible guidance in the protocol or the IB.

The sponsor will report all SUSARs to the competent authorities (the HPRA in Ireland) and the ethics committees concerned. Fatal or life-threatening SUSARs must be reported within **7 days**. SUSARs which are not fatal and not life-threatening are to be reported within **15 days**. The sponsor will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

If the initial report is incomplete, e.g. if the sponsor has not provided all the information/assessment within seven days, the sponsor will submit a completed report based on the initial information within an additional eight days.

If significant new information on an already reported case is received by the sponsor, the clock starts again at day zero, i.e. the date of receipt of new information. This information will be reported as a follow-up report within **15 days**.

In addition to the expedited reporting above, the sponsor shall submit once a year throughout the clinical trial or on request, a safety report to the competent authority (the HPRA in Ireland) and ethics committees. The annual safety report will be presented in the DSUR format as per ICH guideline E2F - Note for guidance on development safety update reports. This is a legal requirement.

14.5 Data Monitoring Committee, Trial Steering Committee

The independent Data Monitoring Committee will review blinded data on a six-monthly basis during the trial and will act according to the DMC Charter which will be ratified at the organisational meeting. Data Monitoring Committee

The DMC will be an independent committee established prior to the commencement of the trial to assess progress, safety data and data security and will meet every six months during the trial. The DMC will hold an organisational meeting prior to recruitment, at which time the DMC. The Trial Management Group and trial statistician will prepare a report for the Committee circulated two weeks prior to each meeting. Charter will be ratified. No member of the DMC will have a conflict of interest with the Sponsor. Blinded data will be presented to the DMC for safety

evaluation every six months. Adverse events will be reported on individually. Should the Committee wish to review unblinded data, this will be provided. The DMC will report to the TSC, which has authority to decide whether the trial should be suspended or ended. Minutes of the DMC meetings including safety evaluations will be presented to the TSC at every meeting.

The advice of the DMC will be notified upon receipt by the sponsor to the REC and CA that approved the protocol. With this notification a statement will be included indicating whether the advice will be followed. The composition of the DMC will include an independent statistician, an independent trial methodologist, and an independent clinical investigator.

The Trial Steering Committee will comprise investigators, clinical experts not directly involved in the trial, a service user representative, and staff nominated by the Sponsor. The committee will include members who are independent of the investigators, SPUH, funders and the Sponsor. The TSC will consider and act, as appropriate, upon the recommendations of the DMC and ultimately carries the responsibility for deciding on premature termination of the trial. The TSC will take responsibility for the scientific validity of the study protocol, assessment of study quality and conduct as well as for the scientific quality of the final study report. This section will be updated with details of the members of the DMC and TSC once established.

14.6 Pregnancy

Pregnancy is not considered an AE or SAE however the investigator will collect pregnancy information for female trial subjects or female partners of male trial subjects who become pregnant while participating in the study.

The investigator will record the information on a Pregnancy Notification Form and submit this to the sponsor. Any pregnancy that occurs in a trial subject or a trial subject's partner during a trial will be followed to outcome. It may be necessary to monitor the development of the new-born for an appropriate period post-delivery.

While ketamine has been shown to be teratogenic in rats, there are no data of its use during human pregnancy, particularly at sub-anaesthetic doses. Insufficient data are available on Midazolam to assess its safety in pregnancy; however other benzodiazepines have been associated with teratogenicity. There have been no studies of sub-anaesthetic doses of midazolam in pregnancy. Date of last menstrual period (LMP) will be recorded at recruitment and the first ketamine infusion. Information regarding the importance of adequate contraception during the trial and informing researchers if there is any possibility of pregnancy is provided in the Participant Information Leaflet (Appendix).

15 STATISTICS

15.1 Description of statistical methods

Descriptive statistics will be used to report: rates of recruitment, willingness to be randomised, willingness to complete assessments, medical/ cognitive/ psychotomimetic/ general adverse events between groups, adherence to allocated treatments, adherence to follow-up between groups, and reasons for drop-outs between groups. Relapse-free survival times will be compared between groups using Kaplan-Meier survival curves and log-rank test.

As this is a pilot trial and insufficiently powered to achieve statistical significance 40, there will be no formal comparison of the two treatment groups. However, Cox proportional hazard regression analysis will provide a 95% confidence interval for an unadjusted hazard ratio for ketamine versus midazolam groups. This will be used to inform a future definitive trial.

Secondary exploratory analyses (Cox proportional hazards regression models) will be used to identify pre-randomisation clinical factors or covariates (e.g. Family history of alcohol dependence; gender; age; presence of psychosis and extent of treatment-resistance in index depressive episode; remission status) that might influence response and help guide randomisation stratification in a future definitive trial.

Comparative descriptive statistics will be used to compare scores on cognitive and tolerability assessments. For the neuroplasticity biomarker studies, changes in protein/activity levels will be compared between groups over different timepoints by ANCOVA; linear models will test relationships between these changes and relapse during six-month follow-up.

15.2 Determination of sample size subjects

We wish to recruit 20 patients per group, a total of 40, an acceptable number for the purposes of a pilot trial. A formal sample size calculation is not appropriate as per the Medical Research Council Guidelines. Response rates to inpatient depression treatment are approximately 80%²⁹. However, as this is a heterogeneous sample with RDD, we expect response rates to be lower at 50-60%. Allowing for a 15% drop-out rate, we seek to recruit 98 patients upon admission, expecting that at least n=51 will meet response criteria and that n=40 will consent to be randomised. Prof McLoughlin's team recently finished recruiting severely depressed inpatients (n=140) to an RCT of ECT at St Patrick's University Hospital (see <http://www.controlled-trials.com/isrctn/pf/23577151>). 60 patients were recruited in the first 16 months. As the proposed study is less intensive, with the attraction of an additional therapy and a wider cohort to recruit from, the recruitment rate is expected to be higher at 98 participants within 16 months.

15.3 Analysis sets

Pilot trial data will be analysed on an intention-to-treat basis for all Phase II participants who complete at least one infusion and one post-infusion evaluation. Data analyses will be performed blinded to allocation, by Prof. Leslie Daly in CSTAR (above).

15.4 Demographic and baseline disease characteristics

Demographic and Baseline disease characteristic data will be summarized for each treatment group by presenting descriptive statistics.

15.5 Efficacy analysis

No formal interim analysis is proposed due to the pilot trial design, short recruitment phase and small trial numbers.

15.5.1 Primary efficacy endpoint

This pilot trial is not designed to assess efficacy. The focus is on trial process with assessment of the primary clinical outcome being secondary. However, efficacy data will be collected in the course of the trial and will be reported as part of the study findings.

The primary outcome relating to efficacy (the assessment of which is not a primary objective) is the relapse rate at six months as measured by HRSD-24. Criteria for relapse are ≥ 10 point increase in HRSD-24 compared to baseline Phase 2 score plus HRSD ≥ 16 ; in addition, increase in the HRSD should be maintained one week later (if indicated, additional follow-ups will be arranged). Hospital admission, further ECT, and deliberate self-harm/suicide also constitute relapse. Timing of these events will be recorded.

15.5.2 Secondary efficacy endpoint

- (i) Subjective mood ratings as measured by scores on QIDS-SR.
- (ii) Tolerability of ketamine vs. midazolam in terms of cognitive outcomes as measured by scores on ACE-R, digit spans, Trails A and B, FCSRT, and AMI.
- (iii) Tolerability of ketamine vs. midazolam in terms of psychotomimetic effects as measured by scores on CADSS, BPRS, YMRS, and PRISE.
- (iv) Number of adverse effects in ketamine vs. midazolam groups.

Secondary efficacy endpoints will be analysed by descriptive methods.

15.6 Safety analysis

Descriptive statistics will be used to report the results of clinical monitoring (heart rate, blood pressure, pulse oximetry, and presence of ECG changes), cognitive assessments (ACE-R, digit spans, Trails A and B), monitoring for psychotomimetic effects (CADSS, BPRS, YMRS, PRISE), and adverse effects between groups. Blinded data will be presented to the DMC for safety evaluation every four months. Minutes of the DMC meetings including safety evaluations will be presented to the TSC at every meeting.

15.7 The level of statistical significance

As this is a pilot trial there will be no formal comparison of the two groups but Cox proportional hazard regression analysis will provide a 95% confidence interval for an unadjusted hazard ratio that will allow interpretation of statistical difference between ketamine and midazolam groups.

15.8 Criteria for the termination of the trial

The trial will be terminated once 20 subjects have been allocated to each arm of the randomised treatment phase.

15.9 Procedure for accounting for missing, unused and spurious data

As this is a pilot trial and small numbers of participants are involved, no missing values will be imputed.

15.10 Procedure for reporting any deviation(s) from the original statistical plan

Deviations from the original statistical plan will be reported to the Sponsor within a timely interval and discussed by the Trial Steering Committee at the next meeting. These will be recorded and justified in the final report. Where a deviation from the original statistical plan is judged by the investigators or Sponsor to comprise a substantial amendment to the trial protocol, the standard procedure for reporting substantial amendments to the HPRA will be followed.

16 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

17 DATA HANDLING AND RECORD KEEPING

Data will be entered, handled and stored at St Patrick's University Hospital. It will be anonymised and then processed by members of the research team at Trinity College Institute of Neuroscience and at the Centre for Support and Training, University College Dublin.

17.1 Data collection, source documents and case report forms (CRF)

Source documents for this study include clinical notes, medication records, and study-specific data collection documents. Information will be extracted from these documents and recorded legibly on CRFs/ secure eCRFs. If an error is made, the error will be crossed through with a single line in such a way that the original entry can still be read. The correct entry will then be clearly inserted, and the alterations will be initialled and dated by the investigator. Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies must be explained.

All documents will be stored safely in a designated locked filing cabinet in a locked office within the Research Building at St Patrick's University Hospital and confidentiality will be observed at all times. With the exception of the informed consent form, subjects will be referred to only by their subject identification number on all study-specific documents, whether hard copies or electronic. Anonymised biological materials will be stored and processed in Professor McLoughlin's laboratory facilities in Trinity College Institute of Neuroscience and the Institute for Molecular Medicine in St James's Hospital. Data analysis will take place in another facility (Centre for Support and Training in Research and Analysis (CSTAR), University College Dublin), however data will be anonymised prior to secure transfer to CSTAR for analysis.

17.2 Data reporting

The trial DMC will be responsible for overseeing data security. Subjects will be identified by a study specific subject number and/or code in the database. The name and any other identifying detail will not be included in any study data electronic file. A trial-specific Data Entry and Quality Assurance Protocol will be followed by all researchers. This involves eight levels of assurance and checking.

18 RETENTION OF ESSENTIAL DOCUMENTS

Biological material will be retained securely following the protocols in place at Trinity College Dublin, for a period of four years following trial termination, and disposed of by staff authorized to do so by Trinity College Dublin and in accordance with the institution's policies and data protection legislation. Data derived from biological material and essential trial documents will be retained for a period of at least five years in accordance with Article 17 of EU Directive 2005/28/EC. These will be retained for no longer than ten years and will then be destroyed in accordance with data protection legislation at that time. This is included in the Participant Information Leaflets and consent forms. The essential documents are defined as those that individually and collectively permit evaluation of the conduct of the trial and the quality of the data produced. These documents will be filed in an organised way that facilitates management of the clinical trial, audit and inspection by competent authorities and will be readily available on request.

As this is an academic study, recommendations regarding retention of essential documents for EMA approval/ clinical development of the IMP are not of concern here. Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirements or by an agreement with the sponsor. The investigator/institution should agree to retain the trial-related essential documents as required by the applicable regulatory requirements and until the sponsor informs the investigator/institution these documents are no longer necessary.

19 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

This study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. The measures taken to ensure data obtained is accurate, complete and reliable include:

- (i) Researchers will attend Good Clinical Practice training
- (ii) Researchers will be trained in administration of the primary assessment tool used in this study, the HRSD-24. Administration will be according to specified guidelines and training will be repeated every 6 months to ensure inter-rater reliability.
- (iii) Quality assurance in the laboratory is assured by adherence to protocols outlined by Molecular Medicine Ireland (<http://www.molecularmedicineireland.ie/libraries/libgroup/8>) and manufacturers' instructions for any laboratory assay products used.
- (iv) A trial-specific Data Entry and Quality Assurance Protocol will be followed by all researchers. This involves eight levels of assurance and checking.

The trial site, laboratory and Sponsor's/research team's offices are subject to GCP inspection at any time. In accordance with the legislation, the trial master file comprising the essential documents which enable both the conduct of the trial and the quality of the data produced to be evaluated will be available to provide the basis for the GCP inspection. Responses to a GCP inspection report will be provided within 30 working days of the date of issue.

20 AUDITS AND INSPECTIONS

This trial may be subject to internal or external auditing or inspections procedure to ensure adherence to GCP. Access to all trial-related documents will be given at that time.

21 ETHICS

21.1 Declaration of Helsinki

The sponsor will ensure that this study is conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

21.2 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and 2005/28/EC.

21.3 Approvals

Required documents including the protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other required documents will be submitted to a recognised research ethics committee (here, the REC of the Mater Misericordiae Hospitals Group) and the competent authority for written approval. The sponsor will submit and obtain approval from the above parties for substantial amendments to the original approved documents.

21.4 Informed consent

Written consent will be obtained by members of the research team using the study-specific consent form (Appendix). Potential participants will be provided with an information leaflet and letter of invitation (Appendix) and verbal information at the first point of contact with a member of the research team. This process will take place following screening. Verbal assent will be sought at each treatment step. Time will be provided to address questions. Every effort will be made to provide adequate time for the participant to consult with family, friends and their general practitioner prior to making a decision, however as it is common for treatment for depression to begin on the same day as admission to hospital for treatment, in some cases, provision of information and the process of obtaining consent may take place on the same day. Participants will be encouraged to reflect on the information provided and ask questions but it is recognised that some participants may prefer to make a decision at the first point of contact and this will also be accommodated.

Prior to any study-related screening procedures being performed on the subject, the informed consent statement will be reviewed and signed and dated by the subject and investigator. Informed consent will be obtained verbally at each intervention e.g. phlebotomy, infusion, by the member of the research team who will perform the intervention

21.5 Benefits and risks assessment

This study may further the understanding of the pathogenesis of depression, a major public health issue, and provide further information about a potential treatment to reduce relapse rates. In designing this study, all efforts have been made to reduce the risk to and burden for participants. It is believed that risk to and burden for the subject will be in proportion to the potential value of this research. There are no guaranteed direct benefits to participants. However all participants may benefit indirectly from participation in terms of increased awareness of mental health issues. Some participants may benefit from the administration of ketamine in terms of reduced six-month relapse rates, but this is not guaranteed. This study will not include incapacitated adults or minors. Issues regarding specific vulnerable populations are addressed individually below.

- (i) This study requires inclusion of **adults with mental illness** to address the research question. Only those who have capacity to provide valid informed consent will be invited to participate. Where there is any concern expressed about the capacity of a person to make his/her own treatment decisions, a capacity assessment is performed by the treating team. This assessment will guide investigators in selecting those who have capacity to consent to enrolment in the trial. The trial is expected to benefit participants who have a mental illness indirectly by improving scientific knowledge of a major mental health issue. There are possible direct and indirect benefits to participants with a mental illness in terms of reduced relapse rate and increased awareness of mental health through participation, however there is no guaranteed direct benefit.
- (ii) **Women of childbearing** age are defined by the Irish Central Statistics Office as women of ages 15-49 (Census 2011 This is Ireland (Part 1) - CSO - Central Statistics Office). Women of this age group will not be excluded from this study as this group constitutes a significant proportion of the population of interest, i.e. people with treatment resistant depression. Irish women are more likely to suffer from depression than men and 25% of women in Ireland will require treatment for depression in their lifetime. Thus the primary study objective cannot be accurately achieved without inclusion of women of childbearing age. Previous studies of ketamine have included women of childbearing age and precautions will be taken as detailed here to ensure adequate contraception is in place throughout the trial.

21.6 Subject confidentiality

The trial staff will ensure that the subjects' anonymity is maintained. The subjects will be identified only by initials and a subject's identification number on the CRF and any database. All documents will be stored securely. The study will comply with the Data Protection Act.

21.7 Other ethical considerations

Use of placebo/active comparator: Participants in this pilot trial will continue usual care as recommended by their responsible clinical team. They may receive an additional treatment as part of this pilot trial but will not be denied any treatment for the purposes of this trial. Participants will be provided with verbal and written information regarding the 1:1 randomisation strategy and the possibility of being randomised to a placebo group. There is no evidence to suggest that subjects who are randomised to the placebo arm of the study will suffer poorer outcomes, as a study of this nature has not yet been performed. The DMC will monitor data for safety parameters throughout the trial including the possibility of a large

discrepancy between placebo and ketamine groups, and in the event of such a circumstance, will follow the specifications in this protocol.

22 FINANCING AND INSURANCE/INDEMNITY

Details of funding will be updated once formal funding has been secured. Insurance is provided by indemnity cover for research in place at St Patrick's University Hospital. This will be in place once the trial is approved by the St Patrick's Mental Health Services REC, an application for which will be submitted following approval by the authorised clinical trials REC of St. James' and Tallaght Hospitals.

Please see Appendix, "St Patrick's Mental Health Services Indemnity Policy". <Describe financing and insurance arrangements.

23 CLINICAL STUDY REPORT AND PUBLICATION POLICY

The publication policy involves formal presentation of preliminary study findings at national and international neuroscience and psychiatry meetings. Final findings will be submitted for peer-review and publication in relevant high-impact scientific journals and upon publication they may be further publicized in national and international print and electronic media through the TCD and SPUH websites and public relations departments. Further knowledge dissemination will include registering the trial in the EudraCT database and publication of the trial protocol in a peer-reviewed journal.

During the trial itself, a six-monthly newsletter will be sent to all participants, detailing progress in recruitment with lay summaries of research findings relevant to the study. Research progress and developments will be regularly presented at medical "grand rounds" in St. Patrick's University Hospital (SPUH), and in-house research meetings and seminars in Trinity College Institute of Neuroscience (TCIN). Information about the research programme and other ongoing related depression research will also be contributed through our group's website (<http://www.medicine.tcd.ie/psychiatry/research/projects/depression-neurobiology.php>) available to the general public.

The clinical study report will be presented to the REC and HPRA within one year of the completion or cancellation of the trial. The format of this summary will comply with the EU Note for Structure and Content of Clinical Study Reports (CPMP/ICH/137/95). The clinical study report will be signed by the principal investigator.

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