Cover Page

NCT02414932

Unique Protocol ID: SPUH 05/14

Brief Title: Ketamine for Depression Relapse Prevention Following ECT (KEEP-WELL)

Date of this document: 24.3.14

Please see the attached Study Protocol and Statistical Analysis Plan for this study

Clinical Trial Protocol

1. **INFORMATION ON CLINICAL TRIAL PROTOCOL**
2. **STUDY TITLE**

Ketamine for depression relapse prevention following ECT: a randomised pilot trial with blood biomarker evaluation (The KEEP WELL Study)

3. **STUDY SPONSOR**

St Patrick's Mental Health Services.

4. DETAILS

4.1 Study title

Ketamine for depression relapse prevention following ECT: a randomised pilot trial with blood biomarker evaluation (The KEEP WELL Study)

4.2 Reference numbers

Protocol identification (code or reference number):	SPMHS REC Reference 05/14
EudraCT number:	2014-000339-18
Date and version number:	24.3.14, Version 1.0

4.3 Applicant details

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Sponsor
Name: St Patrick's Mental Health Services

Contact details: The contact person for the sponsor is: Professor J Lucey,
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Funder: Not yet allocated, as of 23.3.14. Contact details to be updated when funding secured.

4.4 Signatures

1. Sponsor: Prof. J. Lucey, Medical Director, St. Patrick's University Hospital (on behalf of St Patrick's Mental Health Services)

2. Principal Investigator: Prof. Declan McLoughlin, Dept. of Psychiatry. St. Patrick's University Hospital.

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Date:25.08.14

4.5 Other relevant information

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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.

6. TABLE OF CONTENTS

7. DOCUMENT HISTORY

Document	Date of Issue	Summary of Change
Protocol version no.	Version 1.0, 24.3.14 Version 2.0, 10.6.14 Version 3.0	Not applicable Consent for both phases to be obtained pre-ECT, data retained 5-10 years only, effects of ketamine can be unpleasant. SPMHS as sponsor
Original protocol	Version 1.0, 24.3.14	Not applicable

8. SYNOPSIS

Title of study	**Ketamine for depression relapse prevention following ECT: a randomised pilot trial with blood biomarker evaluation (The KEEP WELL Study).**
Name of sponsor/company	St Patrick's Mental Health Services. Contact person: Prof. J. Lucey, Medical Director, St Patrick's University Hospital
Phase of development	Pilot Trial
Objectives	**Primary Objective** This pilot trial has two phases. The primary objective is to conduct a randomised, controlled, patient- and rater-blinded pilot study of ketamine vs. an active comparator (midazolam) for four weeks following successful ECT, to assess trial process to inform a future definitive trial. **Secondary Objectives** 1. To calculate a 95% confidence interval for an unadjusted hazard ratio that will allow interpretation of statistical difference between ketamine and midazolam groups to assess ketamine for reducing six-month relapse rates following successful ECT - Phase II (Trial Phase) 2. To evaluate changes in blood levels of neuroplasticity-associated proteins as biomarkers to predict the effect of ketamine in six-month relapse rates in ECT responders - Phase II (Trial Phase) - this is an exploratory objective 3. To conduct an open prospective cohort study of patients with severe depression being treated with ECT vs. a healthy control group to investigate telomerase activity in depression and response to ECT - Phase I (Open Phase) 4. To investigate epigenetic modulation of depression/stress-related genes in depression and response to ECT in the prospective cohort study of patients with severe depression being treated with ECT vs. a healthy control group above - Phase I (Open Phase) 5. To measure the association between stressful early life experiences and epigenetic modification of gene expression in the prospective cohort study of patients with severe depression being treated with ECT - Phase I

	(Open Phase) 6. To assess for association between scores on a brief personality assessment and response to ECT in the prospective cohort study of patients with severe depression being treated with ECT - Phase I (Open Phase)
Trial design	This pilot trial has two phases. **Phase I** - a prospective open cohort study involving patients with major depressive disorder referred for ECT and a healthy control comparison group for investigation of: telomerase activity in both depression and response to ECT, epigenetic modulation of depression/stress related genes in depressed and non-depressed people, epigenetic modulation of depression/stress-related genes in depression and response to ECT, the association between stressful early life experiences and epigenetic modification of gene expression in depression, and personality factors and response to ECT. Peripheral blood telomerase activity will be measured at pre-ECT/Baseline and on completion of the ECT course or at one month for controls. Whole blood samples to examine epigenetic modulation of depression/stress related genes, including DNA methylation, chromatin activation, and microRNAs will also be collected from patients and controls at Baseline and 4 weeks/ post ECT. Demographic, clinical and cognitive data will be collected at Baseline and at four weeks/end of ECT. Participants having ECT will also complete a brief personality screening test and a questionnaire on early life events. **Phase II** – a two-group parallel-design randomised controlled pilot trial involving Phase I participants who responded to ECT. Participants will be randomly allocated in a 1:1 ratio to a four-week course of either once-weekly ketamine or midazolam infusions. Patients and raters will be blind to treatment allocation (assessed at six months). The anaesthetist administering ketamine/ midazolam infusions will not be blinded but will not be involved in assessments or data analysis. Computerised random allocation, using randomly permuted blocks and stratified for family history of alcohol dependence, will be done independently. Both groups will continue usual care. Participants will be followed-up over six-months following ECT to identify if and when relapse occurs, using repeated clinical and cognitive measures at weeks 6, 8, 12, 20 and 26. Phase II participants will also be involved in a study to assess biomarkers to predict effect of ketamine on six-month relapse rates in ECT responders. Changes in blood levels of neuroplasticity-associated proteins will be assessed at four points before, during and after the first infusion of either ketamine or midazolam.
Key inclusion criteria	**Inclusion Criteria for Phase I:** (i) Patients ≥ 18 years of age referred for ECT (ii) Unipolar major depressive disorder (DSM-IV) (iii) 24-item Hamilton Rating Scale for Depression (HRSD-24) score of ≥21 (iv) Can provide valid informed consent **Inclusion Criteria for Phase II:** (i) Received a course of ECT ≥ 5 sessions in Phase I (ii) Achieved response criteria - ≥ 60% decrease from Baseline HRSD-24 score, and score ≥ 16 on two consecutive weekly ratings (iii) Have a nominated adult who can stay with them for 24hours on outpatient treatment days

	(iv) Mini-Mental State Examination (MMSE) score of ≥ 24 (v) Can provide valid informed consent
Key exclusion criteria	**Exclusion Criteria Phase I and II:** (i) Any condition rendering patient medically unfit for ECT; general anaesthesia, ketamine or midazolam – assessed by physical examination, routine haematology and biochemistry investigations prior to enrolment in Phase I (routine care) (ii) Active suicidal intention (iii) Dementia, intellectual disability, or MMSE <24 (iv) Lifetime history of bipolar affective disorder (v) Current history of post-traumatic stress disorder (vi) Other Axis I diagnosis (DSM-IV) (vii) ECT in the six months prior to recruitment (viii) Alcohol dependence or substance misuse in the six months prior to recruitment (ix) Pregnancy or breast-feeding (x) Residing in a nursing home (xi) Prisoner (xii) Diagnosis of terminal illness (xiii) Inability or refusal to provide valid informed consent
Number of subjects	**Phase I (Open Phase)** Patients referred for ECT, n=78, Healthy controls, n=78 Total, n=156 **Phase II (Trial Phase)** ECT responders randomised to ketamine (n=20) or midazolam (n=20) Total, n=40 (from Phase I cohort) **Total number of participants, n=156**
Test product, dose and mode of administration	**Investigative Medicinal Product** - Ketamine Hydrochloride (Pfizer Healthcare Ireland) 10 mg/ml infusion at 0.5mg/kg made up as 50ml colourless saline solution and administered intravenously over 40 minutes via syringe driver pump. **Active Comparator** - Midazolam Hydrochloride 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.
Duration of treatment	Phase I participants, duration of involvement – 4-6 weeks Phase I and II Participants, duration of involvement – 7.5 months. Duration of **treatment** with IMP in Phase II – 4 weeks.
Statistical methods	Pilot trial data will be analysed on an intention-to-treat basis for all patients who complete at least one infusion and one follow-up assessment. Data analyses will be performed blinded to allocation, by Prof Leslie Daly in CSTAR. Descriptive statistics will be used to report rates of: recruitment (Phases 1 and 2); willingness to be randomised; willingness to complete assessments (Phases 1 and 2); medical/ cognitive/ psychotomimetic/ general adverse events between groups in Phase 2; 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. Cox proportional hazard regression analysis will provide a 95% confidence interval for a hazard ratio for ketamine versus midazolam groups, accounting for the stratification factor "alcohol family history". 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; ECT remission status) that might influence response and help guide randomisation stratification in a future definitive trial. In the telomerase study, Baseline demographic and clinical data will be compared between groups using independent t-tests and chi-square tests as appropriate. Between groups data will be analysed by ANCOVA, with age and gender as covariates. In the depressed group, data will be analysed with linear models to test relationships between both Baseline telomerase activity and pre/post ECT change in activity and both clinical (e.g. HRSD-24) and cognitive measures. Linear regression will be used to investigate the association between epigenetic modulation of depression/stress-related genes and childhood trauma in depressed patients and the healthy control group. For all projects, continuous data will be tested for normality and transformed to enable linear modelling techniques. Where multiple hypotheses are applied to one dataset they will be corrected using Bonferroni or similarly conservative techniques.
Sample size	**Phase I (Open Phase)** In this prospective cohort study, we aim to recruit 78 ECT patients and 78 healthy volunteers, n=156 in total. Data collected in this cohort study will be used in studies of : (i) Telomerase activity in depression – limited data available to inform sample size however sample of n=156 will be adequate to detect meaningful differences at a 0.05 significance level with 80% power. (ii) Studies of epigenetic modulation of depression/stress related genes, and the association between stressful early life experiences with epigenetic modification of gene expression - based on previous studies, sample sizes were estimated to detect meaningful differences at a 0.05 significance level with 80% power. (iii) Personality factors affecting ECT – no formal sample size calculation as this is an exploratory objective. **Phase II (Trial Phase)** A formal sample size calculation has not been performed for this pilot study; numbers are based on previous trial recruitment and completion rates. Please see section 15.2 for further details regarding sample size.

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
AMI	Autobiographical Memory Interview
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
FCSRT	Free and Cued Selective Recall Test
GCP	Good Clinical Practice
GP	General Practitioner
GSK-3	Glycogen Synthase Kinase 3
HRSD-24	Hamilton Depression Rating Scale, 24-item version
HSE	Health Service Executive
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IMB	Irish Medicines Board
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
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, ECT AND RELAPSE

Major depressive disorder (MDD) is a debilitating mental illness with a lifetime prevalence of 12-20%. In Ireland depressive disorders account for 6,000 of 20,000 annual psychiatry admissions (http://www.dohc.ie/statistics/pdf/stats11_psyc.pdf). It's the most costly brain disorder in Europe, accounting for 1% (€118 billion annually) of the total European economy [1]. Indeed, depression is currently the second largest cause globally for *years lived with disability.*[2] . It is thus a public health priority, additionally so in Ireland with worryingly high suicide rates (http://www.3ts.ie/wp-content/uploads/2013/05/Suicide-in-Ireland-Survey-2003-2008-Report.pdf).

About 30% of patients don't respond to antidepressants even after multiple trials with/without psychotherapies [3]. However, electroconvulsive therapy (ECT) offers up to 70% of such treatment-resistant patients complete remission [4, 5]. ECT is a medically safe procedure and is more acutely effective than psychotherapy or antidepressants for severe, often treatment-resistant, depression[4]. The major concerns are cognitive side-effects but for most people these are transient and many cognitive functions improve [6]. Treatments involve passing small electrical charges through the brain to induce a seizure lasting ~30 seconds under anaesthesia with muscle-relaxant. 6-10 treatments are administered in a course, 2-3 times weekly. One million people worldwide annually receive ECT, including nearly 250 people in Ireland (http://www.mhcirl.ie/File/The-Administration-of-ECT-in-Approved-Centres-Activity-Report-2012.pdf).

However, we reported in a recent meta-analysis that relapse rates following successful ECT are high: 27.1% after three months and 37.7% after six months [7]. Even continuation ECT (C-ECT), albeit mostly at unadjustable fixed schedules, did not improve six-month relapse rates (37.2%). Notably, these rates are similar to patients who respond only after ≥3 antidepressant steps and most likely reflect the recurrent nature of treatment-resistant depression[3]. We also found that, compared to placebo, taking antidepressants after ECT halved the risk (risk ratio=0.49, $p<0.0001$, NNT=3.3) for relapse at six months from nearly 80%[7]. Some form of continuation therapy is therefore essential.

To date, the few reported randomised controlled trials for relapse prevention following ECT have focused on antidepressants and/or fixed-schedule C-ECT [7]. Searching www.clinicaltrials.gov, there are just five on-going or yet-to-be-reported trials of relapse prevention following ECT: three comparing various combinations of C-ECT and/or antidepressants; one assessing triiodothyronine; and one studying antidepressants/C-ECT/cognitive behavioural therapy. There is a need for other, potentially better, methods for relapse prevention. One possibility is the anaesthetic/analgesic ketamine.

KETAMINE AS AN ANTIDEPRESSANT

Ketamine is a competitive glutamate *N*-methyl-D-aspartate receptor (NMDAR) antagonist with half-life of 2-3 hours. Ketamine has a remarkably rapid antidepressant effect, targeting core symptoms, in treatment-resistant depression when given as single sub anaesthetic doses, usually a 40 minute 0.5 mg/kg intravenous infusion [8]. Ketamine is psychotomimetic (with abuse potential) but at low dosage it's safe, with patients and healthy controls experiencing mild dissociative and psychotic symptoms that resolve soon after finishing infusions [8]. To control for these effects, and also avoid "carry-over" effects in crossover studies while improving blinding, midazolam is now being used as control in parallel-group design trials rather than inactive placebo saline [9]. Thereafter robust antidepressant effects (~70% responder rates) occur within 2-4 hours and persist for a few days, i.e. beyond immediate NMDAR blockade. Chronic, mostly recreational, high-dose ketamine use can cause uropathy and dependency [8]. However, repeated (e.g. 2-3/week for two weeks) infusions of sub anaesthetic ketamine are safe with more sustained antidepressant effects [8, 10].

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 monaminergic antidepressants [11]. Preclinical studies have shown that within just two hours ketamine increases synaptogenesis and spine formation in rodent prefrontal cortex [12] and rapidly reverses chronic stress-induced depressive behaviours and prefrontal neuronal atrophy [13]. These effects are mediated, at least in part, via Akt/GSK-3/mammalian target of rapamycin (mTOR) signalling and increased dendritic translation of synaptic proteins [12], as well as deactivation of eukaryotic elongation factor 2 (eEF2) kinase, resulting in de-suppression of brain-derived neurotrophic factor (BDNF) translation [14]. BDNF mediates synaptic plasticity and is implicated in mechanisms of antidepressants and ECT [11]. Interestingly, a lesser response to ketamine was found in low-activity Met *BDNF* Val66Met polymorphism carriers [15] while increased plasma BDNF was detectable in responders compared to non-responders four hours post-infusion [16], though this hasn't been found in all studies. Changes in blood mononuclear cell levels of phosphorylated mTOR, eEF2 and GSK-3beta have also been associated with response to ketamine[17], suggesting potential as biomarkers for response.

Ketamine has been used for ECT anaesthesia and is associated with earlier improvement and possibly fewer cognitive side-effects but no overall better response [8]. However, no trials have yet been reported, or registered, for using ketamine as an adjunctive treatment to reduce relapse rates following successful depression treatment - an important potential use of ketamine that this proposal addresses.

NEUROPLASTICITY BIOMARKERS

Identifying a simple peripheral blood-based biomarker for diagnosis of depression and treatment response would be a major step. Based upon the above findings, the most appropriate biomarkers for initial evaluation are neuroplasticity markers, i.e. BDNF [11, 14, 16] as well as phosphorylated species of mTOR, eEF2 and GSK-3beta [12, 14, 17] 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 [18]. Another neuroplasticity peripheral biomarker for predicting treatment response may be the enzyme telomerase which maintains the length of telomeres, regions at chromosome ends that protect them from degradation or fusion. Telomere length provides a measure of biological, rather than chronological, age and reduced leukocyte telomere length occurs in depression and other stress-related conditions [19]. Telomerase functions in neuroplasticity and is critical for adult neurogenesis and antidepressant-like behaviours in a mouse depression model [20]. Interestingly, telomerase activity is elevated in un-medicated depressed patients. Following sertraline treatment, those with lower baseline activity showed a greater activity increase which was associated with superior antidepressant response [21]. Moreover, in a study of dementia caregivers with depressive symptoms, improvement was associated with increased telomerase activity[22].

The purpose of this research programme is to conduct a randomised pilot trial of ketamine for relapse prevention following ECT and develop molecular biomarkers for ketamine response and depression, one of the major health challenges for the 21st Century. Outcomes will include knowledge gained from the pilot trial to inform a future randomised controlled trial, and from analyses of preselected blood molecules. The latter will aid development of clinical biomarkers to help severely depressed patients.

EPIGENETICS, DEPRESSION AND EARLY LIFE STRESS

Epigenetics refers to control mechanisms that work alongside DNA sequences, altering their activity without changing the sequence itself [23] [24] Recent research shows that depressed patients have a different epigenetic profile compared with control subjects [23, 24]We will compare epigenetic modulation of depression/stress-related genes (e.g. *BDNF, FKBP5*) in depressed patients and healthy controls. Studies also suggest that reversing or bringing about compensatory epigenetic alterations in gene expression may be important in the mechanism of action of antidepressant therapies. We will test

the hypothesis that antidepressant therapy with ECT reverses or brings about compensatory epigenetic changes by examining epigenetic modulation of depression/stress-related genes in patients pre- and post-ECT. Epigenetics provides a potential mechanism by which adverse external stimuli such as early life stress can result in lasting changes in expression of depression/stress-related genes thus increasing the risk of depression in adulthood ([23, 24]. To gain insight into the pathogenesis of depression, we will measure the association between early life trauma and epigenetic modification of depression/stress-related genes in depressed patients and the healthy control group [25, 26]. 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 pyrosequencing [27] 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 immunoprecipition [28] 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 [29]. 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.

PERSONALITY AND RESPONSE TO ECT

Personality disorder is a recognised psychiatric diagnosis characterised by enduring maladaptive patterns of behaviour, cognition and inner experience (DSM-IV). Concomitant diagnoses of depression and personality disorder result in a doubling of the risk of a poor outcome for depression compared with no personality disorder [30]. There is evidence that a diagnosis of personality disorder with depression can affect response rates to ECT [31, 32] and also relapse rates following successful ECT [33]. There is also evidence that specific personality traits can affect response to ECT [34]. It has been suggested that clinicians under-prescribe ECT for depressed patients with a personality disorder [35]. Personality disorder or traits can be diagnosed clinically following repeated assessments and collateral history, or using a structured diagnostic interview for personality disorder e.g. SCID-II. Patients referred for ECT are often severely depressed and the extensive patient input required to make a formal diagnosis of personality disorder may be impracticable. Therefore a study assessing for association of a brief personality assessment and response to ECT may be useful. The validated screening tool SAPAS (Standardised Assessment of Personality – Abbreviated Scale) [36] is an eight-item dichotomously-rated screening tool for personality disorder. A score of 3 on the SAPAS correctly identified the presence of DSM IV personality disorder in 90% of participants in one study with sensitivity and specificity of 0.94 and 0.85 respectively. It has been shown that the majority of self-report personality traits are stable pre- and post-ECT treatment [37]. We propose to assess for association between scores on a brief personality screening questionnaire and response to ECT by administering SAPAS to participants in Phase I.

Rationale for the study

As described above, there is evidence of ketamine's antidepressant effect and a need for treatments to prevent relapse of depression following successful ECT. There are also identified needs for biomarkers for depression, response to ECT and response to ketamine, to further the understanding of the biology of depression and mechanism of action of ECT and ketamine in depression.

Based on the background as described above, we hypothesise the following:

1. Ketamine will reduce six month relapse rates following successful ECT

2. Blood molecular changes evident within hours after the first ketamine infusion (e.g. increase in levels of BDNF, phosphorylated mTOR, etc.) will predict lower six-month relapse rates

3. Telomerase levels will be lower in depressed patients than controls but will normalise in response to ECT

4. Depressed patients will have a different epigenetic profile compared with healthy controls and response to ECT will be associated with reversal of these differences or compensatory epigenetic changes in depression/stress-related genes

5. There will be an association between early life trauma and epigenetic modification of depression/stress-related genes in depressed patients and the healthy control group

6. Scores on a brief personality assessment will be associated with response to ECT

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 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.

A recent review of trials of ketamine for use as an antidepressant showed the most commonly used dosage in the past was as described above, i.e. 40 minute infusion of 0.5mg/kg [8]. Bioavailability of ketamine is highest when administered intravenously. There have been insufficient studies of intramuscular, oral or intranasal ketamine for depression to warrant studying these preparations for relapse prevention. Studies comparing ketamine with midazolam have used the sub anaesthetic dose of 0.045mg/kg as this dose mimics some of the effects of ketamine at 0.5mg/kg [8]. The regimen of four weekly infusions was chosen to facilitate subjects travelling for appointments, and because ketamine has proven effects as a rapid-acting antidepressant but has not yet been studied as a series of infusions for relapse prevention. While the half-life of ketamine is 3 hours, in previous studies the antidepressant effect was maintained to up to seven days [10]. It is proposed that by administering a series of four weekly infusions, a cumulative effect may be observed that could be protective during the vulnerable first month post ECT and could affect relapse rates.

Risks and Hazards

Ketamine has a rapid, robust antidepressant effect when administered at low-dosage (0.5mg/kg) intravenously [8]. Ketamine is psychotomimetic (with abuse potential) but is safe at low dosage, with patients and healthy controls experiencing mild dissociative and psychotic symptoms that resolve soon after finishing infusions [8]. Chronic, mostly recreational, high-dose ketamine use can cause uropathy and dependency. However, repeated (e.g. 2-3/week for two weeks) infusions of sub anaesthetic ketamine are safe with more sustained antidepressant effects [8, 10]. 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 [8]. Thus monitoring procedures have been out in place and will be followed as per the clinical 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 [8].

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. As above, strict monitoring procedures will be followed before, during and after infusions. Please see the monitoring procedures as described in section 15.6 of this protocol for more detail.

11 STUDY OBJECTIVE

11.1 Primary objective

This pilot trial has two phases.

The primary objective is to conduct a randomised, controlled, patient- and rater-blinded pilot study of ketamine vs. an active comparator (midazolam) for four weeks following successful ECT, to assess trial process to inform a future definitive trial. This primary objective will be fulfilled in Phase II (Trial Phase), Project 1. Several secondary objectives will be fulfilled in both Phase I (Open Phase) and Phase II (Trial Phase).

11.2 Secondary objective/s

(i) To calculate a 95% confidence interval for an unadjusted hazard ratio that will allow interpretation of statistical difference between ketamine and midazolam groups to assess ketamine for reducing six-month relapse rates following successful ECT - Phase II (Trial Phase)

(ii) To conduct an open prospective cohort study of patients with severe depression being treated with ECT vs. a healthy control group to investigate telomerase activity in depression and response to ECT - Phase I (Open Phase)

(iii) To investigate epigenetic modulation of depression/stress-related genes in depression and response to ECT in the prospective cohort study of patients with severe depression being treated with ECT vs. a healthy control group above - Phase I (Open Phase)

(iv) To measure the association between stressful early life experiences and epigenetic modification of gene expression in the prospective cohort study of patients with severe depression being treated with ECT - Phase I (Open Phase)

(v) To assess for association between scores on a brief personality assessment and response to ECT in the prospective cohort study of patients with severe depression being treated with ECT - Phase I (Open Phase)

11.3 Exploratory objectives

(i) To evaluate changes in blood levels of neuroplasticity-associated proteins as biomarkers to predict the effect of ketamine in six-month relapse rates in ECT responders - Phase II (Trial Phase)

11.4 Primary and secondary/ exploratory endpoints/outcome measures

Phase I

(i) Telomerase activity in depression and response to ECT

The outcome in this project is peripheral blood telomerase activity, measured at pre-ECT/baseline and after completing the ECT course or one month for controls, using the commercially available TRAPeze® Kit RT Telomerase Detection Kit (Chemicon International, Millipore). Response to ECT will be assessed by scores on the Hamilton Depression Rating Scale, 24-item (HRSD-24). HRSD scores are assessed at baseline, weekly during ECT, and at end-of-ECT. To enter Phase 1 patients must score ≥21 on HRDS-24. Response to ECT is defined as achieving ≥60% decrease from baseline HRSD-24 and score ≤16 on two consecutive weekly ratings.

(ii) Epigenetic modulation of depression/stress-related genes in depression and response to ECT

Outcomes for this project include markers of epigenetic modulation of depression/stress related genes, including microRNA and DNA methylation, chromatin activation status and mRNA expression for e.g. FKBP5 and BDNF. RNA and DNA will be extracted from whole blood samples from patients and controls at Baseline and 4 weeks/ post ECT. Blood samples for plasma protein levels of the relevant gene products will also be examined. Response to ECT will be assessed by scores on the Hamilton Depression Rating Scale, 24-item (HRSD-24) as detailed above.

(iii) The association between stressful early life experiences and epigenetic modification of gene expression in depression vs. healthy controls

Outcomes for this project include presence of stressful early life experiences assessed by baseline scores on the validated, 28-item self-rated Childhood Trauma Questionnaire, short form (CTQ-SF)[38], and measures of epigenetic modification of depression/stress-related genes e.g. DNA methylation, chromatin activation status and mRNA expression for FKBP5 and BDNF. These will be measured via whole blood samples from patients and controls at Baseline and 4 weeks/ post ECT.

(iv) Personality factors and response to ECT

The outcomes for this project are baseline scores on the brief personality assessment measure the Standardised Assessment of Personality- Abbreviated Scale (SAPAS)[36], and 8-item self-rated validated scale (ref), and response to ECT assessed by scores on the Hamilton Depression Rating Scale, 24-item (HRSD-24)[39]. HRSD scores are assessed at baseline, weekly during ECT, and at end-of-ECT.

Phase II

(i) Pilot trial of ketamine vs. midazolam

The focus of this pilot trial is on trial process with assessment of the primary clinical outcome being secondary because the pilot itself is not designed to measure efficacy [40].

Process outcomes that will inform a future definitive ketamine relapse prevention trial include information on the following:

recruitment methods and rate
willingness of participants to be randomised
willingness of participants to complete assessments
randomisation
success of blinding
ability to administer a course of ketamine infusions
medical safety and acceptability of ketamine infusions in an ECT responder population
rates of adverse dissociative and psychiatric events
adherence to allocated treatment
adherence to follow-up
reasons for drop-out from treatment
reasons for drop-out from follow-up
a 95% confidence interval for the difference between the ketamine and midazolam groups in six-month relapse rates to help with power calculations for a future definitive trial

Clinical outcomes:

These measures will be assessed at baseline, intra-treatment, end-of-treatment and follow-up time points illustrated in Figure 2 (Schedule of events).

(i) Diagnosis and treatment history: Diagnosis of major depressive disorder will be confirmed using the mood episodes module of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID)[41]. The Maudsley Staging Method for Treatment Resistant Depression (MSTRD)[42] will be used to provide a measure of treatment-resistance. Handedness will be recorded with the Edinburgh Handedness Questionnaire[43]. The National Adult Reading Test (NART) [44] will measure premorbid ability.

Additional baseline data from patient interview and case-note review will include age, sex, weight, height, occupation, educational attainment, duration of index depressive episode, number of previous depressive episodes, previous ECT, history of medical illness and surgical treatments, personal and family history of alcohol/substance dependency, presence of psychotic symptoms (detected by SCID), and current medications and other therapies. Changes in medications during Phases 1 and 2 will be documented at follow-up interviews (see Figure 2, Schedule of events).

(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 Phase 1 patients must score ≥21. *Response* to ECT is defined as achieving ≥60% decrease from baseline HRSD-24 and score ≤16 on two consecutive weekly ratings. *Remission* criteria are ≥60% decrease in HRSD from baseline and score ≤10 on two consecutive weekly ratings. 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.

Patients must achieve at least *response* to participate in the Phase 2 pilot trial. Baseline and weekly intra-treatment course HRSD-24 scores will be obtained during the treatment periods of Phases 1 and 2. During the infusion sessions in Phase 2 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 post-ECT 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 time points using the Quick Inventory of Depressive Symptoms, self-report version ($QIDS\text{-}SR_{16}$)[44].

(iii) Cognitive outcomes: Cognitive testing can be challenging for severely depressed patients. It is vital that patients be encouraged to remain in the trial throughout the whole study period and a great deal of tact is required of raters. Priority will be given to obtaining scores on the HRSD-24. In a recent meta-analysis we identified that ECT has the most pronounced subacute (i.e. 0-3 days post course) adverse effects upon verbal memory (delayed word list recall) and frontal executive function [6]. Sub anaesthetic doses of ketamine can cause problems with orientation, concentration, working and episodic memory but these resolve within two hours of beginning an infusion[8]. There are no published data on effects of ketamine on cognition in ECT responders. We will use the following battery in Phase 1 (pre and post ECT course; the latter will serve as baseline for Phase 2) and Phase 2 (one day after the first and fourth infusions and at six-months; see Tables 1 and 2). Parallel versions will be used to reduce practice effects.

a) Global cognition will be assessed with the revised *Addenbrooke's Cognitive Examination* (ACE-R; three parallel versions [45]) which also generates *Mini-Mental State Examination* (MMSE [46]) and verbal fluency scores. The ACE-R provides a total score (maximum=100) plus subscale scores for different aspects of cognition and has been used to study cognition in depression and by ourselves during ECT.

b) *Forward and Backward Digit Spans(Wechsler, 1989)* - immediate short-term memory, attention and working memory

c) *Trail Making Test (Part A Wechsler, 1989)*- motor and psychomotor speed

d) Frontal-executive function will be rated by *Trail Making Test (Part B, Wechsler, 1989)* plus *letter and category verbal fluencies*.

e) Anterograde verbal memory will be tested using the *Free and Cued Selective Reminding Test* variant (immediate and delayed recall) of the *Buschke Selective Reminding Test* [47].

f) To measure retrograde amnesia for autobiographical information we will use the *Kopelman Autobiographical memory Interview (AMI*[48]*).* This will be administered pre and post ECT, after the fourth ketamine infusion, and at six months.

(iv) Ketamine psychotomimetic effects and adverse events: Outcomes will include scores on the following scales, assessed before, during (+35-40 mins) and after (+240 mins) infusions of ketamine or midazolam:

- Clinician-Administered Dissociative States Scale (CADSS)[49]
- Brief Psychiatric Rating Scale (BPRS; four-item positive symptom subscale)[50]
- Young Mania Rating Scale (YMRS; mood item)[51]
- Patient-Rated Inventory of Side Effects[52]

(ii) Neuroplasticity biomarkers for predicting effect of adjunctive ketamine on relapse prevention

Outcomes for this project include changes in blood levels of neuroplasticity biomarkers including BDNF, pMTOR, pGSK3beta and peEF2 in response to the first infusion of ketamine/midazolam, compared at time points -60, +40, +120 and +240 minutes. Telomerase activity will also be analysed at -60 and +240 minutes. Relapse will be assessed using HRDS-24. Criteria for relapse are ≥10 point increase in HRSD-24 compared to baseline Phase II 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.

12 TRIAL DESIGN

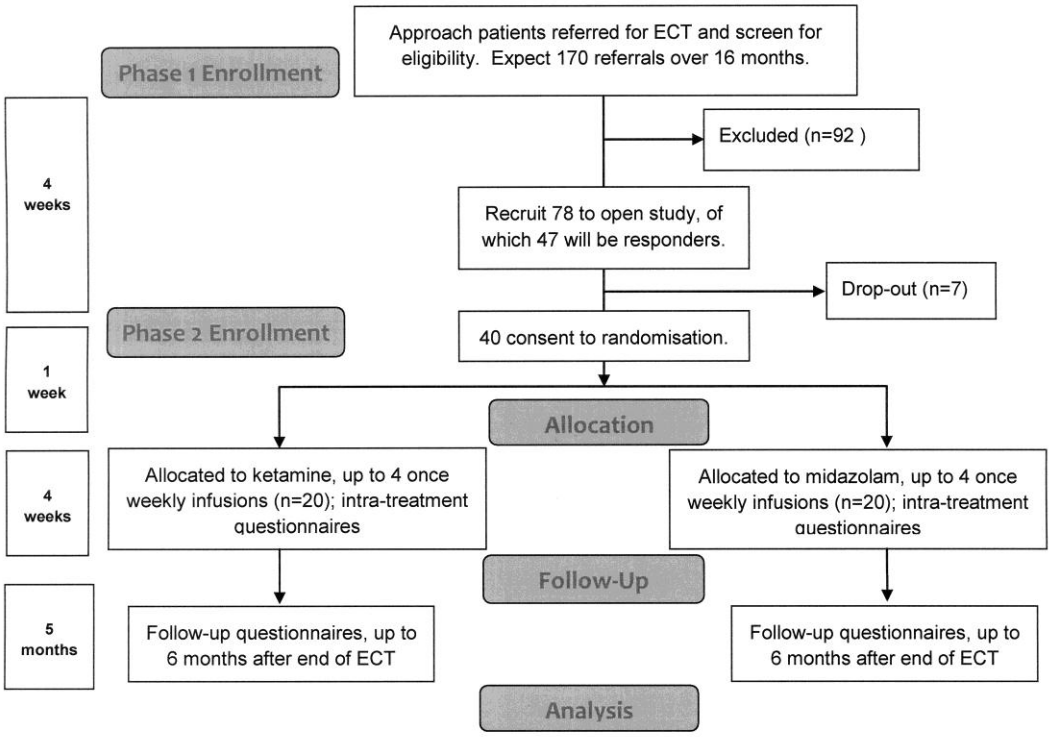
12.1 General considerations

This single-site study will take place in St. Patrick's University Hospital, Dublin.

This pilot trial consists of two phases.

The study design is presented graphically in the form of a CONSORT flow diagram below.

Figure 1. CONSORT Flow Diagram plan for pilot trial of ketamine for relapse prevention following successful ECT



Phase I

A prospective open study that involves recruiting patients with major depressive disorder (DSM-IV criteria) referred for ECT and a healthy control comparison group (recruited using the enclosed advertisement material, Appendix 2) for participation in several concurrent prospective cohort studies described separately below. Trial duration for participants in Phase I is one month.

Phase I, Telomerase in depression and response to ECT

This prospective cohort study will assess telomerase activity in depressed patients referred for ECT compared with a healthy control comparison group. Baseline peripheral blood telomerase activity will be measured via whole blood samples collected from fasting and resting (45 minutes) participants and analysis will take place in the laboratory at Trinity College Institute of Neuroscience, according to the method described in section 0. Telomerase activity will be measured again following completion of ECT or at one month for controls. Response to ECT will be assessed using scores on the Hamilton Depression Rating Scale, 24-item (HRSD-24) as outlined in section 0 and telomerase activity will be compared between responders and non-responders to ECT. Baseline and follow-up demographic, clinical and cognitive assessments will also be performed comprising –

Clinical: Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), Hamilton Depression Rating Scale, 24-item (HRSD-24), Quick Inventory of Depressive Symptoms, self-rated (QIDS-SR)

Background: Demographic data, Edinburgh Handedness Questionnaire, the National Adult Reading Test (NART), Childhood Trauma Questionnaire (CTQ), Standardised Assessment of Personality – abbreviated scale (SAPAS),

Cognitive: Addenbrooke's Cognitive Assessment, Revised (ACE-R), digit spans, Trails A and B, Full and Cued Selective Recall Test (FCSRT), and the Autobiographical Memory Interview.

Please see the detailed schedule and description of all assessments in section 12.3

Phase I, Epigenetic modulation of depression/stress-related genes in depression and response to ECT

This study will examine epigenetic modulation of depression/stress related genes in the cohort of depressed patients referred for ECT and a healthy control comparison group, as above. Whole blood samples will be collected at baseline and on completion of ECT (or at four weeks for controls) and markers of epigenetic modulation will be examined using the method described in section 12.3and compared between responders and non-responders to ECT, defined by scores on the Hamilton Depression Rating Scale, 24-item (HRSD-24) as outlined in section 12.3.

Phase I, The association between stressful early life experiences and epigenetic modification of gene expression in depression

This case-control study will compare baseline scores on the Childhood Trauma Questionnaire, short form (CTQ-SF), and measures of epigenetic modification of depression/stress-related genes between depressed patients referred for ECT and a healthy control comparison group, as above. Whole blood samples will be obtained at baseline and on completion of ECT or at four weeks for controls and markers of epigenetic modulation will be examined using the method described in section 12.3. Presence of depression will be assessed by diagnosis of unipolar major depressive disorder on SCID at screening and scores of ≥21 on HRSD-24 at screening and baseline.

Phase I, Personality factors and ECT

This prospective cohort study of depressed patients referred for ECT will assess for association between baseline scores on the brief personality assessment the Standardised Assessment of Personality- Abbreviated Scale (SAPAS), and response to ECT assessed by scores on the Hamilton Depression Rating Scale, 24-item (HRSD-24).

Phase II

Following Phase I, participants identified as being ECT responders will be invited to participate in Phase II, which consists of a two-group parallel-design randomised controlled pilot trial, and a prospective cohort study of biomarkers for prediction of response to ketamine, both described separately below.

Phase II, Pilot trial of Ketamine vs. Midazolam for relapse prevention following successful ECT

Phase I participants who are successfully treated with ECT and continue to meet inclusion criteria will be randomly allocated in a 1:1 ratio to a four-week course of either once-weekly ketamine at 0.5mg/Kg or the active comparator midazolam at 0.045mg/Kg. The trial will take place under "real world" conditions with both groups continuing usual care (e.g. regular medications, psychological and other therapies, and out-patient review) during the randomised treatment phase and thereafter. Participants will be followed-up over six-months following ECT to identify if and when relapse occurs.

A baseline interview will comprise treatment review, HRSD-24, QIDS-SR, and cognitive outcomes (ACE-R, digit spans, Trails A and B, FCSRT, and AMI). Patients and raters will be blind to treatment. Computerised random allocation, using randomly permuted blocks and stratified for family history of alcohol dependence (may be associated with antidepressant response to ketamine), will be done independently at CSTAR. Allocation information will be provided in two sets of opaque envelopes which will be stored in the ECT department where infusions are to be administered. To ensure patient safety during infusions and the post infusion period, the anaesthetist administering ketamine/ midazolam infusions will not be blinded but will not be involved in assessments or data analysis. 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 first infusion will be administered within two weeks of completing ECT and may be administered as an inpatient or outpatient; further infusions will take place as an outpatient. Each infusion will take 40 minutes, monitoring will take place for 200 minutes post-infusion. During each treatment session, participants will be monitored for heart rate, blood pressure, pulse oximetry, and electrocardiogram changes. Adverse or psychotomimetic effects of either agent will be monitored using the Clinician-Administered Dissociative States Scale (CADSS), Brief Psychiatric Rating Scale (BPRS; four-item positive symptom subscale), Young Mania Rating Scale (YMRS; mood item) and the Patient-Rated Inventory of Side Effects administered before, during and after infusions. Cognitive outcomes will be repeated in week 4. All other assessment measures will be repeated weekly; including treatment review/MSTRD, HRSD-24, and QIDS-SR.

Participants will be advised not to drive or operate heavy machinery for 24 hours post-infusions and will be asked to ensure they have a nominated adult who can stay with them for 24 hours on outpatient treatment days. Participants will be contacted by a researcher 24 hours after each session to enquire about side-effects.

Phase II participants will be followed up for six months following ECT with repeated questionnaires comprising treatment review, HRSD-24 and QIDS-SR at weeks 6, 8, 12 and 20 post ECT. A final follow-up session in week 26 will comprise the above listed measures as well as cognitive outcomes (ACE-R, digit spans, Trails A and B, FCSRT, and AMI).

Phase II, Neuroplasticity biomarkers for predicting effect of adjunctive ketamine on relapse prevention

Consented ECT responders who participate in the randomised controlled trial above will be assessed for changes in blood levels of neuroplasticity-associated proteins during the first ketamine infusion. Blood samples for plasma will be collected at four time points before, during and after the first ketamine/midazolam infusion (-60, +40, +120, and +240 minutes) and changes in biomarkers including BDNF, pMTOR, pGSK3beta and peEF2 will be compared between ketamine and midazolam groups. Six-month relapse rates (assessed as described in section 11.4) between groups will be compared with changes in biomarkers to assess these proteins as predictors of the effect of ketamine on relapse rates in ECT responders.

A detailed description of all assessments and interventions is available in section 12.3.

12.2 Selection of Study Population

12.2.1 Overall description of trial subjects

Participants will include healthy controls of ages 18 and above, who are not being treated for a mental health problem, and current inpatients in St Patrick's University Hospital, who have a diagnosis of unipolar MDD and are referred for ECT. Participants may be male or female, aged 18+, and from a variety of geographical (within Ireland) and socioeconomic backgrounds. Participants will not have any medical condition that would preclude treatment with ECT or ketamine/ midazolam.

12.2.2 Inclusion criteria

To be eligible for inclusion, each subject must meet each of the following criteria at Screening (Visit 1) and must continue to fulfil these criteria at Baseline (Visit 2).

- Subjects must be able and willing to give written informed consent and to comply with the requirements of this study protocol
- Subjects must be male or female, aged 18 years or above at Baseline
- If in the ECT patient group – must be diagnosed with unipolar major depressive disorder (DSM-IV) and with a HRDS-24 of ≥21, and referred for ECT.
- Participants in the ECT patient group must not have any finding on physical examination, routine haematology and biochemistry investigations, and an ECG at Screening that might affect ability to be treated with ECT or ketamine.
- If healthy volunteers, subjects who are judged to be in generally good health by the investigator based upon the results of the medical history and clinical assessments at Baseline (SCID).
- Female subjects of child bearing potential and male subjects whose partner is of child bearing potential must be willing to ensure that they or their partner use effective contraception during the study and for 5 weeks thereafter.
- Female subjects are requested to alert researchers to any possibility that they may be pregnant when asked at Screening, Baseline and all subsequent assessments.
- For inclusion in randomised Phase II, patients must have
    - Received a significant course of ECT (i.e. at least 5 sessions)
    - Achieved at least response criteria (i.e. ≥60% decrease from Baseline HRSD-24 score and score ≤16 on two consecutive weekly ratings)
    - Have a nominated adult who can stay with them for 24-hours on out-patient treatment days
    - Have a Mini-Mental State Examination (MMSE) score of ≥24

- Not currently taking any of the contraindicated medications listed in section 13.7.2 of this protocol

12.2.3 Exclusion criteria

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

- Allergy/sensitivity to study medications or their ingredients
- Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.
- Subjects who have participated in another study and received any other investigational agent within six months.
- Subjects unable to provide valid informed consent
- Any condition rendering patient medically unfit for ECT; general anaesthesia; ketamine or midazolam – assessed by physical examination, routine haematology and biochemistry investigations prior to enrolment in Phase I.
- Subjects who have a history of drug or alcohol use that, in the opinion of the investigator, would interfere with adherence to study requirements.
- Known history of, or documented positive hepatitis B or C or HIV infection.
- Diagnosis of current advanced malignancy or terminal illness.
- Scheduled for non-trial procedures requiring general anaesthesia during the study.
- Active suicidal intention
- Dementia, intellectual disability, or MMSE <24
- Lifetime history of bipolar affective disorder
  Current history of post-traumatic stress disorder
- Other Axis I diagnosis (DSM-IV)
- ECT in the six months prior to recruitment
- Residing in a nursing home
- Current prisoner

12.3 Study assessments and procedures

Participants in the healthy control group in Phase I will undergo:

- Phlebotomy for plasma **and** whole blood performed according to WHO "Best Practice in Phlebotomy"
- An interview comprising Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), National Adult Reading Test (NART), background and demographic data including the Childhood Trauma Questionnaire (CTQ) and Standardised Assessment of Personality-abbreviated Scale (SAPAS), Hamilton Depression Rating Scale, 24-item (HRSD-24), Quick Inventory of Depressive Symptoms, self-report version (QIDS-SR), and cognitive measures -
- Cognitive measures - Addenbrooke's Cognitive Examination- Revised (ACE-R); Digit Spans; Trails A and B; Full and Cued Selective Recall Test (FCSRT) and the Autobiographical Memory Interview (AMI)
- These measures including phlebotomy for plasma and whole blood will be repeated at 4 weeks. Trial duration for healthy controls in Phase I is five weeks.

Participants in the ECT treatment group in Phase I will undergo:

- The above listed measures
- Treatment review/Maudsley Staging method for Treatment Resistant Depression (MSTRD) at Baseline (prior to first ECT session), and at the end of the course of ECT.
- Weekly clinical outcome measures (HRSD-24, QIDS-SR).
- Trial duration for Phase I participants undergoing ECT is maximum eight weeks.

Phase I participants who are successfully treated with ECT and suitable to progress to Phase II will undergo:

- Baseline Interview (following completion of ECT) comprising treatment review/MSTRD, HRSD-24, QIDS-SR
- Baseline cognitive measures (ACE-R, Digit spans, Trails A and B; FCSRT and AMI)

Phase II participants will be randomized to receive four weekly intravenous infusions of either ketamine at 0.5mg/Kg or active comparator midazolam at 0.045mg/Kg. The first infusion will be administered as an inpatient within one week of completing ECT; further infusions will take place as an outpatient. Each infusion will take 40 minutes, monitoring will take place for 200 minutes post-infusion.

During each treatment session, participants will be monitored for:

- Heart rate, blood pressure, pulse oximetry, and ECG changes
- Adverse or psychotomimetic effects of either agent will be monitored using Clinician-Administered Dissociative States Scale (CADSS), Brief Psychiatric Rating Scale (BPRS), Young Mania Rating Scale, and Patient-Rated Inventory of Side Effects (PRISE), administered before, during and after infusions
- Cognitive outcomes will be repeated in week 4
- All other assessment measures will be repeated weekly, including treatment review/MSTRD, HRSD-24, and QIDS-SR

At the first infusion session, phlebotomy for plasma and whole blood will be performed on four occasions (at time points 60 mins pre-infusion and 40, 120 and 240 mins post-infusion). Participants will be advised not to drive or operate heavy machinery for 24 hours post-infusions and will be contacted by a researcher 24 hours after each session to enquire about side-effects. Participants in Phase II will be asked to ensure they have a nominated adult who can stay with them for 24 hours on outpatient treatment days.

Phase II participants will be followed up for six months with repeated questionnaires comprising treatment review/MSTRD, HRSD-24 and QIDS-SR at weeks 6, 8, 12 and 20 post infusions. A final follow-up session in week 26 will comprise the above listed measures as well as cognitive outcomes (ACE-R, digit spans, Trails A and B, FCSRT, and AMI). No phlebotomy will be performed on follow-up. Total trial duration for Phase II participants is nine months.

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

Figure 2. Schedule of events. Abbreviations are as in main text and key on pages 8 and 9.

	Phase 1: ECT patients and healthy controls			**Phase 2: ECT responders randomised to ketamine or midazolam**			
Assessment	Baseline (pre ECT)	Weekly during ECT course (patients only)	End of ECT course (4 weeks for controls)	Pre-infusions	Infusions #1-4; weeks 1-4	Follow-ups: weeks 6, 8, 12, 20	Final follow-up week 26
DIAGNOSIS AND TREATMENT							
Background, SCID, NART	✓						
Treatment review/ MSTRD	✓		✓		✓ (1-4)	✓ (6-20)	✓
CLINICAL OUTCOMES							
HRSD-24	✓	✓✓✓✓✓✓	✓		✓✓✓✓ (1-4)	✓ (6-20)	✓
QIDS-SR	✓	✓✓✓✓✓✓	✓		✓✓✓ ✓ (1-4)	✓ (6-20)	✓
COGNITIVE OUTCOMES							
ACE-R	✓		✓		✓✓1st + 4th		✓
Digit spans	✓		✓		✓✓1st + 4th		✓
Trails A + B	✓		✓		✓✓1st + 4th		✓
FCSRT	✓		✓		✓✓1st + 4th		✓
AMI	✓		✓		✓4th		✓
KETAMINE EFFECTS							
CADSS					✓✓✓ ✓ (1-4)		
BPRS					✓✓✓ ✓ (1-4)		
YMRS					✓✓✓ ✓ (1-4)		
PRISE					✓✓✓ ✓ (1-4)		
BLOOD SAMPLES							
Phlebotomy	✓		✓		✓✓✓✓1st		
CONSENT							
Signed Consent	✓						
Verbal Assent	✓	✓	✓	✓	✓✓✓✓ (1-4)	✓ (6-20)	✓
ELIGIBILTY							
Eligibility check	✓	✓	✓	✓	✓✓✓✓ (1-4)	✓ (6-20)	✓
RANDOMISATION							
Allocation				✓			

The following circumstances will constitute a protocol violation and will be reported to the Sponsor and discussed at the next Trial Steering Committee meeting. Multiple protocol violations may result in premature termination of the trial, at the decision of the Sponsor or investigators.

(i) Eligibility cannot be determined prior to referral for ECT, thus any assessment or intervention performed prior to referral for ECT constitutes a protocol violation
(ii) Baseline phlebotomy performed after ECT session 1
(iii) Phase I follow-up phlebotomy performed more than one week after the end of ECT treatment
(iv) Follow-up questionnaires repeated on more than the five specified occasions in Figure 2
(v) Questionnaires repeated more than seven months after the end of ECT treatment
(vi) Administration of more than four separate infusions of either ketamine or midazolam
(vii) Administration of ketamine or midazolam within six days of the last infusion
(viii) Administration of the first infusion of ketamine or midazolam more than two weeks after the end of ECT treatment

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 for participants in Phase I, but monitoring of vital signs for patients undergoing ECT is part of standard care.

In Phase II, 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 for Phase I. ECG monitoring (rhythm strip) will take place during ECT sessions as per usual care. ECG monitoring will be performed for all Phase II 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. Results of clinical laboratory tests of potential participants in the ECT treatment group of Phase I (who may progress to Phase II) will be reviewed by the anaesthetist as part of routine clinical screening prior to ECT.

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 start of a course of ECT (Phase I, Open Phase), and at the first ketamine infusion (Phase II, Trial Phase). Information relating to the importance of contraception during the trial is provided in the Participant Information Sheet.

Concomitant Medication

For all participants in Phase I, all over-the-counter or prescription medication, vitamins, and/or herbal supplements will be recorded on CRFs. The indication for treatments will be recorded. Please see section 13.7 for further details.

The Maudsley Staging Method for Treatment-Resistant Depression (MSTRD) will be used as a measure of treatment resistance in participants in the ECT treatment group in Phase I and will be performed at baseline Phase I assessment and at end-of-ECT. For Phase II participants, the MSTRD will be repeated at Phase II baseline and again at each infusion and follow-up session.

Cognitive Assessments

Cognitive measures including Addenbrooke's Cognitive Examination- Revised (ACE-R); Digit Spans; Trails A and B; Full and Cued Selective Recall Test (FCSRT) and the Autobiographical Memory Interview (AMI) will be performed at baseline and end-of-ECT or four weeks in Phase I, and at baseline and final infusion session in Phase II, as well as at final Phase II follow-up at week 26. 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) will be performed once by trained researchers at baseline assessment in Phase I. 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 major depressive disorder in participants undergoing ECT in Phase I, and to out rule any psychiatric diagnosis in participants in the healthy control group in Phase I.

Hamilton Depression Rating Scale, 24-item (HRSD-24) is a validated depression rating measure which will be performed on a repeated basis by trained researchers in both Phases I and II as a measure of depressive symptomatology and response to treatment.

Quick Inventory of Depressive Symptoms, self-report version (QIDS-SR) is a validated self-report measure of depressive symptoms which will be used repeatedly in both Phases I and II. It is best practice to include both self- and clinician-rated measures of depressive symptoms to assess treatment response in depression.

National Adult Reading Test

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 in Phase I.

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 in Phase I.

Standardised Assessment of Personality- abbreviated Scale (SAPAS)

SAPAS is a validated brief (8-item) personality assessment questionnaire which will be completed once at baseline assessment in Phase I. It is not diagnostic of DSM-V personality disorder.

Side Effect Measures

During infusion sessions in Phase II, adverse or psychotomimetic effects of either ketamine or midazolam will be monitored using validated scales comprising: Clinician-Administered Dissociative States Scale (CADSS), Brief Psychiatric Rating Scale (BPRS), Young Mania Rating Scale, 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.

Telomerase Assay

Peripheral blood telomerase activity will be measured at baseline assessment in Phase I and after completing the ECT course or one month for controls. Blood will be collected from fasting and resting (45 minutes) participants into heparinised tubes containing a Ficoll separation gradient. Peripheral Blood Mononuclear Cells (PBMCs) will be extracted, pelleted, washed once with phosphate buffered saline (PBS) and repelleted. All traces of PBS will be removed and pellets will be stored at -80°C. The commercially available TRAPeze® Kit RT Telomerase Detection Kit (Chemicon International, Millipore) will be used to assess telomerase activity according to manufacturer's instructions and as used by others. This kit allows fluorometric detection of telomerase activity through using Amplifluor® primers.

Neuroplasticity Biomarkers

At the first infusion session in Phase II, changes in blood levels of BDNF, pMTOR, pGSK3beta and peEF2 in response to the ketamine/midazolam will be compared at time points -60, +40, +120 and +240 minutes. Blood samples for plasma will be collected using EDTA vacutainer tubes and centrifuged to generate plasma that will be aliquoted and stored at -80^{o}C. BDNF levels will be determined using ELISA (ChemiKine, USA) following the manufacturer's instructions. PBMCs will be collected, pelleted and stored at -80^{o}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. PBMCs will be collected and analysed for telomerase activity as described in Project 2 above at -60 and +240 minutes. One whole 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

In Phase I, whole blood samples will be collected using one PAXgene tube and one EDTA tube at baseline and post-ECT/four weeks for controls, to analyse epigenetic modulation of depression/stress-related gene expression. Epigenetic modifications that will be examined may include DNA methylation, measured by pyrosequencing [27], 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 immunoprecipition [28] 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 [29].

12.3.2 Endpoints assessments

Please see detailed information on outcomes and assessment of endpoints in section 12.3.

Efficacy Assessment

This pilot trial is not designed to assess efficacy [40]. 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.

Secondary efficacy endpoints

(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.

Safety Assessment

The following safety evaluations will be performed during the study: adverse event monitoring, vital signs, cognitive and clinical assessments. Regular laboratory investigations and physical examinations are not indicated. Blinded data will be presented to the DSMB for safety evaluation every four months. Minutes of the DSMB meetings including safety evaluations will be presented to the TSC at every meeting.

12.3.3 Screening procedure

Screening

Screening will take place prior to recruitment to Phase I, Open Phase

Screening for both healthy volunteers and patients referred for ECT will involve the following:

- HRDS-24
- MMSE
- Brief medical history
- Brief psychiatric history – to out rule bipolar affective disorder, alcohol or substance dependence, post traumatic stress disorder
- Review of clinical notes to out rule exclusion criteria e.g. resident in a nursing home

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. There will be one decision point regarding eligibility, according to the inclusion criteria specified in section 12.2.2. There are no laboratory screening tests or physical examinations. The maximum duration between screening and randomisation is one month. Informed consent will be obtained prior to any study related procedures being undertaken.

Recruitment

Phase I, Open Phase

Healthy volunteers will be recruited by advertising in volunteer organisations with an interest in mental health, using the advertisement material in Appendix 2.

Participants for the ECT treatment cohort will be recruited by verbal approach on the ward by a researcher, following their referral for ECT treatment.

Phase II, Trial Phase

Phase II participants will be recruited from Phase I participants who have received ECT. ECT responders who consent to randomisation will be allocated to one of two treatment groups.

12.3.4 Baseline assessments

The following pre-treatment Baseline assessments will be performed prior to enrolling in **Phase I**:

- Confirmation of eligibility (review inclusion/exclusion criteria)
    - In the case of Phase I participants having ECT, this involves examining the documented results of physical examination, laboratory investigations, ECG and chest X-ray which are routinely performed by the team referring the participant for ECT
- Recording of demographics, medical history and concomitant medications
- For women of child-bearing potential the LMP will be recorded
- Phlebotomy for plasma **and** whole blood performed according to WHO "Best Practice in Phlebotomy"
- An interview comprising:
    - Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), National Adult Reading Test (NART), Childhood Trauma Questionnaire (CTQ) and Standardised Assessment of Personality- abbreviated Scale (SAPAS), Hamilton Depression Rating Scale, 24-item (HRSD-24), Quick Inventory of Depressive Symptoms, self-report version (QIDS-SR)
    - Cognitive measures - Addenbrooke's Cognitive Examination- Revised (ACE-R); Digit Spans; Trails A and B; Full and Cued Selective Recall Test (FCSRT) and the Autobiographical Memory Interview (AMI)
- Phase I Participants **undergoing ECT** will also undergo a treatment review/Maudsley Staging Method for Treatment Resistant Depression (MSTRD)

Phase I Participants who respond to ECT will be invited to participate in Phase II and upon clarification of their assent, will undergo the following **Phase II baseline assessment**:

- Baseline Interview (following completion of ECT) comprising treatment review/MSTRD, HRSD-24, QIDS-SR and eligibility review
- Baseline cognitive measures (ACE-R, Digit spans, Trails A and B; FCSRT and AMI)

12.3.5 Subsequent study visits and procedures

Phase I, Open Phase

Subsequent visits for Phase I participants in the **healthy control group** will involve:

- One follow-up visit with repeat of baseline assessments (above) including phlebotomy and eligibility review at four weeks
    - SAPAS and CTQ will not be repeated

Subsequent visits for participants in the **ECT treatment group** will involve:

- Weekly repeat of HRSD and QIDS-SR during course of ECT
- End-of-ECT assessment with repeat of baseline measures (above) including phlebotomy, eligibility check and treatment review
    - SAPAS and CTQ will not be repeated

Phase II, Trial Phase

Following randomisation, participants in Phase II will attend on four occasions for infusions of ketamine or midazolam and will also have follow-up assessments over a period of six months.

1. The **first infusion session** will take place within one week of completing ECT and will comprise:

- Phlebotomy for plasma and whole blood on four occasions (at time points 60mins pre-infusion and 40, 120 and 240 mins post-infusion)
- Eligibility check and treatment review/MSTRD
- HRSD-24 and QIDS-SR
- 40-minute infusion of either ketamine or midazolam
- Regular monitoring of heart rate, blood pressure, pulse oximetry, and ECG changes before, during and for 200 minutes after infusion
- Clinician-Administered Dissociative States Scale (CADSS), Brief Psychiatric Rating Scale (BPRS), Young Mania Rating Scale, and Patient-Rated Inventory of Side Effects (PRISE), administered before, during and after infusions
- Telephone contact by a researcher in 24 hours to enquire about side-effects

2. **Three subsequent infusion sessions** will involve the above measures except phlebotomy.
3. The **final infusion session** will also include repeat of cognitive assessments including Addenbrooke's Cognitive Examination- Revised (ACE-R); Digit Spans; Trails A and B; Full and Cued Selective Recall Test (FCSRT) and the Autobiographical Memory Interview (AMI)

Phase II participants will be followed up for six months with repeated questionnaires comprising eligibility review, treatment review/MSTRD, HRSD-24 and QIDS-SR at weeks 6, 8, 12 and 20 post infusions. A final follow-up session in week 26 will comprise the above listed measures as well as cognitive outcomes (ACE-R, digit spans, Trails A and B, FCSRT, and AMI). No phlebotomy will be performed on follow-up.

A detailed description of each of the assessments is available in section 12.3.

10.4.6 Method of assigning Subjects to treatment groups

This is a two-phase pilot trial. There are no treatment groups in Phase I, Open Phase.

In Phase II, Trial Phase, ECT responders will be randomised after Baseline data collection. Subjects will 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). Blocks will be stratified for family history of alcohol dependence as this has been associated with antidepressant response to ketamine [53]. Allocation information will be provided in a set of sealed numbered envelopes prepared by CSTAR. The information in these will be made available only to the anaesthetist to ensure allocation concealment. A second identical set of envelopes will also be provided by CSTAR and these may be accessed for emergency purposes.

Blinding

Study treatment assignment will be blinded for both the raters and the participants. Two sets of opaque envelopes containing allocation information will be provided by CSTAR following randomisation as above. 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 second 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 Phase II participant. End-of-trial will be reported to the REC and IMB within 90 days, or 15 days if the study is terminated prematurely. A summary report of the study will be provided to the joint REC of St. James' and Tallaght Hospitals, the REC of St Patrick's Mental Health services, and IMB 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, FCSRT, AMI) outcomes
- Assessment of safety - check for any adverse effects
- Recording of concomitant medication

12.4.1 Premature termination of the study

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

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 IMB and Trial Steering Committee and justified in the final report

12.5 Discontinuation/withdrawal of subjects from study treatment

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 section 11.3 of 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 in an infusion session, monitoring of vital signs and mental health must be performed for 200 minutes following the end of the infusion, comprising the measures detailed in section 11.3.

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 treatment(s)

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.

This dose and administration was chosen based on a previous randomised controlled trial of ketamine and midazolam [10] in which this dosage was well-tolerated.

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:
(i) Use of the treatment in this trial
(ii) Name of sponsor, and principal investigator;
(iii) 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(s)

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 per section 13.2, 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 in Phase II as they may significantly alter the pharmacokinetics of ketamine.[54]. 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 [55].

Contraindicated medications:

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

Participants taking any of these medications at Screening for Phase II will be excluded from the trial. Medication history will be checked at Baseline in Phase II 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 in section 12.3.5. 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. Please see exclusion criteria in 12.2.3.

14. SAFETY REPORTING

Safety and tolerability will be evaluated throughout the study according to the framework presented in section 12.3.

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 Evaluations of AEs and SAEs

14.2.1 Assessment of seriousness

The investigator should make an assessment of seriousness as defined in section 14.1.

14.2.2 Assessment of casualty

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.

13.7.1 Assessment of expectedness

The exepctedness of an adverse reaction will be determined by the sponsor according to the investigator's brochure.

13.7.2 Emergency unblinding procedures

Emergency unblinding can be performed by any investigator by opening one or all of the second 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.

13.8 Reporting procedures for all adverse events

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

13.9 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 (IMB) and the ethics committees concerned (Joint REC of St James' and Tallaght Hospitals and SPUH REC). 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 IMB 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.

13.10 Data safety monitoring board (DSMB)

The DSMB 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 four months during the trial. No member of the DSMB will have a conflict of interest with the Sponsor. Blinded data will be presented to the DSMB for safety evaluation every four months. Minutes of the DSMB meetings including safety evaluations will be presented to the TSC at every meeting.

The DSMB may review unblinded data on request for individual or all participants. Additionally, the DSMB may request to terminate the trial according to section 13.9. The advice of the DSMB will be notified upon receipt by the Sponsor to the joint REC of St. James' and Tallaght Hospitals, the REC of

St Patrick's Mental Health Services, and the IMB. With this notification a statement will be included indicating whether the advice will be followed.

The Trial Steering Committee will comprise investigators, clinical experts not directly involved in the trial, 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 DSMB 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 DSMB and TSC once established.

13.11 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 newborn 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 the start of ECT treatment (Phase I, Open Phase) and the first ketamine infusion (Phase II, Trial Phase). 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 4).

15. STATISTICS

15.1 Description of statistical methods

Descriptive statistics will be used to report: rates of recruitment (Phases I and II), willingness to be randomised (Phase II), willingness to complete assessments (Phases I and II), medical/ cognitive/ psychotomimetic/ general adverse events between groups in Phase II, 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 in Phase II, Trial Phase. 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; ECT remission status) that might influence response and hep guide randomisation stratification in a future definitive trial.

Data will be analysed using IBM PASW (SPSS) version 20, and "R" (R Foundation for Statistical Computing, Austria).

15.2 Determination of sample size

Phase I, Open Phase - sample size

Phase I will consist of a prospective cohort study of depressed patients receiving ECT and a healthy control group. We aim to recruit 78 ECT patients and 78 healthy volunteers, n=156 in total. Data collected in this cohort study will be used in the studies of telomerase activity in depression and studies of epigenetic modulation of depression/stress related genes, and the association between stressful early life experiences with epigenetic modification of gene expression, described in section 12.

(i) Only limited data are currently available to inform sample size calculations for the study of telomerase activity and so should be interpreted as being preliminary. For example, Wolkowitz et al (2012) found mean (SD) baseline telomerase activity was significantly increased in depressed patients (10.8(5.7) units) compared to controls (7.9(5.0) units).[21] A sample size of 47 per group will have 80% power to show a similar difference between pre-ECT depressed and control groups with a two-sided t-test and α=0.05. Lavretsky et al (2013) reported mean telomerase activity to increase from 2.7(1.3) units to 3.6(0.2) following yogic meditation for depressive symptoms.[22] A sample size of 47 will have 91% power to detect such a difference pre/post ECT in a two-sided t-test and α=0.05. As we plan to recruit above these numbers, sample size will be adequate.

(ii) Phase I (Open Phase) involves a prospective cohort study of epigenetic modulation of depression/stress related genes in depression and response to ECT, and a study examining the association between stressful early life experiences and epigenetic modification of gene expression within this cohort. There have been few studies of this nature previously. Similar studies examining DNA methylation profiles in depressed people vs. controls reported sample sizes of n=38 (Fuchikami, 2011), n=85 (D'Addario et al., 2013) and n= 46 Cruceanu et al., (2013). Other studies of epigenetic modulation in antidepressant treatment have used samples of n=25 (Lopez et al., 2012), n=9 (Belzeaux et al, 2012), and n=10 (Bocchio-Chiavetto, 2013). Previous studies examining associations between early life adversity and epigenetic modification of gene expression have reported sample sizes from n=215 (Perroud, 2011), n=99 (Tyrka, 2012) to n=24 (McGowan, 2009). Based on previous studies including those mentioned above, sample sizes were estimated to detect meaningful differences at a 0.05 significance level with 80% power.

(iii) Participants in Phase I who are having ECT will also complete a brief personality assessment as part of a study to assess for association between response to ECT and scores on a brief personality assessment. Although there are studies which have examined diagnosis o personality disorder and response to ECT, there are no previous studies of a brief personality assessment such as the SAPAS and response to ECT to inform sample size, therefore this study is considered exploratory.

Phase II, Trial Phase - sample size

Phase II involves a pilot trial of ketamine vs. midazolam for relapse prevention following successful ECT and a study of biomarkers to predict response to ketamine.

(i) There are no available data to inform the biomarker study in Phase II as this is an exploratory objective.

(ii) For the pilot trial, in line with recommendations for pilot studies, a formal sample size calculation has not been performed. The sample size described (n=156 in Phase I, of which n=40 progress to Phase II), was determined through previous experience of this

research department of recruitment to a large (n=140) randomised controlled trial of depressed patients undergoing ECT, in which 60 patients were recruited in the trial's first 16 months. 80% of participants were retained after 12 months, and one participant withdrew during treatment. For this pilot trial we wish to recruit 20 patients per group, a total of 40, which is an acceptable number for the purposes of a pilot trial [40]. Response rates to ECT are ~60%, so at least 66 patients need to be recruited to Phase I. Allowing for a 15% drop-out rate, we will therefore seek to recruit 78 patients in Phase I, and the same number of healthy volunteers as a control group, i.e. Phase I total, n=156. As this study is a less intensive trial than the one described above, with the attraction of an additional therapy, we expect the recruitment rate to be higher than that reported above and to recruit 78 participants to Phase I within 16 months. We expect that 47 of the ECT patient group of 78 will meet response criteria following ECT, and that 40 of these will additionally consent to be randomised and participate in the Phase II pilot trial.

This section will be updated regularly to reflect recruitment rates on commencement of the trial.

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

15.5.1 Primary efficacy endpoint

This pilot trial is not designed to assess efficacy [40]. 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 endpoints

(v) Subjective mood ratings as measured by scores on QIDS-SR.

(vi) 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.

(vii) Tolerability of ketamine vs. midazolam in terms of psychotomimetic effects as measured by scores on CADSS, BPRS, YMRS, and PRISE.

(viii) 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, FCSRT, AMI), monitoring for psychotomimetic effects (CADSS, BPRS, YMRS, PRISE), and adverse effects between groups in Phase II. Blinded data will be presented to the DSMB for safety evaluation every four months. Minutes of the DSMB 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 [40] 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 Phase II.

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 IMB 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, ECT booklets, 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 Data Safety Monitoring Board 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.

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 Control and 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.

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 GCP training, e.g. Martha Finnegan attended GCP training provided by Molecular Medicine Ireland on 11.3.14.
(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 [56] 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.

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

An application for approval for this trial will be submitted to the joint REC of St. James' and Tallaght Hospitals for ethical review. An application will also be submitted to the local site REC (St Patrick's Mental Health Services).

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 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 5). Potential participants will be provided with an information leaflet and letter of invitation (Appendix 4) and verbal information at the first point of contact with a member of the research team. This process will take place following screening for Phase I, at which time informed consent for both Phases I and II will be completed. If invited to partake in Phase II, verbal assent will be confirmed after completion of ECT.

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 referral for ECT to take place the day before administration of the first ECT 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 accomodated. 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 Phase II 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. An assessment of capacity to make treatment decisions is made by the treating team prior to ECT treatment. 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) Healthy volunteers will be used as a comparison group in order to investigate the role of telomerase in depression and response to ECT. This objective cannot accurately be achieved without a healthy control group. The trial is not expected to benefit healthy volunteer participants directly, there may be indirect benefits in increased awareness of mental health, and increased scientific knowledge gained. It is not anticipated that healthy volunteer participants would experience any adverse effects other than lifestyle inconvenience and discomfort during phlebotomy, as a result of participation. Risks and burden are considered minimal in this population.

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 both ECT and ketamine have included women of childbearing age and precautions will be taken as detailed in point 12.3 to ensure adequate contraception is in place throughout the trial. Women of childbearing age may be in the healthy volunteer group or adults with mental illness group with expected benefits to these participants as listed above.

21.6 Subject confidentiality

Investigators 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 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 DSMB 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

Financing at the time of Version 1.0 of this protocol is through pre-existing research grants under the supervision of Prof. D. McLoughlin, Principal Investigator. This funding is from sources not connected with the design, completion or reporting of this trial. Funding is also currently being sought from the HRB. Applications for further funding will continue and funding details will be updated as necessary.

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 6, "St Patrick's Mental Health Services Indemnity Policy".

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 IMB 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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