Title Page

A Randomised Double-Blinded Placebo-Controlled Trial to Assess the Efficacy and Safety of Scopolamine Compared to Placebo in Individuals with Bipolar Disorder who are Experiencing a Depressive Episode (SCOPE-BD)

Protocol Version no:	3	Date: 18th December 2020
Test Drug:	Scopolamine	
Clinical Phase:	IIb	
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Sponsor Number:	NUIG-2017-002	

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The study will be conducted in compliance with the protocol, International Conference on Harmonization – Good Clinical Practice (ICH-GCP) and any applicable regulatory requirements.

1. (i) Sponsor Protocol Agreement Page

I, the undersigned, agree to the content of the final clinical trial protocol, as presented.

Signed:

Vice-President Research, NUIG

Signature	Print name	Date
	LOKESH JOSHI	Jan 11 2024

1. (ii) Chief Investigator Agreement

I, the undersigned, agree to the content of the final clinical trial protocol, as presented.

Signed:

Chief Investigator

Signature	Print name	Date
	B MALLAHAN	12/01/21

2. Site Investigator Agreeement

I, the undersigned, am responsible for the conduct of the trial at this site and agree to the following:
I understand and will conduct the trial according to the protocol, any approved protocol amendments, ICH GCP and all applicable regulatory authority requirements and national laws.
I will not deviate from the protocol without prior written approval from the HPRA and the Ethics Committee, except where necessary to prevent any immediate danger to the participant.
I have sufficient time to properly conduct and complete the trial within the agreed trial period, and I have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely. I will ensure that any staff at my site(s) who are involved in the trial conduct are adequately trained regarding the protocol and their responsibilities.

Signed:

Principal Investigator

Signature	Print name	Date
	B. MALLAHAN	12/01/21

TABLE OF CONTENTS

3. Document History

Document	Date of Issue	Summary of Change
1.0	03-Sept-2019	N/A-first release of document.
2	11-Oct-2019	• Date/version number updated. • Table of contents/page numbers updated. • Minor changes to format and spacing. • The word "subject" has been updated to "participant" throughout the protocol for consistency. • Abbreviations edited to remove RNA Ribonucleic Acid and ROI Republic of Ireland. • Section 9.1 Description of study treatments, updated to reference that the placebo will be an authorised product, commercially sourced through the Galway University Hospital pharmacy. • Exclusion criterion 12 updated to include acute porphyria. Section 8.11, Discontinuation/withdrawal of participants from study protocol has additionally been updated to account for this. • Exclusion criterion 13 updated to provide clarification that patients on anticholinergic medications will be excluded from enrolling in the study. • Reference to Buprenorphine has been removed due to its lack of anti-cholinergic activity (exclusion criterion 13, section 8.11 (Discontinuation/withdrawal of participants from study protocol) and 9.8.2 (Prohibited medications) updated. • Additional exclusion criterion 20 added stating that any disorder, unwillingness or inability, not covered by any of the other exclusion criteria, which in the investigator's opinion, might jeopardise the participant's safety or compliance with the protocol. • Section 8.4.2, Medical and Surgical History, updated to align to SmPc information, warnings and precautions that, medical history of prostatic hypertrophy, asthma, hepatic or renal impairment, gastrointestinal obstruction, epilepsy and thyrotoxicosis should be sought. • Section 8.4.4.1, Vital signs has been updated to indicate that Clinical support includes ready access to resuscitation equipment and clinical expertise and participant should be asymptomatic of potential IMP related symptoms as well as having satisfactory vital signs before discharge post infusion.

		• Section 9.8.1 has been updated to replace reference to section 9.7.2 with section 9.8.2. • To align with the SmPC warnings and precautions, blood tests for U&Es, LFTs and TFTs are required to be within acceptable ranges in the previous 4 months at screening (visit 1). If necessary blood tests will be performed at Visit 1 or Visit 2 and confirmed as acceptable prior to first infusion. Updates to inclusion criteria, abbreviations, Table 2 (table and footnote), section 8.3.3 & 8.3.4 (assessments) and 8.4.5 (Laboratory tests) have been made. • Duplicated paragraph in section 8.6 removed and HDRS score agreed at <23. • Vital signs included as part of Visit 1 assessments in protocol version 2. Updates made to Table 2 and section 8.3.3 Screening assessments. • Vital signs have been removed from placebo run-in exclusion criteria at randomisation visit. Updates made to Exclusion criteria, section 8.3.5 (Psychometric instruments). • The version of SCID questionnaire has been updated to SCID-RV from the DSM-V Axis I disorder. The following sections updated to account for this: Section 6.1.3, 8.3.3, 8.5, inclusion criterion 1, Table 2 (table and footnote). • The WAIS questionnaire is replacing the WASI questionnaire in protocol Version 2. The following sections updated to account for this: Section 8.3.4, 8.3.5, 8.3.6, 8.5, abbreviations, and Table 2 (table and footnote). • A pregnancy urine dipstick test has been introduced to confirm pregnancy status at Visit 2, 3, 4, 5 prior to each infusion. This test is in addition to serum pregnancy test, at Visit 2 but for which the results are not immediately available and will confirm pregnancy status prior to infusion at Visit 3. The following sections updated to incorporate these changes: Section 8.4.5 (Laboratory Tests), exclusion criterion 18, Table 2 (Table and footnote), section 8.3.4, 8.3.5, 8.4.7. • Table 2, Visit 3 column heading, * beside Randomisation removed. • Reference section updated for version 2 to remove references no longer required and additional references added.

3	18-Dec-2020	• Update in Investigative Medicinal Product from 400micrograms/ml to 600micrograms/ml (section 9.1), product supplier and dose to participant remaining the same • Deletion of section 10.2.6, replication of section 10.2.5 • Section 8.4.2, Medical and Surgical History, updated to align to SmPC information

4. Synopsis

Title of study	A Randomised Double-Blinded Placebo-Controlled Trial to Assess the Efficacy and Safety of Scopolamine Compared to Placebo in Individuals with Bipolar Disorder who are Experiencing a Depressive Episode (SCOPE-BD)
Name of sponsor	Prof. Lokesh Joshi (Vice President of Research) National University of Ireland Galway University Road Galway
Phase of development	Phase IIb Trial
Objectives	Primary Objective: 1. The primary objective is to investigate the efficacy and safety of IV Scopolamine, compared to placebo, in reducing severity of depression in individuals with bipolar disorder who are experiencing a depressive episode of at least moderate severity. Secondary objectives: 1. To investigate if IV Scopolamine compared to placebo improves mood, cognition and functioning as measured on a number of objective and subjective psychometric instruments in individuals with bipolar disorder experiencing a depressive episode of at least moderate severity. 2. To investigate the safety and tolerability of repeated (x3) IV Scopolamine versus placebo in individuals with bipolar disorder experiencing a depressive episode of at least moderate severity.
Trial design	This is a single-site, randomised, double-blind, placebo-controlled, parallel, phase IIb clinical trial. The trial will be sponsored by the National University of Ireland (NUI) Galway and the sponsorship role coordinated by the HRB-Clinical Research facility Galway (CRFG). The site will be University Hospital Galway, Galway and the site activities will also be coordinated by the CRFG. Participants will be attending the Galway-Roscommon Mental Health Services, including a specialised bipolar clinic in Galway and referred due to experiencing a depressive episode by their treating clinical team; or patients who have previously attended NUI Galway/HRB-CRFG with an interest to participate/or have participated in research and have indicated a willingness to engage in future research; or patients who approach the research group directly requesting participation. Once participants have consented to the trial they will be reviewed for eligibility, with inclusion and exclusion criteria at the screening visit (Visit 1).

	At the next visit, Visit 2, all participants will receive placebo run-in (100mls of Saline IV). The maximum time between screening (Visit 1) and Visit 2 is 14 days. The screening visit and Visit 2 can be completed on the same day. Within 7 days of Visit 2, participants will be assessed against the placebo run-in criteria and if these criteria are met they will be randomised (visit 3) to receive either placebo (n=25) or 4 µg/kg Scopolamine (n=25) IV in 100mls of Saline over 15 minutes at • Visit 3 (Day 0), • Visit 4 (days 2-6) and • Visit 5 (days 6-10), At least two days will elapse between IV infusions (Visits 2-5). Two follow-up visits (Visits 6 and 7) will occur at days 15 (+/- 5 days) and days 29 (+/- 7 days). At least two days will elapse between Visits 5 and 6.
Key inclusion criteria	To be eligible each participant must meet all of the following eligibility criteria at Screening (Visit 1) and must continue to fulfil these criteria at Visit 2 to take part in the trial: 1. Diagnosis of Bipolar Disorder according to Diagnostic Statistics Manual (DSM)-V criteria 2. Experiencing an episode of depression of at least moderate severity at Visit 1 (Screening) and Visit 2 based on clinical interview by a trained clinician and a Hamilton Depression Rating Scale (HDRS) score ≥ 14. 3. ≥ 18 years old at Visit 2 (male or female) 4. In the opinion of the Principal Investigator or Sub Investigator's, be able and willing to provide written informed consent and to comply with the requirements of this study protocol. 5. Written informed consent prior to participating in the study 6. U&Es, LFTs and TFTs laboratory tests within acceptable ranges in the previous 4 months of the Screening Visit (Visit 1). Placebo run-in inclusion criteria at **Randomisation visit (Visit 3):** 7. In addition to above participants must be experiencing an episode of depression of at least mild severity (having previously experienced an episode of moderate depression at Visit 2 with HDRS ≥ 14), based on clinical interview by a trained clinician and a HDRS score of ≥ 8.

Key exclusion criteria	Participants who meet <u>any</u> one or more of the following exclusion criteria at Screening (Visit 1) or Visit 2 will not be eligible to take part in the trial: 1. History of other Axis I diagnosis (including Recurrent Depressive Disorder or Psychotic Disorders such as schizo-affective disorder, conditions that can also present with depressive episodes) 2. History in the three months prior to Visit 2 of alcohol dependence syndrome or substance dependence syndrome. 3. Current use of oral steroid at Visit 1 4. A confirmed diagnosis of dementia 5. A diagnosis of intellectual disability (IQ < 70) 6. Participants with bipolar disorder that are euthymic in the investigator's opinion, at screening or Visit 2. 7. Participants with bipolar disorder that are hypomanic or manic (Young Mania Rating Scale (YMRS) > 6) at screening or Visit 2. 8. Presence of an established neurological disorder or other serious demyelinating conditions as determined by the treating physician (e.g. space occupying lesion, multiple sclerosis) 9. Current involuntary detention under the Mental Health Act (MHA) 2001 in an acute psychiatric inpatient unit 10. Severity of Bipolar Disorder is such that participation in a clinical trial is not appropriate because of the risk of imminent self-harm (based on clinical note review and review at screening visit by experienced clinician) 11. A history of an allergic reaction or sensitivity to Scopolamine (Hyoscine Hydrobromide). Participants will be asked at the screening visit about any previous treatment with scopolamine (Hyoscine Hydrobromide) to ascertain any previous allergic reaction or sensitivity to this agent. 12. A clinical diagnosis of narrow angle glaucoma, myasthenia gravis, paralytic ileus, pyloric stenosis, toxic megacolon and acute porphyria. 13. Individuals will be excluded from the study if currently prescribed anticholinergic medications including Physostigmine, Biperiden and Procyclidine. Individuals will additionally be excluded if currently prescribed Tricyclic Antidepressants which are associated with significant anticholinergic properties (e.g. Amitriptyline and Nortriptyline) that are currently causing the participant to experience anticholinergic side effects (e.g. blurred vision, constipation, urinary retention, cognitive difficulties). No individuals will have anticholinergic medications stopped to allow them enter the trial. 14. Bradycardia < 50 bpm, tachycardia > 100bpm or hypotension (systolic BP <90 and / or diastolic BP < 60) prior to IV administration of placebo or Scopolamine

	15. A recent history in the last 6 months of symptomatic orthostatic hypotension or syncope. 16. Previous participation in this trial. Participation is defined as randomised. Participation in another trial within 3 months prior to Visit 1. Receipt of any investigational medicinal product (IMP) within 3 months prior to Visit 1. 17. Participants concurrently being administered Electroconvulsive Therapy (ECT). 18. Pregnancy, as determined by a positive urine dipstick at Visits 2, 3, 4, 5, positive blood serum result executed at Visit 2 and confirmed prior to infusion at Visit 3 or participants who are actively breastfeeding (female only). 19. Women of child-bearing potential are defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during dosing of study treatment. Highly effective contraception methods include: • Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. • Male partner sterilization • Combination of any two of the following: a. Barrier methods of contraception e.g. Condom b. Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception c. Placement of an intrauterine device (IUD) or intrauterine system (IUS) • Women who are considered post-menopausal i.e. amenorrhea at least 12 months or undergone hysterectomy/bilateral oophorectomy 20. Any disorder, unwillingness or inability, not covered by any of the other exclusion criteria, which in the investigator's opinion, might jeopardise the participant's safety or compliance with the protocol Placebo run-in exclusion criteria at **Randomisation visit (Visit 3):** 21. In addition to having completed Visit 2, participants must not be experiencing a hypomanic, or manic episode (YMRS >6). 22. A Serious Adverse Event (SAE) experienced during infusion which required medical intervention and whereby attending physician deemed it inappropriate for the participant to engage in future infusions.
Number of participants	The total sample size of patients randomised is 50 participants, with 25 individuals in each arm.
Test Drug Dose Mode of administration	Scopolamine (Hyoscine Hydrobromide) 4µg/kg in 100ml Saline 15 minutes IV infusion

Placebo Dose Mode of administration	Saline (Sodium Chloride 0.9%) 100 ml 15 minutes IV infusion
Duration of treatment	Run-in period will be a maximum 21 days. The treatment period will be a maximum 10 days and the maximum number of days for follow up is 28 days
Primary endpoint	The primary endpoint will be the change in severity of objective depressive symptoms as measured by change in HDRS score from pre-randomisation (pre-IV infusion at Visit 3) compared to Visit 6.
Secondary endpoints	These include change in severity of depressive symptoms at study Visits 3, 4, 5, 6 and 7 and changes in cognitive scores at Visit 6
Statistical methods	The primary analysis of the primary outcome will estimate the difference in change in HDRS score between groups from pre-randomisation (pre-IV on Visit 3) to Visit 6 (with treating scores as continuous variables). Secondary outcomes will be analysed as appropriate to the distribution of each outcome, comparing the difference between groups at the specified follow-up visits. Pre-randomisation variables predictive of each outcome; and stratifying variables will be included in ANCOVA or generalised linear models of outcome variables. Inverse probability weighting will be used to account for missing outcome data and loss to follow-up at a given follow-up visit.
Sample size	The primary outcome in this study is the Hamilton Depression Rating Scale score (HDRS). A decrease of 50% or more in the HDRS score between randomisation and Visit 6 is considered clinically meaningful, and sufficient to consider a patient to have experienced a response. We anticipate that the average HDRS score in the proposed population pre-randomisation will be 24 units and that this outcome variable will be approximately normal. Based on a review of the literature, it is estimated that a range of plausible values for the standard deviation is between 5 to 13 units. Using this information, a sample of 44 participants (22 in each group) will have 85% power to detect a difference of 12 units in mean score change between the treatment and control arms, i.e. an expected HDRS mean score of 12 in the treatment group vs. an expected HDRS mean score of 24 in the control group after Visit 6. The sample size is based on a 2-sample t-test (two-sided) with a standard deviation of 13 units (conservative approach) and a significant level of 0.05. Allocating 25 participants to each arm will ensure power >=85% when loss to follow-up is <12% (6 participants missing at Visit 6 follow up).

5. Abbreviations

Ach	Acetylcholine
AE	Adverse event
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APA	American Psychiatric Association
AR	Adverse reaction
AUDIT	Alcohol Use Disorder Identification Test
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BP	Blood Pressure
BPD	Bipolar Disorder
bpm	beats per minute
CA	Competent authority
CANTAB	Cambridge Neuropsychological Test Automated Battery
CDMS	Clinical Data Management System
CGI-I	Clinical Global Impression-Improvement
CGI-S	Clinical Global Impression-Severity
CHRMs	Muscarinic cholinergic receptors
CI	Chief investigator/Co-ordinating investigator
CRA	Clinical research associate
CRF	Case report form
CRO	Contract research organisation
CT	Clinical trial
CTA	Clinical trial authorisation
DMF	Data Management File
DMP	Data Management Plan
DSM	Diagnostic Statistics Manual
DSMB	Drug Safety Monitoring Board
ECG	Electrocardiogram
ECT	Electroconvulsive Therapy
EU	European Union
GAF	Global Assessment of Functioning
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GP	General Practitioner
HDRS	Hamilton Depression Rating Scale
HPRA	Health Products Regulatory Authority
HR	Heart Rate
HRB-CRFG	Health Research Board - Clinical Research Facility Galway
HSE	Health Service Executive
ICF	Informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IL	Interleukin
IMP	Investigational medicinal products
IMPD	Investigational medicinal product dossier
IQ	Intelligence Quotient
IV	Intravenous
LFTs	Liver Function Tests
MADRS	Montgomery and Asberg Depression Scale
MDD	Major Depressive Disorder
MEHI	Modified Edinburgh Handedness Inventory
MHA	Mental Health Act

mTOR	mammalian Target of Rapamycin
NCHD	Non-Consultant Hospital Doctor
NEO PI-FFI	NEO Personality Inventory-Five Factor Inventory
NMDA	N-methyl-D-aspartate
NUI Galway	National University of Ireland Galway
PAL	Paired Associates Learning
PI	Principal Investigator
PIL	Patient/participant information leaflet
POMS	Profile of Mood States
PRISE	Patient Rated Inventory of Side Effects
REC	Research Ethics Committee
ROI	Republic of Ireland
RR	Respiratory Rate
SAE	Serious adverse event
SAR	Serious adverse reaction
SCID	Structured Clinical Interview for DSM
SmPC	Summary of product characteristics
SMRI	Stanley Medical Research Institute
SOC	System Organ Class
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TFTs	Thyroid Function Tests
TNF	Tumor Necrosis Factor
TSC	Trial Steering Committee
t1/2	Half-life
U&Es	Urea and Electrolytes
VAS	Visual Analogue Scale
WAIS	Wechsler Adult Intelligence Scale
WHO	World Health Organisation
YMRS	Young Mania Rating Scale

6. Introduction

6.1 Background information

6.1.1 What is Bipolar disorder?

Bipolar disorder is a chronic disabling psychiatric disorder characterized by recurrent episodes of mania or hypomania and depression. Bipolar disorder can be separated into bipolar 1 and bipolar 2 disorders with bipolar 1 disorder characterizing individuals who have episodes of mania and depression, and bipolar 2 disorder characterizing individuals who have episodes of depression with periods of hypomania, but not mania (DSM-IV; APA, 1995). Bipolar disorder has an estimated prevalence of approximately 1% and a roughly equal gender ratio (Belmaker, 2004). Bipolar disorder is one of the top 20 leading causes of disability worldwide, with a similar disability adjusted life year rate (138.3 per 100,000) to more prevalent conditions such as asthma (147.9 per 100,000) and Alzheimer's disease (108.5 per 100,000) (Ferrari et al., 2016).

Current pharmacological treatments for bipolar disorder and in particular for the management of depressive episodes in bipolar disorder remain sub-optimal, with pharmacological strategies employed to date often only partially effective (Fournier et al., 2010; Yildiz et al., 2011). In addition to the time duration for treatment response, multiple treatment trials are also often required, thus increasing patient discomfort and distress (Insel and Wang, 2009). Consequently, pharmacological mechanisms that potentially alleviate depressive symptomatology in bipolar disorder and ameliorate patient functioning could present an additional pharmacological strategy for the management of bipolar disorder. A number of recent studies have suggested that Scopolamine, a pan muscarinic (M) receptor antagonist can elicit a rapid anti-depressant response in both major depressive disorder (MDD) and bipolar disorder (Ellis et al., 2014; Furey et al., 2006; Drevets et al., 2010) and thus may present a novel therapeutic strategy, particularly for the management of bipolar disorder, in individuals experiencing depressive episodes (Janowsky, 2011). No significant adverse effects were noted with treatment with Scopolamine (intravenously) in the above-named studies.

6.1.2 Scopolamine trials and putative antidepressant effect

A number of double-blind placebo-controlled trials of Intravenous (IV) Scopolamine, have been associated with a rapid-acting anti-depressant effect in MDD or bipolar disorder (Drevets and Furey, 2006; Furey and Drevets, 2010; Furey et al., 2010; Ellis et al., 2014), but other trials have not reported a significant treatment effect (Newhouse et al., 1988; Gillin et al., 1991) (See Table 1). Of note, those clinical trials where efficacy has been demonstrated have been undertaken in the same centre (Mood and Anxieties Disorder Program at the National Institute of Mental Health in Bethesda, MD) with the more recent trials of larger numbers from that centre including individuals that participated in the previous clinical trials. Although beneficial effects have been noted for depressive episodes in both MDD and bipolar disorder, the actual numbers of participants with bipolar disorder has been limited with only 14 participants experiencing a depressive episode included in these studies described to date (Ellis et al., 2014; Furey et al., 2010; Furey and Drevets, 2006). Fewer studies have utilised other potential modes of administration for Scopolamine. A previous study of intramuscular scopolamine demonstrated no antidepressant effect (Gillin et al., 1991), whilst one trial of oral scopolamine as an augmentation agent demonstrated an antidepressant effect, albeit this study did not demonstrate a rapid-acting effect as demonstrated in the studies of IV Scopolamine and did not include individuals with bipolar disorder (Khajavi et al., 2012). In addition, oral Scopolamine has very limited bioavailability of ~4 %).Trials relating to administration of Scopolamine utilising a transdermal patch for the management of depressive episodes have yet to be published.

Compared to clinical studies, few non-clinical animal model studies have been conducted. However, both mouse and rat models (Helpless Mouse and Flinders Sensitive Rat) that demonstrate behavioural phenotypes consistent with a model of depression have noted associations with reduced activity with cholinergic agonism (Daws & Overstreet, 1999; Popa et al., 2006).

Thus, a putative effect of scopolamine is suggested from animal studies and clinical trials in humans, with a suggestion that the effect was most evident with IV administration of scopolamine. However, studies of IV scopolamine as a treatment agent for depressive episodes to date have had limited numbers of participants and have included heterogeneous populations. This study will be the first to exclusively examine depressive episodes in bipolar disorder to ascertain if a rapid antidepressant effect is demonstrated.

6.1.3 Population, including sample size and power calculation

Trial participants will be adults (both male and female) who have bipolar disorder and are experiencing a depressive episode of at least moderate severity.

A diagnosis of bipolar disorder (bipolar 1 or 2 disorder) will be determined by interview with the Structured Clinical Interview for DSM-V (SCID-RV) with severity of the depressive episode assessed using the Hamilton Depression Rating Scale (HDRS) (HDRS ≥14 for study inclusion) at screening. Participants will all be required to provide informed consent and will fulfil the inclusion and exclusion criteria as detailed in Section 8.2.2 and section 8.2.3.

Participants will be attending the Galway-Roscommon Mental Health Services for the management of bipolar disorder or will be participants who have previously engaged in research into bipolar disorder at NUI Galway and expressed a preference to be contacted again in relation to future research projects.

The primary outcome in this study is the change in severity of depressive symptoms as measured on the Hamilton Depression Rating Scale score (HDRS). Allocating 25 participants to each arm will ensure power ≥85% to detect a 50% lower score on the Hamilton Depression Rating Scale score (HDRS) in the Scopolamine group compared to placebo group. This assumes a loss to follow-up of ≤12%. It is also anticipated that approximately 60 participants will need to be recruited to ensure 50 participants are eligible for randomisation after a placebo run-in. Further details of this sample size calculation are given in section 11.2.

Table 1. Scopolamine trials for depressive episodes in BPD and MDD

Author	Agent and Diagnosis	N	Assessment	Trial findings
Newhouse et al., 1988	0.1, 0.25, 0.5mg IV, placebo, 1mg lorazepam — given 2 days apart Cross-over study Baseline HDRS = 29 (SD = 7)	9 = MDD	BDI, VAS, POMS	No effect of any dose of Scopolamine on depressive symptoms
Poland et al., 1989	4.5mcg/kg IV x2 given one week apart (at night-time)	14 = MDD 16 = past MDE (now asymptomatic) 18 = HC	EEG	Mood effects not measured Scopolamine increased REM sleep latency, reduced REM density and activity in all groups
Gillin et al., 1991	0.4mg IM	10 = MDD 10 = HC	MADRS	No effect on depressive symptoms

Author	Agent and Diagnosis	N	Assessment	Trial findings
Furey and Drevets , 2006	Depressive Episode: MADRS mean = 32 (SD = 6) Unmedicated > 3 weeks 7 IV sessions 3-5 days apart Single-blind lead in 4ug/kg IV or placebo x 3 Cross-over design Interview 3-5 days post last session Sessions every 3-5 days Sessions every 3-5 days	9 = MDD 9 = BPD	MADRS, HARS, YMRS, CGI Self-Report VAS and POMS during session 20, 60, 120, 150 minutes	56% remission rate, 61% response rate Effect persisted for 2/52 No elation BPAD, MDD groups — similar response rates
Drevets and Furey, 2010*	Depressive Episode Unmedicated > 3 weeks 7 IV sessions 3-5 days apart Single-blind lead in 4ug/kg IV or placebo x 3 Cross-over design Interview 3-5 days post last session Sessions every 3-5 days	22 = MDD	MADRS, HARS, YMRS, CGI Self-Report VAS and POMS during session 20, 60, 120, 150 minutes	50% remission and 64% response Antidepressant effect within 3-4 days of Scopolamine administration
Furey et al., 2010*	Depressive Episode: MADRS BPAD baseline =30, SD=9, MDD = 30, SD = 8) Female = 31, Male =21 Unmedicated > 3 weeks 7 IV sessions 3-5 days apart Single-blind lead in 4ug/kg IV or placebo x 3 Cross-over design Interview 3-5 days post last session Sessions every 3-5 days	38 = MDD 14 = BPD	MADRS, HARS, YMRS, CGI Self-Report VAS and POMS during session 20, 60, 120, 150 minutes	58% remission and 71% response in females, 28% remission and 42% response in males No effect of diagnosis (BD v MDD)
Khajavi et al., 2012	1mg oral x 6 weeks Patients treated with citalopram	40 = MDD	HDRS	Antidepressant effect (65% remission and response rate with Scopolamine compared to 30% and 20% in placebo group) No improvement in speed of onset of antidepressant effect

Author	Agent and Diagnosis	N	Assessment	Trial findings
Ellis et al., 2014**	Treatment naïve (MDD = 31; BPD = 4) (MADRS 31, SD = 5), Treatment resistant (MDD = 31; BPAD = 9) (MADRS = 33, SD = 4). 7 IV sessions 3-5 days apart Single-blind lead in 0.4mcg IV or placebo x 3 cross-over Interview 3-5 days post last session Sessions every 3-5 days Unmedicated for > 2 weeks	62 = MDD 13 = BPD (1 = BPD1)	MADRS	Both treatment naïve(58% response, 29% remission) and treatment resistant (42% response, 19% remission)

BDI = Beck Depression Inventory; BPD = Bipolar Disorder; EEG = Electroencephalogram; HARS = Hamilton Anxiety Rating Scale; HDRS = Hamilton Depression Rating Scale; MADRS = Montgomery and Asberg Depression Rating Scale; MDD = Major Depressive Disorder; MDE = Major Depressive Episode; POMS = Profile of Mood States; REM = Rapid Eye movement; SD = Standard Deviation; VAS = Visual Analogue Scale

* Included participants from Furey & Drevets (2006)
** Included participants from Furey & Drevets (2006), Furey et al., (2010) and Drevets & Furey (2010)

6.2 Rationale for the study

6.2.1 Muscarinic Cholinergic Receptors (CHRMs)

Muscarinic cholinergic receptors (CHRMs) are a family of seven-transmembrane domain receptors consisting of five receptor subtypes (M1-5) and are associated with heterotrimeric G-proteins to translate a transduction cascade (Bonner et al., 1987, Hulme et al., 1990). M1 and 3 receptors act as postsynaptic receptors in the central nervous system (CNS), where they are mainly localized in the cortex and hippocampus and play an important role in cognitive function (Porter et al., 2002). M2 and M4 receptors have been demonstrated to act as presynaptic autoreceptors in the cerebral cortex (Lai et al., 2001), basal ganglia and hippocampus (Lai et al., 2001; Dean et al., 2000; Scarr et al., 2007), having an inhibitory effect on the release of acetylcholine (Ach). M5 receptors are predominantly distributed in the hippocampus and substantia nigra and have been demonstrated to have a role in the modulation of dopamine release (Vilaro et al., 1990; Weiner et al., 1990).

6.2.2 Cholinergic System and Mood Disorders

Despite the paucity of human studies targeting the cholinergic system in mood disorders, this system has long been implicated as a contributory factor in their aetiology. In 1950, the organophosphorus insecticide disopropyl-fluorophosphate (DFP), a cholinesterase inhibitor (increasing synaptic ACh) was demonstrated to have a depressive effect on healthy controls and individuals with mania (Rowntree et al., 1950), with these findings also noted by other authors (Gershon and Shaw, 1961). Physostigmine, a cholinesterase inhibitor (and thus an opposite effect to Scopolamine) has been demonstrated to exacerbate depressive symptomatology in individuals with bipolar disorder (Davis et al., 1978; Janowsky et al., 1972). These, and other observations, have led to the hypothesis that an imbalance between central

cholinergic and adrenergic neurotransmitter activity, released from the cholinergic nerves in the parasympathetic, and adrenergic nerves in the sympathetic systems could induce mania and depression (Janowsky et al., 1972). Thus, over-activity and oversensitivity of the cholinergic system has been associated with depressive symptomatology, with a reduction in M2 receptors demonstrated *in vivo* during depression (Cannon et al., 2006), partially explained by variation in the M2 gene (Cannon et al., 2011). M2 antagonism by Scopolamine (a cholinergic antagonist) may potentially regulate activity of this system and therefore reduce the oversensitivity associated with depression.

6.2.3 Other proposed mechanisms for Scopolamine's putative antidepressant effect

In addition to modulation of the cholinergic system, several other potential mechanisms have been considered to explain the observed antidepressant effect of Scopolamine. This is likely given the persistence of the antidepressant effect beyond the plasma half-life of Scopolamine (t1/2 = ~ 3 hours) (Drevets and Furey, 2010). These include modulation of N-methyl-D-aspartate (NMDA) receptor activity, with increased NMDA receptor gene expression and reduced mRNA concentrations of NMDA receptors 1A and 2A in rodents' brain (Liu et al., 2004) resulting in reduced neuronal damage in the hippocampus secondary to glutamate medicated neurotoxicity (Rami et al., 1997). Further evidence for modulation of the glutamatergic system is derived from the enhancement of extracellular glutamate concentrations in the striatum in rodents with Scopolamine administration (Rawls and McGinty, 1988) which may additionally activate synaptic plastic pathways. Activation of synaptic plasticity has been proposed as an underlying biological effect for "scopolamine's rapid antidepressant activity, with scopolamine rapidly increasing mammalian target of rapamycin (mTOR) signalling and synaptogenesis (Voleti et al., 2013), with pre-treatment with mTOR and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonists in animal trials (Voleti et al., 2013) blocking "Scopolamine's antidepressant effects. The mTOR pathway has similarly been implicated in the rapid antidepressant effects of ketamine (Dwyer et al., 2012).

6.2.4 Scopolamine administration in study

At Visit 2, all participants will receive placebo run-in (100mls of Saline IV) (see section 8.1.3 for rationale). The screening visit (Visit 1) and Visit 2 may occur on the same day. Within 7 days of Visit 2, participants will be randomised (Visit 3) to receive either placebo (n=25) or 4 µg/kg Scopolamine (n=25) IV in 100mls of Saline over 15 minutes at Visits 3 (day 0), 4 (days 2-6) and 5 (days 6-10), with at least 2 days between IV infusions (Visits 2, 3, 4 and 5). Two follow-up visits (Visits 6 and 7) will occur at day 15 (+/-5 days) and day 29 (+/- 7 days).There will be at least 2 days between Visits 5 and 6 and 3 days between Visits 6 and 7.

7. Study Objectives

7.1 Primary Objective

The primary objective is to investigate the efficacy and safety of IV Scopolamine, compared to placebo, in reducing severity of depression in individuals with bipolar disorder who are experiencing a depressive episode of at least moderate severity.

7.2 Secondary Objectives

There are a number of secondary objectives.

1. To investigate if IV Scopolamine compared to placebo improves mood, cognition and functioning as measured on a number of objective and subjective psychometric instruments in individuals with bipolar disorder experiencing a depressive episode of at least moderate severity.

2. To investigate the safety and tolerability of repeated (x3) IV Scopolamine versus placebo in individuals with bipolar disorder experiencing a depressive episode of at least moderate severity.

7.3 Primary and Secondary/exploaratory endpoints/outcome measures

7.3.1 Primary Endpoint

The primary endpoint will be the change in severity of objective depressive symptoms as measured by change in HDRS score from pre-randomisation (pre-IV infusion on Visit 3) compared to Visit 6.

7.3.2 Secondary Endpoints

1. Remission of depressive episode measured with the HDRS at Visit 6 compared to Visit 3.
2. Response to a depressive episode measured with the HDRS at Visit 6 compared to Visit 3.
3. Reduction of depressive symptoms (MADRS) at Visit 6 compared to Visit 3.
4. Improvement in objectively measured overall functioning (GAF) at Visit 6 compared to Visit 3.
5. Reduction in depressive symptoms as measured using the POMS after all IV infusion visits compared to before IV infusions at the same visit (Visits 2, 3, 4, 5).
6. Response and remission data measured with the HDRS at Visit 7 compared to pre-randomisation HDRS scores at Visit 3.
7. Reduction in subjectively measured mood symptoms using the POMS at follow-up visits (Visits 6 and 7) compared to pre-randomisation.
8. Reduction in depressive symptoms, measured with the MADRS at Visit 7 compared to MADRS scores at Visit 3.
9. Improvement in global functioning, measured with the GAF at Visit 7 compared to pre-infusion GAF scores at Visit 3.
10. Psychiatric inpatient admissions due to depressive episodes over the duration of the study.
11. Antidepressant medication usage or change in antidepressant medication dose due to depressive episodes over the duration of the study.
12. Adverse events over the duration of the study.
13. Post infusion side effects at all 3 IV treatment visits: Bradycardia, Hypotension, Dizziness and Sedation (Visits 3, 4 and 5)
14. Occurrence of a hypo (manic) episode over the duration of the study.
15. Improvement in motor processing, visual and spatial memory at Visit 6 compared to initial scores measured at Visit 2 or Visit 3 as measured on CANTAB.

8. Trial Design

8.1 Design Summary

This is a single-site, randomised, double-blind, placebo-controlled, parallel, phase IIb clinical trial. The trial will be sponsored by the National University of Ireland (NUI) Galway and the sponsorship role coordinated by the HRB-Clinical Research facility Galway (CRFG).

The site will be University Hospital Galway, Galway and the site activities will also be coordinated by the CRFG. Participants will be attending the Galway-Roscommon Mental Health Services, including a specialised bipolar clinic in Galway and referred due to experiencing a depressive episode by their treating clinical team; or patients who have previously attended NUI Galway/HRB-CRFG with an interest to participate/or have

participated in research and have indicated a willingness to engage in future research; or patients who approach the research group directly requesting participation.

Once participants have consented to the trial they will be reviewed against the inclusion and exclusion criteria at the screening visit (Visit 1). Eligible participants will be randomised to one of two groups; treatment group (n ~ 25) or placebo group (n ~ 25). Participants will receive Scopolamine or placebo in addition to their current treatment regimen.

At the next visit, Visit 2, all participants will receive placebo run-in (100mls of Saline IV). This has been utilised in other IV Scopolamine treatment studies (Furey & Drevets, 2006; Drevets and Furey, 2010; Furey et al., 2010; Ellis et al., 2014) and has the advantage of reducing the subsequent placebo effect (when receiving active intervention) noted between groups and reducing the loss to follow-up rate post-randomisation (Davis et al., 1995; Rosenkranz, 2016).

The maximum time between screening (Visit 1) and Visit 2 is 14 days. The screening visit and Visit 2 can occur on the same day and thus some data attained on the 'screening visit' will not require replication on the same day including a review of inclusion and exclusion criteria, the Hamilton Depression Rating Scale (HDRS) and the Young Mania Rating Scale (YMRS) and pre-infusion vital signs.

Within 7 days of Visit 2, participants will be assessed against the placebo run-in criteria and if these criteria are met they will be randomised (visit 3) to receive either placebo (n=25) or 4 µg/kg Scopolamine (n=25) IV in 100mls of Saline over 15 minutes at:

- Visit 3 (Day 0),
- Visit 4 (days 2-6) and
- Visit 5 (days 6-10),

At least two days will elapse between IV infusions (Visits 2-5).

Two follow-up visits (Visits 6 and 7) will occur at days 15 (+/-5 days) and days 29 (+/- 7 days).

At least two days will elapse between Visits 5 and 6.

8.1.1 Treatment Group

Participants randomised to the scopolamine group will receive one 15-minute infusion of IV Scopolamine in 100ml saline, repeated over 3 visits (Visit 3, 4 & 5).

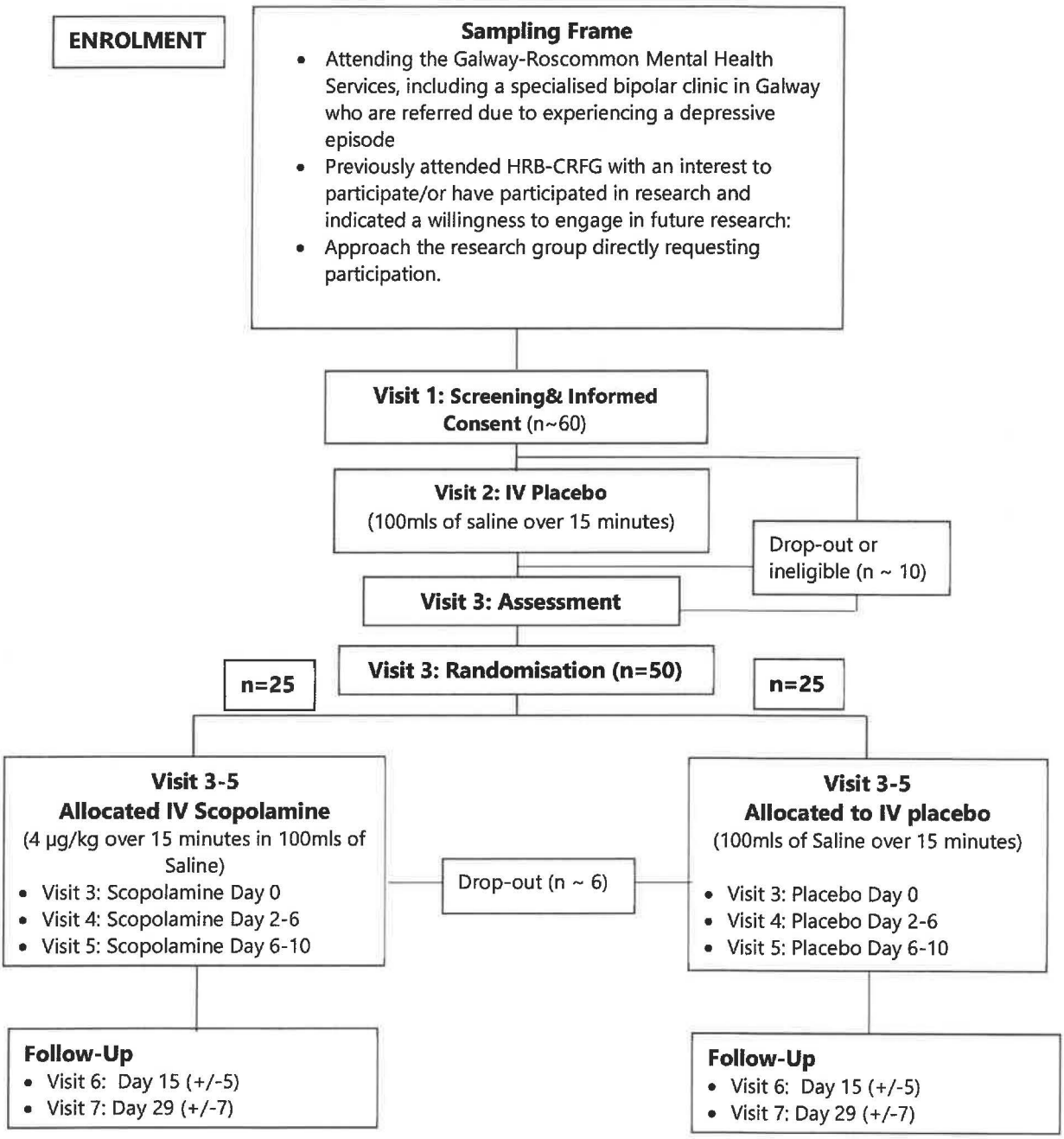
8.1.2 Placebo Group

Participants randomised to the placebo group will receive one 15-minute infusion of IV saline, repeated over 3 visits (Visit 3, 4 & 5).

8.1.3 Placebo run-in

All participants will receive placebo at Visit 2, known as a placebo run-in. In addition, at Visit 3 pre-randomisation, participants must ensure they continue to fulfil placebo run-in inclusion criteria.

Figure 1: CONSORT Trial Flow Diagram: A Randomised Double-Blind Placebo-Controlled Trial of Scopolamine in Bipolar Disorder



Note timeline between visits:
Visit 1 and 2: ≤14 days. Can also occur on the same day and thus data does from screening visit does not need to be replicated at Visit 2.
Visit 2 and 3: ≥2 days and ≤7 days
Visit 3 and Visit 4 and Visit 5 and Visit 6: ≥2 days
Visit 6 and Visit 7: ≥3 days

8.2 Selection of study population

8.2.1 Population

There will be 50 adult trial participants (both male and female) who have bipolar disorder (bipolar disorder 1 or 2) and are experiencing a depressive episode of at least moderate severity as measured by a HDRS of ≥ 14 at Visit 2; with 25 participants randomised to receive Scopolamine and 25 randomised to receive placebo. It is anticipated that approximately 60 individuals will be recruited into the study at Visit 2, and that approximately 10 individuals will withdraw prior to study randomisation. Thus, the target for recruitment is approximately 60 patients with 50 patients to be randomised into the trial.

8.2.2 Inclusion criteria

To be eligible for inclusion, each participant must meet all of the following inclusion criteria at Screening (Visit 1) and must continue to fulfil these criteria at Visit 2 to take part in the trial:

1. Diagnosis of Bipolar Disorder according to Diagnostic Statistics Manual (DSM)-V criteria
2. Experiencing an episode of depression of at least moderate severity at Visit 1 (Screening) and Visit 2 based on clinical interview by a trained clinician and a Hamilton Depression Rating Scale (HDRS) score ≥ 14.
3. ≥ 18 years old at Visit 2 (male or female)
4. In the opinion of the Principal Investigator or Sub Investigator's, be able and willing to provide written informed consent and to comply with the requirements of this study protocol.
5. Written informed consent prior to participating in the study
6. U&Es, LFTs and TFTs laboratory tests within acceptable ranges in the previous 4 months of the Screening Visit (Visit 1).

Placebo run-in inclusion criteria **at Randomisation visit (Visit 3):**

7. In addition to above, participants must be experiencing an episode of depression of at least mild severity (having previously experienced an episode of moderate depression at Visit 2 with HDRS ≥14), based on clinical interview by a trained clinician and a HDRS score of ≥ 8.

8.2.3 Exclusion criteria

Participants who meet <u>any</u> one or more of the following exclusion criteria at Screening (Visit 1) or the Visit 2 will not be eligible to take part in the trial:

1. History of other Axis I diagnosis (including Recurrent Depressive Disorder or Psychotic Disorders such as schizo-affective disorder, conditions that can also present with depressive episodes)
2. History in the three months prior to Visit 2 of alcohol dependence syndrome or substance dependence syndrome.
3. Current use of oral steroid at Visit 1
4. A confirmed diagnosis of dementia
5. A diagnosis of intellectual disability (IQ < 70)
6. Participants with bipolar disorder that are euthymic in the investigators opinion at screening or Visit 2.

7. Participants with bipolar disorder that are hypomanic or manic (Young Mania Rating Scale (YMRS) > 6) at screening or Visit 2.
8. Presence of an established neurological disorder or other serious demyelinating conditions as determined by the treating physician (e.g. space occupying lesion, multiple sclerosis)
9. Current involuntary detention under the Mental Health Act (MHA) 2001 in an acute psychiatric inpatient unit
10. Severity of Bipolar Disorder is such that participation in a clinical trial is not appropriate because of the risk of imminent self-harm (based on clinical note review and review at screening visit by experienced clinician)
11. A history of an allergic reaction or sensitivity to Scopolamine (Hyoscine Hydrobromide). Participants will be asked at the screening visit about any previous treatment with scopolamine (Hyoscine Hydrobromide) to ascertain any previous allergic reaction or sensitivity to this agent.
12. A clinical diagnosis of narrow angle glaucoma, myasthenia gravis, paralytic ileus, pyloric stenosis, toxic megacolon and acute porphyria.
13. Individuals will be excluded from the study if currently prescribed anticholinergic medications, including Physostigmine, Biperiden and Procyclidine. Individuals will additionally be excluded if currently prescribed Tricyclic Antidepressants which are associated with significant anticholinergic properties (e.g. Amitriptyline and Nortriptyline) that are currently causing the participant to experience anticholinergic side effects (e.g. blurred vision, constipation, urinary retention, cognitive difficulties). No individuals will have anticholinergic medications stopped to allow them enter the trial.
14. Bradycardia < 50 bpm, tachycardia > 100bpm or hypotension (systolic BP <90 and / or diastolic BP < 60) prior to IV administration of placebo or Scopolamine
15. A recent history in the last 6 months of symptomatic orthostatic hypotension or syncope.
16. Previous participation in this trial. Participation is defined as randomised. Participation in another trial within 3 months prior to Visit 1. Receipt of any investigational medicinal product (IMP) within 3 months prior to Visit 1.
17. Participants concurrently being administered Electroconvulsive Therapy (ECT).
18. Pregnancy, as determined by a positive urine dipstick at Visits 2, 3, 4, 5, positive blood serum result executed at Visit 2 and confirmed prior to infusion at Visit 3 or participants who are actively breastfeeding (female only).
19. Women of child-bearing potential are defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during dosing of study treatment. Highly effective contraception methods include:
    - Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment.
    - Male partner sterilization
    - Combination of any two of the following:
        a. Barrier methods of contraception e.g. Condom
        b. Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception
        c. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
    - Women who are considered post-menopausal i.e. amenorrhea at least 12 months or undergone hysterectomy/bilateral oophorectomy
20. Any disorder, unwillingness or inability, not covered by any of the other exclusion criteria, which in the investigator's opinion, might jeopardise the participant's safety or compliance with the protocol

Placebo run-in exclusion criteria at **Randomisation visit (Visit 3):**

21. In addition to having completed Visit 2, participants must not be experiencing a hypomanic, or manic episode (YMRS >6).
22. A Serious Adverse Event (SAE) experienced during infusion which required medical intervention and whereby attending physician deemed it inappropriate for the participant to engage in future infusions.

8.3 Recruitment, Study visits and procedures

8.3.1 Treatment duration

Run-in period will be a maximum 21 days. The treatment period will be a maximum 10 days and the maximum number of days for follow up is 28 days

8.3.2 Identification and Recruitment

Potential participants for this study will be identified as follows:

1. Patients currently attending the Galway-Roscommon Mental Health Services, including a specialised bipolar clinic in Galway who are referred due to experiencing a depressive episode by their treating clinical team; or,
   Or,
2. Patients who have previously attended NUI Galway/HRB-CRFG with an interest to participate/or have participated in research and indicated a willingness to engage in future research:
   Or,
3. Patients who approach the research group directly requesting participation.

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

8.3.3 Screening Assessments (Visit 1)

- Informed consent
- Review of inclusion/exclusion criteria
- Demographic Data (Gender, Age, Race)
- Handedness MEHI questionnaire
- Review of relevant medical and surgical history
- Review clinical history of bipolar disorder
- Recording of current medications
- Vital Signs recorded (BP, HR, RR)
- U&Es, LFTs and TFTs blood tests (if required)
- Completion of SCID-RV for DSM-V Disorders
- Completion of HDRS questionnaire
- Completion of YMRS questionnaire
- AUDIT screening for alcohol use
- Verbal confirmation received from female participants that they are not pregnant.
- Adequate contraception advice given to female and male participants (see 8.4.7)

The maximum amount of time between screening and Visit 2 will be 14 days, inclusive of weekend days and bank holidays. The screening visit and the Visit 2 may occur on the same day and thus case data for screening visit does not need to be replicated for the Visit 2.

8.3.4 Visit 2 Assessments

The following pre-treatment assessments will be performed at Visit 2.

- Confirmation of eligibility (review inclusion/exclusion criteria)
- Confirmation of pregnancy status (pregnancy tests) and contraception advice
- U&Es, LFTs and TFTs blood tests (if required)
- Review of current medications, including changes in medications since Visit 1
- Physical measurements (height and weight)
- Vital Signs recorded (BP, HR, RR) (pre-infusion but post cannulation)
- ECG to be undertaken
- Completion of HDRS (pre-infusion)
- Completion of the YMRS (pre-infusion)
- Completion by participant of the POMS (pre-infusion) (optional)
- Completion of CGI-S (pre-infusion)
- Completion of Fagerstrom (pre-infusion)
- Completion of MADRS questionnaire (pre-infusion)
- Completion by participant of a VAS (pre-infusion)
- Cannulation site check (pre-infusion)
- IV cannulation

The following post-treatment assessments will be performed at Visit 2:

- Cannulation site check (post-infusion)
- 15-minute infusion of IV placebo (100ml saline)
- Completion by participant of the POMS (minimum 15 minutes post infusion) (optional)
- Completion of WAIS (post-infusion)
- Completion of NEO (post-infusion)
- Completion by participant of a VAS (minimum 15 minutes post-infusion)
- Undertake CANTAB tests (post-infusion) (may occur at Visit 3 either)
- Vital Signs recorded (BP, HR, RR) within 15 minutes of when infusion stopped and 50 to 90 minutes post-infusion
- Completion of PRISE questionnaire by participant (post-infusion, optional)
- Assessment for other adverse effects (see section 10.1) (pre, during and post infusion)

8.3.5 Treatment (Visit 3, Visit 4 and Visit 5)

At Visit 3, after assessment, participants will be randomised to placebo (n=25) (100mls IV Saline) or 4 µg/kg Scopolamine (n=25) IV in 100mls of Saline over 15 minutes and receive further infusions at Visits 4 (days 2-6) and 5 (days 6-10), with at least 2 days between IV infusions (see Table 2).

Assessments at Visits 3 prior to randomisation to ensure trial eligibility

- As per inclusion criterion 7 and exclusion criteria 21 and 22.
- HDRS ≥ 8 (indicating depressed mood of at least mild severity
- YMRS <6 (indicating patient is not (hypo)manic)
- Medication review to ensure no participants have been prescribed medications which would exclude the participant from the trial between Visit 2 and Randomisation.
- Confirmation of pregnancy serum result (females only)

Randomisation via Interactive Web Response System (IWRS) (Visit 3 only)
Assessments at Visits 3, 4 and 5 are:

- Review of any medication changes
- Vital Signs recorded (BP, HR, RR) (pre-infusion but post cannulation)

- Dispensing via IWRS (Visit 3, 4, and 5)
- Completion of HDRS questionnaire (Visit 3 (completed as part of eligibility assessment), Visit 4, and 5, pre-infusion)
- Completion of MADRS questionnaire (Visits 3, 4 and 5, pre-infusion)
- Completion of YMRS (Visit 3 (completed as part of eligibility assessment), Visit 4, and 5, pre-infusion)
- Confirmation of pregnancy status (Urine dipstick) and contraception advice (Visit 3, 4 and 5, pre-infusion)
- Completion by participant of VAS (Visit 3, 4, and 5, pre-infusion)
- Completion by participant of the POMS (Visit 3, 4, and 5, pre-infusion) (optional)
- Completion of GAF questionnaire (Visits 3 and 5, pre-infusion)
- Completion of CGI-S (Visit 3, 4 and 5, pre-infusion)
- Completion of CGI-I (Visits3, 4 and 5, pre-infusion)
- IV cannulation

- Cannulation site check (pre-infusion and post-infusion)
- Completion by participant of the POMS (minimum 15 minutes post-infusion (optional)
- Completion by participant of VAS (minimum 15 minutes post-infusion)
- Completion by participant of CANTAB subscales (Visit 3, post-infusion) (optional)
- Completion of WAIS (post infusion at Visit 3,4, or 5) (optional)
- Completion of NEOPI-FFI (post infusion at Visit 3,4, or 5) (optional)
- Vital Signs recorded (BP, HR, RR) (within 15 minutes of infusion stopping and 50 to 90 minutes post-infusion)
- Completion by participant of the PRISE questionnaire (post-infusion at Visit 3, 4 or 5 optional)
- Assessment for other adverse effects (see section 10.1) (pre, during and post infusion)

8.3.6 Follow up visits (Visit 6 and 7)

Visits 6 [15 (+/-5 Days)] and 7 [29(+/-7)] have no treatment component. There will be at least 2 days between Visit 5 and 6 and 3 days between Visits 6 and 7.

Assessments at Visit 6 and 7 are:

- Review of any medication changes
- Completion of HDRS questionnaire
- Completion of YMRS questionnaire
- Completion by participant of the POMS questionnaire (optional)
- Completion of CGI-S
- Completion of CGI-I
- Completion by participant of CANTAB subscales (Visit 6) (optional)
- Completion of MADRS questionnaire
- Completion of GAF questionnaire
- Completion of WAIS (optional)
- Completion of NEOPI-FFI (optional)
- Adverse event assessment (see section 10.1)
- Participant assessment of treatment group (IV Scopolamine or IV Saline) (Visit 7)

Visits 6 and 7 maybe conducted over the telephone if necessary but only in limited circumstance such as participant withdrawn or unwilling /unable to attend clinic for scheduled visit.
An outline of scheduled study assessments and procedures are outlined below in table 2.

Table 2: Schedule of Assessments

Procedures	Visit 1 Screening	Visit 2 PLACEBO IV Infusion			Visit 3 Randomisation Day 0 Scopolamine or Placebo IV Infusion			Visit 4 Day 4 (± 2 Days) Scopolamine or Placebo IV Infusion			Visit 5 Day 8 (± 2 Days) Scopolamine or Placebo IV Infusion			Visit 6 Day 15 (± 5 Days) Follow-Up	Visit 7 Day 29 (± 7 Days) Follow-Up
		Pre	During	Post	Pre	During	Post	Pre	During	Post	Pre	During	Post		
Signed informed consent	X														
Inclusion/Exclusion	X	X			X***										
Randomisation					X										
IWRS					X			X			X				
Demography	X														
MEHI	X														
Medical/Surgical History/History BPD	X														
Current Medication	X	X			X			X			X			X	X
Vital Signs – HR, BP & RR	X	X		X	X		X	X		X	X		X		
SCID-RV	X														
HDRS	X	X			X			X			X			X	X
YMRS	X	X			X			X			X			X	X
AUDIT	X														
Pregnancy Discussion	X	X			X			X			X				
Contraception Advice	X	X			X			X			X				
Serum Pregnancy Test$^{\Delta}$		X													
Pregnancy urine dipstick$^{\Delta}$		X			X			X			X				
U&Es, LFTs, TFTs °	X	X													
Fagerstrom		X													
CGI-S		X			X			X			X			X	X
CGI-I					X			X			X			X	X
VAS		X		X	X		X	X		X	X		X		
MADRS		X			X			X			X			X	X
GAF					X						X			X	X
ECG		X													
Height (cm) & Weight (kg)		X													
IV Cannulation		X			X			X			X				

Procedures	Visit 1 Screening	Visit 2 PLACEBO IV Infusion			Visit 3 Randomisation Day 0 Scopolamine or Placebo IV Infusion			Visit 4 Day 4 (± 2 Days) Scopolamine or Placebo IV Infusion			Visit 5 Day 8 (± 2 Days) Scopolamine or Placebo IV Infusion			Visit 6 Day 15 (± 5 Days) Follow-Up	Visit 7 Day 29 (± 7 Days) Follow-Up
		Pre	During	Post	Pre	During	Post	Pre	During	Post	Pre	During	Post		
Infusion administration			X			X			X			X			
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cannulation Site Check		X		X	X		X	X		X	X		X		
POMS (Optional)		X		X	X		X	X		X	X		X	X	X
CANTAB ** (Optional)				X			X							X	
WAIS * (Optional)				X*			X*			X*			X*	X*	X*
NEO-PI-FFI * (Optional)				X*			X*			X*			X*	X*	X*
PRISE (Optional)				X			X			X			X		
Subjective Assessment															X

Randomisation will only occur at Visit 3 after HDRS is demonstrated to be ≥ 8 and YMRS < 6. These measures will be conducted pre-randomisation (and consequently also pre-infusion).
Pre-infusion vital signs should be taken post cannulation. Post-infusion vital signs within 15 minutes of infusion stopping and 50 to 90 minutes post-infusion.
Visit 6 and 7 can be conducted by telephone in limited circumstances if a participant has withdrawn or is unwilling/unable to attend clinic at the scheduled time.
* Can be undertaken at any of the marked visit
** These include Paired Associates Learning (PAL), Spatial recognition, Delayed Match to Sample, ID-ED Shift, motor screening and Eyes of the Mind tests.
***Only inclusion criterion 7 and exclusion criteria 21 and 22 are required to be met for randomisation at Visit 3 only
Δ Pregnancy test (serum pregnancy test and pregnancy urine dipstick), when required, for female participants only. Serum result confirmed prior to Visit 3.
° Blood tests for U&Es (Urine and Electrolytes), LFTs (Liver Function Tests) and TFTs (Thyroid Function Tests) should be confirmed within acceptable ranges in the previous 4 months of the Screening (Visit 1). Can be performed if required at Visit 1 or 2 (results must be confirmed as acceptable prior to infusion).

AUDIT = Alcohol Use Disorder Identification Test; **BP** = Blood Pressure; **CANTAB** = Cambridge Neuropsychological Test Automated Battery, **CGI-I** = Clinical Global Impression – Improvement, **CGI-S**= Clinical Global Impression- Severity, **ECG**= Electrocardiograph; **Fagerstrom**: Test for Nicotine Dependence, **GAF** = Global Assessment of Functioning; **HDRS** = Hamilton Depression Rating Scale; **HR** = Heart Rate; **IV** = Intravenous; **IWRS** = Interactive Web Response System; **MADRS** = Montgomery and Asberg Depression Rating Scale; **MEHI** = Modified Edinburgh Handedness Inventory; **NEO PI-FFI** = NEO Personality Inventory-Five Factor Inventory; **POMS** = Profile of Mood States; **PRISE**= Patient Rated Inventory of Side Effects; **RR** = Respiratory Rate; **SCID-RV** = Structured Clinical Interview for DSM; **VAS** = Visual Analogue Scale; **YMRS** = Young Mania Rating Scale; **WAIS** = Wechsler Adult Intelligence Scale.

Note timeline between visits:
Visit 1 and 2: ≤14 days. Can also occur on the same day and thus data does from screening visit does not need to be replicated at Visit 2.
Visit 2 and 3: ≥2 days and ≤7 days
Visit 3 and Visit 4 and Visit 5 and Visit 6: ≥2 days
Visit 6 and Visit 7: ≥3 days

8.4 Description of Study Procedures

8.4.1 Informed Consent

Eligible participants may only be included in the trial after providing written informed consent. Informed consent must be obtained prior to conducting any trial specific procedures and the process for obtaining informed consent must be fully documented in the patient's medical records.

The central ethics committee (EC) approved Patient Information Leaflet and Informed Consent Form (PIL and ICF) will be provided to potential participants, which the member of the research team, Principal Investigator and/or Sub Investigator's will explain and discuss the nature of the study. Potential participants will have ample time to ask and have answered any questions prior to making a decision regarding participation.

When the participant is ready to do so, the ICF will be initialled, signed and dated by the participant, and the Principal Investigator and/or Sub-Investigators who administered the informed consent form. The complete original ICF will be filed by the site in the site file, a copy of the ICF will be given to the participant and a copy will be filed in the participant's notes.

8.4.2 Medical and Surgical History

Details of current and previous relevant diagnoses and treatments will be recorded as deemed relevant by PI / Sub PI or research team. In particular, medical history of cardiovascular disease, prostatic hypertrophy, asthma, hepatic or renal impairment, gastrointestinal obstruction, epilepsy and thyrotoxicosis should be sought.

8.4.3 Demographics

The date of birth, gender, race and handedness will be recorded. Handedness will be accurately measured utilising the Modified Edinburgh Handedness Inventory (MEHI, Oldfield, 1971; Tan, 1988).

8.4.4 Physical Examination

Height (cm) and weight (kg) will be recorded at Visit 2. The weight at Visit 2 will be used for the duration of the study to inform dose calculations.

8.4.4.1 Vital signs

Vital signs will be recorded for all participants and will include: blood pressure (BP), heart rate (HR) (beats per minute (bpm)), and respiratory rate (RR) (respirations per minute). These will be recorded at screening, Visit 2 and at Visits 3, 4 and 5. Patients will rest for 5 minutes before measurements are obtained. Vital signs will be recorded prior to infusions and post infusions. Prior to infusion vital signs should be obtained post cannulation.

For post infusion vital signs will be taken within 15 minutes post-infusion administration and fifty to 90 minutes post-infusion, resting HR and BP will be measured, with a requirement for a HR ≥50bpm, and BP ≥90mm Hg Systolic and ≥60mm Hg diastolic prior to visit completion. Clinical support is present to support and advise research staff and participants in relation to the management or treatment strategies that are required if the participants HR is < 50 bpm

or > 100bpm or if participant is hypotensive (BP < 90/60). Clinical support includes ready access to resuscitation equipment and clinical expertise.

Where BP and / or HR are abnormal when measured post-infusion the participants respiratory rate will additionally be recorded, and this information will be provided to an on-site clinician.

All participants will be required to remain in the HRB-CRFG for a period of observation by trial staff for a minimum of 90 minutes post infusion. Participants should be asymptomatic of potential IMP related symptoms as well as having satisfactory vital signs before discharge post infusion.

8.4.4.2 ECG Test

An ECG will be performed at Visit 2. Abnormal findings will be noted for clinical significance and the report will be signed by the investigator. If deemed required by the treating physician, additional input will be obtained by an additional registered medical practitioner prior to continuation in the study.

8.4.5 Laboratory Tests

Prior to engagement in the clinical study, trial participants will demonstrate no renal/hepatic impairment and no evidence of thyrotoxicosis. This will be based on an evaluation of medical history and blood tests including Urea and Electrolytes (U&Es), Liver Function Tests (LFTs) and Thyroid Function Tests (TFTs). The most recent U&Es, LFTs and TFTs baseline must be demonstrated to be within acceptable range in the previous 4 months of the Screening visit (visit 1). If no blood tests have been completed within the last 4 months, these will be performed at Visit 1 or Visit 2 and confirmed as acceptable prior to first infusion. Where results are obtained that are outside the laboratory stated normal range, this will be discussed with the PI and where required, the PI will further liaise with clinical colleagues to ensure that any findings are not clinically significant and are acceptable prior to administration of IMP or placebo. If clinically significant, in the opinion of the investigator, the participant will not receive IMP or placebo.

Women of child-bearing potential must have a negative pregnancy test. A urine pregnancy dipstick test will confirm pregnancy status at Visits 2, 3, 4 and 5 prior to each infusion. A serum pregnancy blood test will additionally be conducted at Visit 2 and this will confirm pregnancy status prior to infusion at Visit 3 (placebo/Scopolamine).

8.4.6 Current Medications

All medications will be recorded and changes in psychotropic medication recorded at each study visit. All over-the-counter or prescription medication, vitamins, and/or herbal supplements will be recorded in the patients notes. The indication for treatments will be recorded. Psychotropic medications prescribed over the previous 6 months will additionally be recorded.

8.4.7 Pregnancy and contraception Advice

Women of childbearing potential can only be included in the study if they have a negative urine dipstick test at Visits 2, 3, 4, 5 prior to infusions and a negative blood serum pregnancy test executed at Visit 2 and confirmed prior to infusion at Visit 3.

Women of child-bearing potential are defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during dosing of study treatment (Visits 1-5). Highly effective contraception methods include:

- Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment.
- Male partner sterilization
- Combination of any two of the following (advice will be provided to all appropriate female participants):
    a. Barrier methods of contraception e.g. Condom
    b. Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception
    c. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
- Women who are considered post-menopausal i.e. amenorrhea at least 12 months or undergone hysterectomy/bilateral oophorectomy
- All males participating in this study should similarly be provided with adequate contraception advice as detailed above.

8.5 Psychometric Instruments

The timings of administration of the below questionnaires is provided in Table 2. The SCID-RV, HDRS, YMRS, MADRS, GAF, AUDIT, Fagerstrom and VAS should be completed on all participants. The other listed psychometric instruments are desirable but not compulsory and are indicated as optional (POMS, CANTAB, PRISE, WAIS, NEO-PI-FFI). The POMS and VAS should be completed a minimum of 15 minutes post-infusion. The WAIS and NEO-PI-FFI can be completed at any of the scheduled visits. Details of each psychometric instrument is provided below:

- Alcohol Use Disorder Identification Test (AUDIT, Babor et al., 2001). A 10-item questionnaire to identify harmful use or dependence on alcohol.
- The Cambridge Neuropsychological Test Automated Battery (CANTAB; Owen et al., 1995; Sahakian et al., 1988) will be undertaken with participants. Tests conducted from this battery will entail the Paired Associates Learning, Spatial recognition, ID-ED shift, delated match to sample, motor screening and eyes to mind test. These tests will be used to evaluate visual, new and spatial memory, motor speed and social cognition.
- Clinical Global Impression (CGI, Guy, 1976). A rating scale that measures illness severity (CGI-S) (1-7), and global improvement (CGI-I) (1-7)) and has an efficacy index (which relates therapeutic effect to adverse effects) (1-4).
- Fagerstrom Test for Nicotine Dependence (Heatherton et al., 1991). A 6-item questionnaire examining nicotine dependence.
- Global Assessment of Functioning (GAF, Hall, 1995). A rating scale from 1-100 that measures an individuals' functioning and is observer rated.
- Hamilton Depression Rating Scale (HDRS, Hamilton, 1960). A 21-item observer rated instrument that measures symptoms of depression.
- Montgomery and Asberg Depression Rating Scale (MADRS, Montgomery & Asberg, 1979). A 10-item observer rated scale that measures symptoms of depression.
- NEO Personality Inventory-Five Factor Inventory (NEO PI-FFI, Costa & McCrae, 1992). This 60 item personality inventory measures personality traits in five dimensions (neuroticism, extraversion, openness to experience, agreeableness and conscientiousness).
- Patient Rated Inventory of Side Effects (PRISE). A 9 item self-report instrument measuring adverse effects associated with the gastrointestinal, genitourinary, nervous,

cardiac systems, sense organs, skin, sexual functioning, sleep and other general adverse effects.

- Profile of Mood State (POMS, McNair et al., 1971). A rating scale where 65 adjectives are rated by participants on a 5-point Likert scale. Six factors are derived from these adjectives (tension, depression, anger, fatigue, vigour and confusion).
- Structured Clinical Interview for DSM-V Axis I Disorders (SCID-RV, First et al., 2015). This is a semi-structured interview that evaluates pathology and has a demographic section followed by nine diagnostic modules, including two that examine mood disorders.
- Visual Analog Scale (VAS). A series of scales will be created to indicate on a 10-point Likert how participants feel they are on the following items (happy, restless, sad, anxious, anger, drowsiness and alertness). These items are consistent with that used by Furey et al. (2012) in their study of Scopolamine in mood disorders.
- Wechsler Adult Intelligence Scale (WAIS, Wechsler, 1955) is a structured instrument to estimate IQ.
- Young Mania Rating Scale (YMRS, Young et al., 1978). An 11–item observer rated instrument that measures symptoms of mania.

8.6 Randomisation

Participants will be randomly assigned to receive either scopolamine or placebo in a 1:1 ratio. Randomly permuted blocks of sizes 4 and 6 will be used to ensure similar numbers of participants in each arm of the trial.

Randomisation will be stratified by the HDRS score at trial entry (a score of <23 indicating a mild-moderate depressive episode and a score ≥23 indicating a severe depressive episode) to ensure greater balance between arms in the final trial sample and increase the efficiency of our treatment effect estimates.

A validated randomisation system will be used at Visit 3, (after HDRS and YMRS are completed) to randomise patients to either arm. This centralised system will ensure allocation concealment; preventing blinded trial staff from knowing which treatment group will be allocated. Blocks of randomly varying length will also reduce the predictability of the allocation sequence.

8.7 Discontinuing/Stopping Infusions

If a trial participant requires medical intervention during or post infusion, the treating physician must assess if it is appropriate to proceed with the current and/or future infusions. If infusions are not completed participants would be asked to complete all other aspects of scheduled visits.

8.8 Blinding

This trial will be conducted in a double-blind fashion with placebo control identical to Scopolamine solution to avoid bias in the assessment of outcomes.
Site Investigators, site personnel, participants, and outcome assessors will be blinded to treatment allocation. Placebo infusion must be prepared, labelled and administered to match procedures for Scopolamine (hyoscine hydrobromide) infusion in order to maintain the blind. Placebo and Scopolamine are identical, colourless solutions and infusions are identical once prepared. Blinding will be maintained as per the process steps and controls outlined in the study specific Investigational Medicinal Product (IMP) Handling Manual. The unblinded study member will be unblinded throughout the length of the study and will not be involved in any

patient assessments or data analysis. Study personnel who administer the infusions to the participants must remain blinded. Discussions regarding treatments between the unblinded team member and the blinded team member must be avoided where possible.

Unblinding will be as per the instructions outlined in the study specific procedure provided to site by the sponsor. 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 participant, a caring physician may unblind a single participant. Any breaking of the blind, whether intentional or unintentional will be recorded and reported to the sponsor as soon as possible. Unblinding will be recorded and justified in the final report.

Circumstances in which unblinding for multiple or all participants may take place include SUSARs, reporting for multiple SAEs or SARs, or if there is new information regarding the safety of the IMP as determined by the caring physician.

8.9 Definition of end-of-trial

The end of trial will be the date of the last visit of the last participant (50th randomised participant). The Sponsor, the funder (Stanley Medical Research Institute (SMRI) and/or Data safety and monitoring board (DSMB) /Trial steering committee (TSC) have the right at any time to terminate the study for clinical or administrative reasons. The end of the study will be reported to the approving EC and HPRA within 90 days of the end of the clinical trial, or within 15 days if the study is terminated prematurely by the Sponsor or the Sponsors representative. The EU Declaration of the End of Trial form must be used for this. The investigators will inform participants and ensure that the appropriate follow-up is arranged for all involved. A summary report of the study will be provided to the REC and HPRA within 1 year of the end of the study.

8.10 Premature termination of the study

The trial may be terminated prematurely if:

- new information about safety or efficacy becomes available
- there is unsatisfactory progress of the study, including unsatisfactory progress in relation to participant recruitment.
- if deemed necessary by the Sponsor, Chief investigator, DSMB, TSC, and / or SMRI

If the trial ends prematurely then the HPRA and the approving EC will need to be informed as required.

8.11 Discontinuation/withdrawal of participants from study protocol

Study participants 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 participant from study treatment or withdraw a participant from the study at any time if it is in the best interest of the participant. In this instance, participants must discontinue the investigational medicinal product (Scopolamine) and be withdrawn from the study. Reasons for investigator to discontinue a participant from study treatment include but are not limited to:

- Pregnancy,
- Involuntary detention under the MHA 2001 in an acute psychiatric inpatient unit
- Treatment with ECT
- Severity of symptoms such that participation in a clinical trial is not appropriate because of the risk of imminent self-harm (based on review by clinician at study visit)
- Development in the study of narrow angle glaucoma, myasthenia gravis, paralytic ileus, pyloric stenosis, toxic megacolon and acute porphyria.
- Development post-infusion of bradycardia (HR < 50 bpm), tachycardia (HR > 100bpm) or hypotension (Systolic BP <90mm HG or Diastolic BP <60mm Hg) that required clinical treatment (e.g. administration of additional medications, review in Emergency Department)
- Treatment at day of infusion with anticholinergic medications (e.g. Physostigmine, Biperiden, Procyclidine)

If a participant withdraws before completing the study, the reason for withdrawal must be documented in their medical notes. Participants who request to have no further infusions, will be invited to complete pre-infusion questionnaires for remaining treatment visits. Ideally, all participants who discontinue the study should comply with specified follow-up procedures (days 15 +/-5, days 29 +/-7) as detailed in this protocol (i.e. assessments via interview compromising psychometric instruments, discussion of adverse effects and medication and phlebotomy – Table 2, Section 8.3). If the participant is unable to attend for follow-up procedures, a telephone interview will be offered to attain relevant study data. The only exception to this requirement is when a participant withdraws consent for all study procedures. If a study participant withdraws consent after just receiving or during an infusion, monitoring for 50-90 minutes post infusion for bradycardia, hypotension, dizziness and sedation must occur, with the study specific Adverse Event Form completed.

If a participant withdraws due to an adverse event, the investigator will arrange for follow-up visits until the adverse event has resolved or stabilised.

8.12 Outcome measures

8.12.1 Effectiveness Assessment

8.12.1.1 Primary outcome measure

The primary outcome measure will be the change from Visit 2 in severity of objective depressive symptoms after the final scheduled treatment as measured by change in the HDRS depression score from Visit 3 (pre-infusion and randomisation) to Visit 6.

8.12.1.2 Secondary outcome measure

1. Remission of depressive episode after the last IV treatment (measured objectively at Visit 6), and is defined as occurring when an individual has:
   (i) a HDRS score ≤7
   (ii) a MADRS score <6
2. Remission of depressive episode at follow-up (measured objectively at Visit 7)
   (i) a HDRS score ≤7
   (ii) a MADRS score <6
3. Response to a depressive episode after the last IV treatment (measured at Visit 6) is defined as:
   (i) a 50% reduction in MADRS score at Visit 6 compared to Visit 3

4. Response to a depressive episode at follow-up (measured objectively at Visit 7)
   (i) a 50% reduction in MADRS score at Visit 6 compared to Visit 3
5. Improvement in mood symptoms measured with the HDRS and MADRS at visits 5, 6 and 7 compared to Visit 3.
6. Improvement in objectively measured overall functioning, measured with the GAF at Visit 5, 6 and at follow-up at Visit 7 compared to Visit 3
7. Improvement in objectively rated illness severity, measured with the CGI-S at visits 4, 5, 6 and 7 compared to Visit 3 and CGI-I at visits 4, 5, 6 and 7 compared to Visit 3.
8. Changing subjective measured depressive symptoms with the POMS and VAS
   (i) a reduction in POMS score post-infusion compared to pre-infusion at visits 2, 3, 4 and 5 across the six different factors of the POMS (tension, depression, anger, fatigue, vigour and confusion).
   (ii) a reduction in VAS scores post-infusion compared to pre-infusion at visits 2, 3, 4 and 5 across 8 items (happy, restless, sad, anxious, anger, drowsiness and alertness).
9. A change in subjectively measured depressive symptoms at Visit 6 compared to Visit 3 as measured with the POMS and VAS.
10. A reduction in subjectively measured depressive symptoms at follow-up (Visit 7) compared to Visit 3 as measured with the POMS and VAS.
11. Psychiatric inpatient admission of a participant due to depressive episodes between Visit 2 and Visit 7.
12. Antidepressants medication use or change by a participant due to depressive episodes over the duration of the study (Visit 2 to Visit 7):
    (i) Introduction of a new antidepressant (yes / no)
    (ii) Increase in dose of an existing antidepressant (yes / no)
13. Change in motor processing, visual and spatial memory (3 categorical variables) at Visit 6 compared to Visit 2 based on the CANTAB battery of tests.
14. Occurrence of a hypo (manic) episodes at visits 3, 4, 5, 6 or 7, as defined by a score of >6 on the YMRS.
15. Occurrence of other adverse effects as observed on monitoring of vital signs, reported by participants or measured utilising the PRISE questionnaire

9. Treatment of Trial Participants

9.1 Description of study treatment(s)

Investigative Medicinal Product: (Active treatment): Scopolamine (Hyoscine Hydrobromide 600 micrograms/ml, 1 ml) Dosage = 4µg/kg in 100mls of Saline administered intravenously over 15 minutes via pulsed infusion. The Scopolamine dose is µg of drug per kg weight of the participant.

Scopolamine supplier: Martindale Pharmaceuticals, Essex, UK.

Placebo: Commercially available 0.9% sodium chloride 100ml infusion bag will be used as the placebo control in this trial. It will be administered intravenously over 15 minutes via pulsed infusion. The volume addition of active drug (hyoscine hydrobromide) will on average be less than 1ml (100kg patient or less) for each infusion bag, therefore no volume addition is required for the preparation of the placebo infusion bag as the volume difference to the active treatment infusion will be negligible to the total volume to be infused (100mls + volume overage in each bag) and will not be noticeable to personnel administering the infusion, thereby maintaining the blind.

Placebo supplier: Commercially available 0.9% Sodium Chloride 100ml infusion bag.
This is an authorised product which will be approved stock sourced through the Galway University Hospital pharmacy. The details will be outlined in the IMP handling manual and the product number and batch number will be recorded for the duration of the trial.

9.2 Formulation, packaging and handling

Both placebo (0.9% sodium chloride) and Scopolamine are clear, colourless solutions and infusions are identical once prepared. The pharmacist will dispense the study drug to an unblinded research healthcare professional who will prepare the Scopolamine infusion as per the IMP handling manual (the unblinded healthcare professional will not be involved in assessments or data analysis). Blinding will be maintained as per the process steps and controls outlined in the study specific Investigational Medicinal Product (IMP) Handling Manual.

The Clinical Trials Pharmacy Unit at the HRB-CRFG, will label commercial stock of Scopolamine as per Annex 13 and per approved study instructions. Labels will indicate:

(i) Use of the treatment in this trial
(ii) Name of sponsor and CI
(iii) Trial reference code allowing identification of the trial, site, investigator and participant number
(iv) The product is 'For Clinical Trial Use Only'

These labels will be added by pharmacy staff on receipt of delivery of the IMP to the HRB-CRFG in accordance with Annex 13 (EU Guidelines to Good Manufacturing Practice, Investigational Medicinal Products).

9.3 Storage and disposition of study treatment(s)

Scopolamine (and placebo) will be stored securely at the HRB-CRFG as per manufacturer's guidelines. Scopolamine (hyoscine hydrobromide) will be stored in Pharmacy Clinical Trials Unit (PCTU) as per manufactures storage instructions. Placebo will be stored in clinical area as per manufactures instructions. Active and control treatments will be prepared into infusions by an appropriately trained and delegated unblinded site team healthcare professional based in the HRB-CRFG as per institutions guidelines/applicable SOPs in a separate clinical area away from the blinded study personnel and participant treatment area. The unblinded staff member will not be involved in any patient assessments or data analysis. Infusions should be administered to the participant immediately with minimal delay. The entire contents of the infusion bag must be administered to the participant. Empty infusion bags and any unused product will be disposed of according to institutions guidelines/applicable SOPs

An accurate and timely record of IMP & placebo inventory must be maintained through the duration of the trial (including receipt, dispensing, return if applicable and destruction of unused product). A temperature log must be maintained for documentation of appropriately IMP and placebo storage areas.

9.4 Preparation/Reconstitution of Dosage Form

Administration instructions and dose preparation instructions will be provided as per the IMP handling manual.

9.5 Accountaibility of the study treatment(s)

The study medication will be supplied by Martindale Pharmaceuticals, UK. Upon delivery, receipt of the products will be recorded by pharmacy staff and labels applied as described in IMP Handling Manual with products then transferred to the secure storage area. Unopened products which are unused by end-of-trial will be destroyed on site as per site destruction policies.

9.6 Assessment of compliance

In this study, interventions will be administered intravenously by the research team and thus treatment non-compliance will be low-risk. Non-compliance may result however from participants refusing further infusions after attaining an initial infusion(s), or asking for an infusion to be discontinued during an infusion.

The investigator is responsible for ensuring that the study treatment is administered in compliance with the protocol. Participant compliance will be assessed by maintaining dispensing records.

9.7 Overdose of Study treatment

It is deemed unlikely that an overdose will occur in this study, given the safeguards in place with careful preparation of each infusion. The Scopolamine drug will be prepared by approved study specific instructions as per the IMP Handling Manual to ensure appropriate preparation of the drug and accuracy of calculations performed as part of drug preparation. In the very improbable event of an overdose, the study participant will be monitored for any change in vital signs by the research team members. If adverse symptoms arise secondary to an overdose, local site policy will be followed in relation to management of any medical emergency. Infusions will be administered over a 15 minute period, with this controlled by use of a calibrated IV infusion pump. Thus, it is unlikely that an infusion will be administered in a time-frame of less than 15 minutes, however if this occurs and adverse symptoms arise, local policy will similarly be followed in relation to the management of a medical emergency.

9.8 Prior and concomitant therapy

All current medication, in addition to the study medication taken during the study will be recorded in the patient's notes. Medications will be documented at the point of consent, at Visit 2 with changes in treatment notes at every assessment appointment thereafter. Psychotropic medications prescribed over the previous six months will also be documented.

9.8.1 Permitted medications/non-investigational medicinal products

All medications including herbal treatments aside from those listed in section 9.8.2 are permitted. Standard of care will continue for all participants during this study.

9.8.2 Prohibited medications

The following medications are contraindicated during the randomised treatment period as they may potentially alter the pharmacokinetics of Scopolamine and any participants newly prescribed these medications at randomisation visit will be excluded from the trial prior to any infusion being administered.

- Physostigmine
- Biperiden
- Procyclidine
- Tricyclic Antidepressants with significant anticholinergic properties (e.g. Amitriptyline and Nortriptyline) that are causing the participant to experience anticholinergic side effects (e.g. blurred vision, constipation, urinary retention, cognitive difficulties).

Participants prescribed any of the above agents during the trial will not receive any further intervention (infusions of Scopolamine or placebo), however will be followed up for the remainder of the trial with data collected included in intention-to-treat analyses if one infusion and one follow-up assessment have been completed.

Participants cannot participate in any other IMP studies while participating in this study (Visits 2-7).

10. Safety reporting

Adverse events will be captured for all study participants from the time of consent for the duration of their participation in the study i.e. from the time of consent to their final study visit. A full AE review at each study visit will ensure that a complete evaluation of the safety and tolerability of the IMP is conducted.

All participants will have their heart rate and blood pressure measured prior to infusion and post-infusion to measure haemodynamic changes, with normal results (see section 8.4.4.1) required post-infusion prior to the participant completing their visit (minimum 90 min monitoring period).

If the readings have not returned to normal within this required 90-minute monitoring period a further period of monitoring will be conducted. A treating investigator must assess and decide if medical intervention is required based on their clinical judgement. Additionally, the PRISE questionnaire (this is optional for participants) will be used where participants agree to document other adverse effects prior to participants completing their clinical visits 2, 3, 4, and 5 (see Section 8.5). All adverse events will be reported to the Sponsor on the AE case report.

10.1 Definitions

10.1.1 Adverse event (AE)

Any untoward medical occurrence in a patient or clinical trial participant 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.

Each individual unintended sign, symptom or disease is considered a separate adverse event unless an overarching diagnosis can be made for a collection of signs or symptoms that are clinically linked and temporally related. The overarching diagnosis should be as specific as possible, using all available clinical data.

All events must undergo an assessment to determine if any of the seriousness criteria are met and each event must be reported to the Sponsor.

10.1.2 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.

10.1.3 Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

Any untoward medical occurrence or affect that at any dose meets one or more of the following criteria:

- results in death,
- is life-threatening*,
- requires hospitalisation (defined as >24 hour hospital stay or admission to inpatient hospital area) or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect
- is medically important**

* This refers to an event in which the participant 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 participant 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.

10.1.4 Suspected unexpected serious adverse reactions (SUSAR)

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (i.e. section 4.8 of the summary of product characteristics).

10.1.5 Servere Adverse Event

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

10.2 Evaluation of AEs and SAEs

The investigator or delegate will report all AEs and SAEs to the Sponsor as outlined in section 10.3, except for those identified as outcomes identified below.

10.2.1 Events exempt from reporting

10.2.1.1 Canulation related

The following commonly experienced events related to cannulation are exempt from AE reporting if they are reported at the site of cannulation and are deemed non-clinically significant by a study investigator.

(a) Discomfort
(b) Small haematoma
(c) Bruising
(d) Transient light headedness
(e) Irritation to plaster/dressing used over or around cannulation site.

If the nature or severity of the event exceeds what is commonly experienced or if the event is deemed clinically significant by the investigator the event should then be reported as an AE. Events related to cannulation must be reported as SAEs/SARs if they meet one or more of the seriousness criteria as defined above.

10.2.1.2 Scopolamine infusion related

The following events are considered expected, transient side effects of scopolamine therapy and are therefore exempt from safety reporting if they occur in the 90 minutes post IMP infusion (visit 3, visit 4 or visit 5). These events will be captured on a study outcome form.

(a) Drowsiness
(b) Blurred vision
(c) Dry mouth
(d) Thirst
(e) Urinary retention

The above occurrences are considered Adverse events and therefore reportable if any of the following occur:

- medical intervention or treatment
- prolonged monitoring in addition to the 90 minutes post infusion required by the protocol
- deemed medically significant by an investigator
- cessation of the study drug (current or subsequent infusions)

These events must be reported as SAEs/SARs if they meet one or more of the seriousness criteria.

10.2.1.3 Change in depressive or (hypo)manic symptoms

Change of baseline depressive symptoms or development of (hypo)manic symptoms based on clinical review are exempt from safety reporting unless they:

(a) are associated with self-harm
(b) are associated with uncharacteristic erratic behaviour
(c) meet any one or more of the seriousness criteria

These events are collected on case report forms.

10.2.1.4 Psychotropic medications

Changes in psychotropic medications are exempt from safety reporting. These changes are collected on case report forms.

10.2.1.5 Psychometric measures

Variations in psychometric assessments captured on HDRS, YMRS, CGI-S, CGI-I, VAS, MADRS, GAF and POMS are exempt from safety reporting. Psychometric assessments are collected on case report forms.

10.2.2 Assessment of seriousness

An appropriately qualified, delegated member of the site team should make an assessment of seriousness as defined in section 10.1.3.

10.2.3 Assessment of causality

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

The investigator 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.
- Unlikely: where a temporal relationship to the study medication makes a relationship improbable (but not impossible) and disease or other drugs provide plausible explanations.
- Possible: 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.
- Probable: The temporal relationship and absence of a more likely explanation suggest the event could be related to the study medication.
- Definite: Plausible temporal relationship and cannot be explained by disease or other drugs.

All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g. definite, possible, probable) 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.

The causality assessment provided by the investigator will also be reviewed by the sponsor. The investigator causality assessment should not be downgraded by the sponsor.

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.

10.2.4 Assessment of severity

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

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.
- Severe or medically significant: An event that prevents normal everyday activities.
- Life threatening: An event that has life-threatening consequences

Note: the term 'severe', should not be confused with 'serious' which is a regulatory definition based on participant/event outcome or action criteria.

10.2.5 Assessment of expectedness

An expectedness assessment will be carried out by the sponsor for each serious adverse reaction to the IMP. The expectedness of a serious adverse reaction will be determined by the sponsor according to the reference safety information (RSI) as contained within section 4.8 of the SmPC for scopolamine.

10.3 Reporting responsibilities of the investigator

10.3.1 Adverse events/serious adverse events

Any AE whose onset occurred between the time of informed consent and the last completed visit, observed by the investigator or reported by the participant, whether or not attributed to the study medication, will be recorded on the AE case report form. The Site Investigator or delegate will follow AE's and SAE's reported during the treatment period until resolved, considered stable, or completion of patient participation in the Scopolamine trial (i.e. final follow up Visit). Follow up information will be sought and submitted as it becomes available. All SAEs will be followed up until resolution or they are clearly determined to be due to a participant's stable or chronic condition or intercurrent illness(es).

The following information will be recorded in the adverse event form: Adverse event term, description, date of onset, outcome, date of resolution, seriousness, severity, assessment of relatedness to the study medication, and action taken with study drug. The Site Investigator is responsible for the assessment of severity (intensity) and causality/relatedness to IMP for all AEs and SAEs. A SAE should also be substantiated by a source document(s). Follow-up information should be provided as necessary.

AEs considered related to the study medication as judged by an investigator or the sponsor will be followed up until the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present post-infusion will be followed up until a satisfactory resolution occurs. Participants will continue to be monitored for AEs throughout the study period up to and including visit 7.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily withdraw

from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end-of-study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.
Any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed-up for congenital anomaly or birth defect.

Any SAE in a participant that the investigator becomes aware of occurring after they have finished the study follow up period, which are deemed by the investigator to be causally related to the IMP should be forwarded to saereport@nuigalway.ie.

10.3.2 Timelines for reporting

10.3.2.1 Adverse events

Adverse event information will be reported by site personnel in a timely fashion from the time the site becomes aware of the event.

10.3.2.2 Serious adverse events

An appropriately qualified member of the site team will report all serious adverse events (SAEs) to the sponsor immediately but no later than **24** hours after site awareness of the SAE. The site team are considered aware of an adverse event from the time of first notification of the first member of the Scopolamine site team, as per the Site Delegation Log. All SAE's will be submitted by the site by completing the required fields on the AE eCRF within 24 hours of site awareness of the event. If the eCRF is unavailable a paper copy of the eCRF should be completed and submitted to HRB-CRFG within 24 hours of site awareness of the event. A valid SAE report must include all of the following:

- Adverse event term (based on what is known at the time of reporting)
- Seriousness criteria
- Severity
- Causality assessments (for Scopolamine/placebo)
- Investigator sign-off

The immediate report will be followed by detailed, written reports. The immediate and follow-up reports will identify participants by unique code numbers assigned to the latter.
All SAE information must be recorded on an SAE forms submitted to sponsor. Additional information received for an event (follow-up or corrections to the original event) should be detailed and submitted to the sponsor in an expedited manner.

The Site Investigator or delegate will follow AEs and SAEs reported during participation in the trial until resolved, considered stable or completion of participation in the Scopolamine trial. Follow up information will be sought and submitted as it becomes available. All SAEs should be followed up until resolution or they are clearly determined to be due to a participant's stable or chronic condition or intercurrent illness(es).

10.4 Responsibilities of the Sponsor

10.4.1 Regulatory Authorities

The sponsor will keep detailed records of all adverse events which are reported to them by the investigator or investigators. The sponsor will report all SUSARs to the competent authority (HPRA) or EudraVigilance (as required), the approving ethics committees concerned, and all principal investigators. Fatal or life-threatening SUSARs must be reported within 7 days.

If the initial report is incomplete, e.g. all the information/assessment has not been provided; the sponsor will submit a completed follow up report within an additional eight days.

SUSARs which are not fatal or life-threatening are to be reported within 15 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.

10.4.2 Safety Reports

The sponsor will distribute masked expedited SUSAR reports, to each participating Site Investigator, as appropriate. SUSARs of which the treatment allocation of the participant is un-blinded should be reported by the sponsor to the national competent authority or EudraVigilance (as required) and the approving EC.

10.4.3 Annual Reports

In addition to the expedited reporting above, the sponsor shall submit annually throughout the clinical trial or on request, a Development Safety Update Report to the competent authority (HPRA) and ethics committees. The annual safety report will be presented in the DSUR format as per the requirements of ICH-GCP and applicable regulatory, HPRA and internal sponsor requirements.

10.4.4 Pregnancy

Pregnancy is not considered an AE or SAE however the investigator must collect pregnancy information for female trial participants or female partners of male trial participants who become pregnant while participating in a study to determine the outcome of the pregnancy. If a congenital anomaly or birth defect is recorded following a pregnancy which occurred during the study the congenital anomaly or birth defect will be reported as a SAE. In some circumstances, it may be necessary to monitor the development of the new-born for an appropriate period post-delivery. The investigator will record the information and submit this to the sponsor.

10.5 Data Safety Monitoring Board (DSMB)

A DSMB is established and members will serve in an individual capacity and provide their expertise and recommendations. The primary responsibilities of the DSMB will be to:

1. Become familiar with the research protocol and the procedures for data safety/monitoring.
2. Review interim analyses of outcome data/adverse event reports.
3. Make written recommendations to the TSC concerning the continuation, modification, or termination of the trial.
4. Consider any requests for release of interim trial data and make recommendations to the TSC or TWG on the advisability of this.
5. Review major proposed modifications to the study prior to their implementation (e.g., termination, increasing target sample size).
6. Maintain confidentiality during all phases of DSMB review and deliberations.
7. Review SAEs and SUSARs as appropriate

The responsibilities of the DSMB are outlined further in the DSMB charter. The membership of the DSMB reflects the professions necessary to interpret the data and results from the study and to evaluate participant safety fully.

10.6 Trial Steering Committee (TSC)

The purpose of the TSC is to provide strategic oversight for the overall direction and strategy for a clinical trial. The primary responsibilities of the TSC are:

1. To contribute to the design of the study
2. Increase information exchange at an early stage of trial development
3. Increase the efficiency of clinical trial collaboration
4. To monitor and review a) Recruitment progress, b) Quality control, c) Ethical amendments, d) Financial aspects, and e) Publications
5. To determine action points to facilitate the satisfactory progress of the Scopolamine study.

This committee includes investigators, other experts not otherwise involved in the trial, and representative of the sponsor.

11. Statistics

11.1 General considerations

Descriptive statistics, graphs and tables will be used to summarise baseline variables, follow-up outcome variables, and operational variables including rates of recruitment, willingness to be randomised, willingness to complete assessments, adherence to allocated treatments and follow-up and reasons for drop-out between groups.

Suitable numerical and graphical summaries will be generated to for patient characteristics. Continuous variables will be summarized by mean, median, standard deviation (SD), minimum and maximum. Categorical variables will be summarized by counts and percentages.

Data analyses will be performed blinded to allocation. Full specification of the statistical analysis to be conducted and its rationale will be given in the statistical analysis plan, along with detailed tables, listings and graphs will be provided separately. This plan will be finalized prior to the end of enrolment.

11.2 Determination of sample size

The primary outcome in this study is the Hamilton Depression Rating Scale score (HDRS). It is judged that a decrease of 50% or more in the HDRS score between randomisation and Visit 6 will be sufficient to consider a patient to have experienced a response. We anticipate that the average HDRS score in the proposed population pre-randomisation will be 24 units and that this outcome variable will be approximately normal. Based on results from the literature, it is estimated that a range of plausible values for the standard deviation is between 5 to 13 units. Using this information, a sample of 44 participants (22 in each group) will have 85% power to detect a difference of 12 units between the treatment and control arms, i.e. an expected HDRS mean score of 12 in the treatment group vs. an expected HDRS mean score of 24 in the control group after Visit 6. The sample size is based on a 2-sample t-test (two-sided) with a standard deviation of 13 units (conservative approach) and a significant level of 0.05. Allocating 25 participants to each arm will ensure power >=85% when loss to follow-up is <12% (6 participants missing at Visit 6 follow up).

11.3 Analysis sets

Effectiveness outcomes will be analysed on an intention-to-treat basis for all participants randomised and with available follow-up data as per their randomised allocation.

A per-protocol dataset is not well-defined under multiple treatments, here it could reasonably be defined as those participants who either received at least 1, at least 2 or all 3 IV treatments after randomisation. Sensitivity analyses will thus be performed on the primary outcome to assess efficacy of the treatment compared to placebo by incorporating the original allocation of participants and their level of adherence to treatment. This will involve examining the short term-effect of treatment in a longitudinal model and the overall effect of number of IV treatments received at follow-up visits after the final IV treatment.

11.4 Demographic and baseline disease characteristics

Demographic, baseline outcome variables (primary and secondary), and additional baseline disease characteristic data will be summarized for each treatment group using descriptive statistics.

11.5 Effectiveness analysis

11.5.1 Primary effectiveness analysis

For the primary effectiveness analysis of the primary outcome, it is expected that pre-randomisation measurements of the HDRS score will be correlated with the scores obtained at Visit 6 of treatment. The mean scores at Visit 6 will be compared across the study arms using an ANCOVA model. The response variable will be the change in HDRS score from Visit 3 to HDRS at Visit 6 including the HDRS score at Visit 3 as a covariate in the model. The addition of stratifying variables and other variables as covariates will be considered as appropriate and specified in the statistical analysis plan. This analysis will increase the power to detect significant differences between the groups.

Inverse probability weighting will be applied to the primary outcome analysis. These weights will be derived based on the inverse of the probability of a patient's data being missing given their pre-randomisation measurements. This will ensure the estimate and inference is more representative of all patients randomised, reducing bias in the estimation of the treatment effect due to participants lost to follow-up and missing data.

Inference regarding the treatment effectiveness will focus on the point estimate, confidence interval and p-value for hypothesis confirmation.

11.5.2 Secondary effectiveness analysis

Secondary outcomes will be analysed as appropriate (according to the distribution of each outcome), comparing the difference between groups at the specified follow-up visit and relative to baseline value (where specified/measured). Baseline variables predictive of each outcome (the measure at baseline where available), and stratifying variables will be included in ANCOVA or generalised linear models of outcome variables. The statistical analysis plan will give complete specification of these models.

For secondary outcomes, the focus will be on the point estimates and confidence intervals for hypothesis generation.

Additional analysis of the primary outcome will compare the time course of HDRS under placebo and treatment using a mixed-effects model including fixed-effects terms for time since randomisation/study visit, trial arm and their interactions, and a random effect to account for correlation of multiple observations per participant.

11.6 Safety analysis

The results of clinical monitoring including blood pressure or heart rate changes, and AEs secondary to phlebotomy or cannulation will be examined between the groups. Adverse events will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent adverse events will be summarized by system organ class (SOC) and preferred term.

Safety data will be presented primarily in a descriptive fashion. The small sample size and likely low adverse event rates will mean any inference is likely underpowered to detect a range of meaningful differences.

Blinded data will be presented to the DSMB for safety evaluation to coincide with scheduled DSMB meetings.

11.7 The level of statistical significance

The level of statistical significance will be set at $p < 0.05$, relating only to the primary analysis of effectiveness. Inference for the treatment effect estimate other outcomes will focus on the point estimate and the confidence interval.

11.8 Procedure for accounting for missing, unused and spurious data

Any missing data which occurs will be summarised and reasons given. As per the statistical analysis specification, an inverse probability weighting will be performed in the primary outcome analysis (weights based on the inverse probability of a patient's data being missing

given their pre-randomisation measurements). This will ensure the estimate and inference is more representative of all patients randomised.

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

Deviations from the original statistical plan will be recorded and justified in the final report.

12. Direct Access to Source Data/Documents

Direct access will be granted to authorised representatives from the sponsor, and the regulatory authorities to permit trial-related monitoring, audits and inspections. Consent from patients/legal representatives for direct access to data will also be obtained. The patients' confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

13. Data handling and record keeping

Data will be entered, handled and stored at the HRB-CRFG. It will be pseudo-anonymised and then processed by members of the research team at the HRB-CRFG. Data will also be submitted to a data repository at the National Institute of Mental Health (NIMH) based in Maryland, Bethseda, USA on a twice-yearly basis to house data on behalf of SMRI.

13.1 Data collection, source documens and case report forms

Source documents for this study will include hospital records and procedure reports and data collection forms. These documents will be used to enter data on the case report forms. Once registered to a trial the patient will be provided with a unique, study-specific participant identifier and this and their initials will be the only way the patient will be identified in the database. Data will be directly entered into the Clinical Data Management System (CDMS) by the site staff. Data entry is by single data entry. Data queries will be generated within the CDMS for the investigational site as required to clarify data discrepancies or request missing information. The designated site staff will be required to respond to these queries and these responses with be reviewed by the Data Management Team. Any amendments to the data will be tracked within the audit trail of the CDMS.

Data reported on the case report forms 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 confidential conditions. On all study-specific documents other than the signed consent, the participant will be referred to by the study participant identification number/code.

Patient identification on the CRF and questionnaires will be through participant initials and their unique trial identifier allocated at the time of enrolment. No names or other identifying details will be recorded on the CRF or in any other format.

13.2 Data reporting

Central data management will be performed by the Data Management Centre at the HRB-CRFG. The Lead Data Manager will develop a Data Management Plan (DMP) which will detail all activities relating to the management of the clinical data. All project specific data management documentation will be filed in a Data Management File (DMF). The Data management team will also develop a CDMS to store the clinical data. This will be developed

following the relevant Data Management SOPs and adhering to regulatory and appropriate legislative requirements.

Local user access to the electronic CRF will be controlled via assigned usernames and passwords, approved by the study Data Manager based at NUIG. Access to the central study database will be governed by HRB-CRFG standard operating procedures (SOP)s and signed off by the Lead Site Investigator. Audit trails will log all transactions of data into and out of the system including time, date, user ID and the records involved. All external electronic communication with the central database will be protected by using Secure Socket Layer technology. The main database will be hosted in a secure enterprise scale data centre.

The research team will take every precaution to respect privacy in accordance with relevant legislation and EU Directives on protection of individuals with regard to the processing of personal data.

The data in the study database will be pseudo-anonymised, so that a number will be assigned to each patient which will be mapped to identifiable patient details at each hospital site only. This means that the data in the database is non-identifiable but will permit re-identification by the local site investigator in case of emergencies and requirement to follow up the patient.

The data for this study may be transferred within and/or outside the EU in line with reporting requirements. For data transferred outside the EU, the data controller must be assured of the legality and privacy safeguards of the transfer and ensure adherence to all other applicable legislative and regulatory requirements including GDPR and Clinical Trial legislation pertaining to such data transfer.

14. Retention of essential documents

The investigator will maintain all trial records according to GCP and the applicable regulatory requirements. The trial master file (TMF) will be established at the beginning of the trial by the sponsor. The investigator site files will be maintained at the investigators site. These will contain the essential documents in line with ICH-GCP. On completion of the trial the essential documents will be maintained by the investigator for a period of at least 15 years or as otherwise specified in the regulations.

Following confirmation, the sponsor will notify the investigator when they are no longer required to maintain the files. If the investigator withdraws from the responsibility of keeping the trial records, custody must be transferred to a person willing to accept responsibility and this must be documented in writing to the sponsor.

15. Quality control and quality assurance procedures

This study will be conducted in accordance with the current approved protocol, International Conference on Harmonisation Good Clinical Practice (GCP), relevant regulations and SOPs.

15.1 Protocol compliance

The investigators will conduct the study in compliance with the protocol given approval/favourable opinion by the Ethics Committee and the appropriate regulatory authority and as per investigator responsibilities outlined in ICH-GCP E6 R2. Substantial changes to the protocol will require HPRA authority and ethics committee approval/favourable opinion prior to implementation, except when modification is needed to eliminate an immediate

hazard(s) to patients. All protocol modifications will be submitted to the competent authority/research ethics committees for review in accordance with the governing regulations.

Site investigator should report any trial-related deviations, violations or serious breaches of GCP and/or the trial protocol to the sponsor/delegate. The PIs will report any serious breaches of GCP to the sponsor immediately after becoming aware of them.

15.2 Monitoring arrangements

The sponsor will be responsible for trial monitoring. The frequency and type of monitoring will be detailed in the monitoring plan and agreed by the trial sponsor/sponsor delegate. Protocol compliance will be monitored by a monitor who will undertake site visits in accordance with the study monitoring plan to ensure that the protocol is adhered to. The monitor will check the completeness of patient records, the accuracy of entries on CRFs, the adherence to the protocol, SOPs and GCP, and the progress of patient recruitment. The Principal Investigator should ensure that access to all investigation related documents including source documents to confirm their consistency with CRF entries) are available during monitoring visits. The extent of source data verification (SDV) will be documented in the monitoring plan. Any deviations from the protocol will be reported to a sponsor representative as per the process and timelines communicated to site during investigator site initiation visit training.

16. Audits and inspections

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

17. Ethics

17.1 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.

17.2 Good Clinical Practise

The trial will be carried out in accordance with the principles of the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines.

17.3 Approvals

Required documents including the protocol, informed consent form, participant information leaflet, and any other required documents will be submitted to a recognised research ethics committee (REC) (Galway University Hospitals Research Ethics Committee) and the HPRA for written approval, as per submission requirements.
The sponsor will submit and obtain approval from the above parties for substantial amendments to the original approved documents.

17.4 Scopolamine benefits and risks assessment

Bipolar disorder is one of the top 20 leading causes of disability worldwide, with a similar disability adjusted life year rate (138.3 per 100,000) to more prevalent conditions such as asthma (147.9 per 100,000) and Alzheimer's disease (108.5 per 100,000) (Ferrari et al., 2016).Current pharmacological treatments for bipolar disorder and in particular for the

management of depressive episodes in bipolar disorder remain sub-optimal, with pharmacological strategies employed to date often only partially effective (Fournier et al., 2010; Yildiz et al., 2011). In addition to the time duration for treatment response, multiple treatment trials are also often required, thus increasing patient discomfort and distress (Insel and Wang, 2009). Consequently, pharmacological mechanisms that potentially alleviate depressive symptomatology in bipolar disorder and ameliorate patient functioning could present an additional pharmacological strategy for the management of bipolar disorder. A number of recent studies have suggested that scopolamine, the agent being investigated in this study (a pan muscarinic (M) receptor antagonist) can elicit a rapid anti-depressant response in both major depressive disorder and bipolar disorder (Ellis et al., 2014; Furey et al., 2006; Drevets et al., 2010) and thus may present a novel therapeutic strategy, particularly for the management of BPD, in individuals experiencing depressive episodes (Janowsky, 2011).

It is envisaged that the potential burden of the study will be less than the potential value of this research, but there are no guaranteed direct benefits. Participants may benefit indirectly from participation in terms of increased awareness of mental health issues. Some participants may benefit from scopolamine administration in attaining a rapid treatment for their depressive episode.
This study will not include incapacitated adults or children (<18 years of age). Issues regarding specific vulnerable populations are addressed individually below.

(i) The study requires inclusion **of adults with mental illness** to address the research question. Only those who have the capacity to provide valid informed consent will be invited to participate. Where there is concern expressed about the capacity of a person to make a decision regarding treatment, a capacity assessment will be performed by the principal investigator or sub-investigator to ensure that participants are able to provide written informed consent.

(ii) **Women of childbearing age** are defined by the Irish Central Statistics Office as women of ages 15-49 years (Census 2011, This is Ireland (Part 1) – 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 (1% of all women) experience bipolar disorder with this disorder usually have its onset in individuals aged 20-45 years of age. Bipolar disorder is equally common in men and women (Kroon et al., 2013) and thus the primary study objective cannot be accurately achieved without including women of childbearing age. At screening visit, precautions will be taken, as detailed in section 8.4.7, in relation to advice on adequate contraception use between Visit 1 to Visit 5.

17.4.1 Physical Risks

17.4.1.1 Tolerability and Adverse Effects of IV Scopolamine

Pulsed IV infusion (4 µg/kg over 15 minutes) has to date been well tolerated with no significant adverse effects (AEs) encountered in other studies that have administered this agent on a similar time regime and at a similar dose to the proposed study (Fury and Drevets, 2010, Fury et al., 2012)

17.4.1.2 Risks secondary to Phlebotomy

Phlebotomy is rarely associated with serious adverse effects and will be performed in this study by experienced delegated staff member of the HRB Clinical Research Facility with phlebotomy experience. Serious side effects such as loss of consciousness and seizures and injury to adjacent structures are rare and vasovagal attacks may occur occasionally. Bruising and small haematoma may occur in approximately 12% of individuals (WHO, 2010). All efforts will be made to reduce the likelihood of adverse events due to phlebotomy, however discomfort during the procedure is common.

17.4.1.3 Risks and hazards secondary to peripheral venous cannulation and intravenous administration of investigative medicinal product (e.g. Scopolamine and 100mls of normal saline or 100mls of normal saline)

Complications that can potentially arise following the procedure of cannulation include infiltration, extravasation, venous spasm, phlebitis, thrombophlebitis, large haematoma, nerve injury, arterial puncture, embolism and needle stick injury (HSE, 2013). In this study, peripheral venous cannulation will be performed using aseptic technique and in accordance with local venous cannulation policy. Cannulation will be utilized for a 15 minute infusion, via a calibrated infusion pump, and be removed prior to discharge from the clinical research facility. The cannulation site will be monitored during the infusion and after cannula removal. Discomfort is common during the insertion of a peripheral venous cannula, however every effort will be made to minimize pain or discomfort, including the use of topical anaesthetics, where indicated.

17.4.1.4 Risks and hazards secondary to IV Scopolamine infusion

AEs that have occurred have been transient in nature (<3 hours) and have included light headedness, sedation, a transiently reduced heart rate and blood pressure (requiring no intervention) and the recognised anticholinergic adverse effects of dry mouth and blurred vision (Drevets and Furey, 2010; Furey and Zarate, 2013).
Pulsed IV administration has not been associated to date with potential AEs noted with high oral dosages including delirium, (hypo)mania, psychosis or disorientation (Furey and Zarate, 2013).

17.4.1.5 Psychological risks

Distress
Some participants may find questionnaires distressing or anxiety-provoking. This is difficult to predict, however researchers will be vigilant for possible negative psychological effects and seek to minimise these wherever possible. Most participants will have experience in answering questions similar to those on the questionnaires and many will previously have completed similar questionnaires. Researchers are all experienced in conducting clinical trials and in research with individuals with bipolar disorder and other major mental illnesses. If participants are distressed, they will be supported and given time out from completion of the questionnaires. If the distress continues, they will be advised that any remaining questionnaires are not required. Where significant distress is present, the clinical team of the participant will be informed by the research team, so that additional support after the clinical visit can be provided.

17.4.1.6 Psychosocial risks

Inconvenience
Attending for assessments or interventions may cause inconvenience to participants. It is anticipated that participants will predominantly reside in Galway or close to Galway. Participant visits will be organised so they will not have to wait prior to review by the researcher.

17.5 Participant confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be pseudo-anonymised and identified by initials and a participant's identification number on the CRF and any database. All documents will be stored securely. The study will comply with the Data Protection Act and the General Data Protection Regulation (GDPR).

17.6 Other Ethical considerations

The vulnerability of this study group is fully appreciated, and every effort will be undertaken to protect their safety and well-being. During their time on the trial, participants will continue usual care, as recommended by their responsible clinical team. In line with the applicable regulatory requirements, consenting processes will be standardised and a robust SOP for consenting participants will be adhered to.

18. Financing and insurance/indemnity

Indemnity is provided by indemnity cover for research in place from NUI Galway/State Claims agency (Clinical Indemnity scheme) once the trial is approved by a Clinical Research Ethics Committee and regulatory authority.

Funding has been attained from the Stanley Medical Research Institute (SMRI) -Treatment Trials. The SMRI is a non-profit organisation supporting research in the cause of, and treatments for, schizophrenia and bipolar disorder based in the United States of America.

19. Clinical study report and publication policy

The publication policy involves formal presentation of study findings at national and international neuroscience and psychiatry meetings including potentially the College of Psychiatrists of Ireland Winter Conference, Biological Psychiatry annual conference and the International Society for Bipolar Disorder annual conference, following consultation with the sponsor and the HRB-CRFG.

Final findings will be submitted for peer-review and publication in relevant high-impact scientific journals and upon publication may be further publicised in national and international print and electronic media though public relations departments and the NUI Galway website.

Further knowledge dissemination will include registering the trial in the EudraCT database and publication of the trial protocol in a peer-reviewed journal.

Research progress and developments will be presented at in-house research meetings with the Department of Psychiatry at Galway University Hospitals and at the HRB-CRFG.

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