Abnormal ventilatory response to carbon dioxide in epilepsy patients a potential biomarker for seizure induced respiratory depression & modification by SSRI- a pilot study

Protocol Identifying Number: 201607761

NCT02929667

Principal Investigator: Rup Sainju, MBBS

IND/IDE Sponsor: NA

Funded by: CURE Epilepsy

Draft or Version Number: v 01.2

June 28th, 2017

Table of Contents

Abnormal ventilatory response to carbon dioxide in epilepsy patients a potential biomarker for seizure induced respiratory depression & modification by SSRI- a pilot study

Version 01.2
Protocol:201607761 06/28/2017

LIST OF ABBREVIATIONS

SUDEP	Sudden unexpected and unexplained death in patients with epilepsy
HCVR	Hypercapnic Ventilatory Response
VE	Minute Ventilation
ETCO2	End tidal carbon dioxide
VRTCO2	Ventilatory recruitment threshold for carbon dioxide
EMU	Epilepsy Monitoring Unit

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by the following (use applicable regulations depending on study location and sponsor requirements; examples follow): • United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812) • ICH E6 All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: **Rup Sainju, MD**
Print/Type Name

Signed: _____ Date: 6/28/2017

PROTOCOL SUMMARY

Title:

Abnormal ventilatory response to carbon dioxide in epilepsy patients: a potential biomarker for seizure induced respiratory depression & modification by SSRI- a pilot study

Précis:

Double blind randomized phase II clinical trial.
Patients with epilepsy either undergoing video EEG study or being planned for one, will be screened by HCVR test. Fourty subjects with a depressed response (slope <2.0 L/mim/mm Hg) will be randomized to either fluoxetine or placebo (1:1)

Intervention will be for 6 weeks.

Subjects will be enrolled in two different locations, EMU or neurology clinic. A follow up HCVR testing will be done at one month.
Continuous VEEG, and comprehensive respiratory monitoring (chest & abdominal belts, oro-nasal pressure & thermistry, pulse oximeter and trancutanesous CO_2 monitor) will be collected.
Change in the HCVR before and after intervention in two groups will be compared to determine effect of the drug. Similarly, differenc in severity of peri-ictal respiratory abnormalities will be compared between two arms.

Objectives:

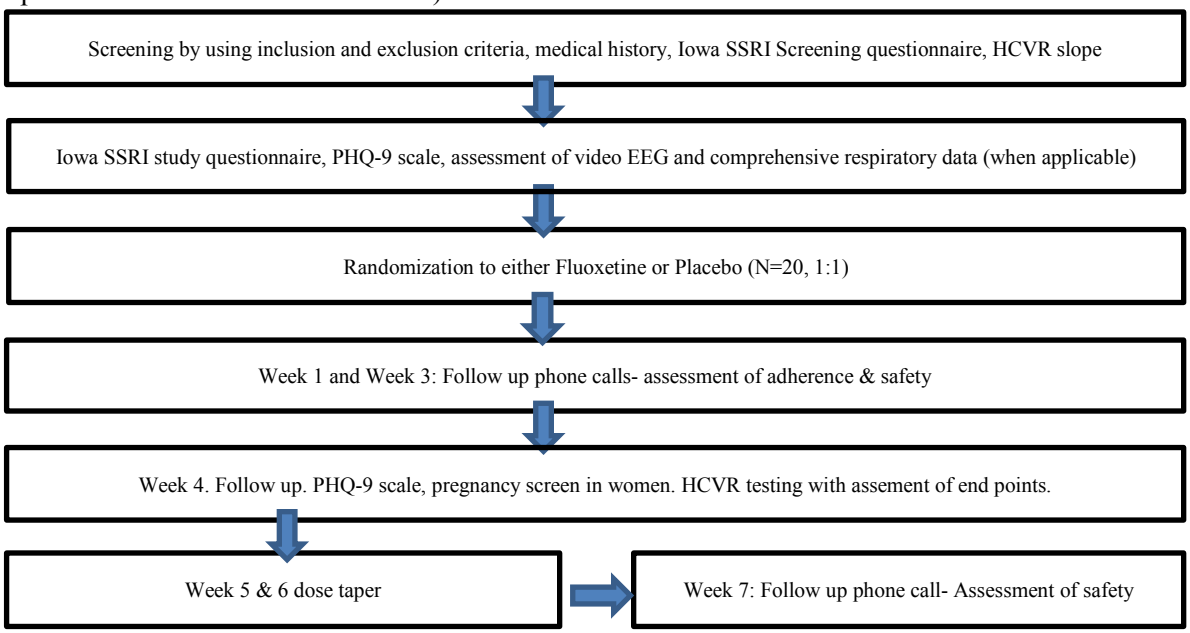
Primary Objective:
To assess feasibility of successfully enrolling subjects

Secondary Obective:
To evaluate effect of fluoxetine on HCVR and peri-ictal respiratory depression in drug resistant epilepsy patients.

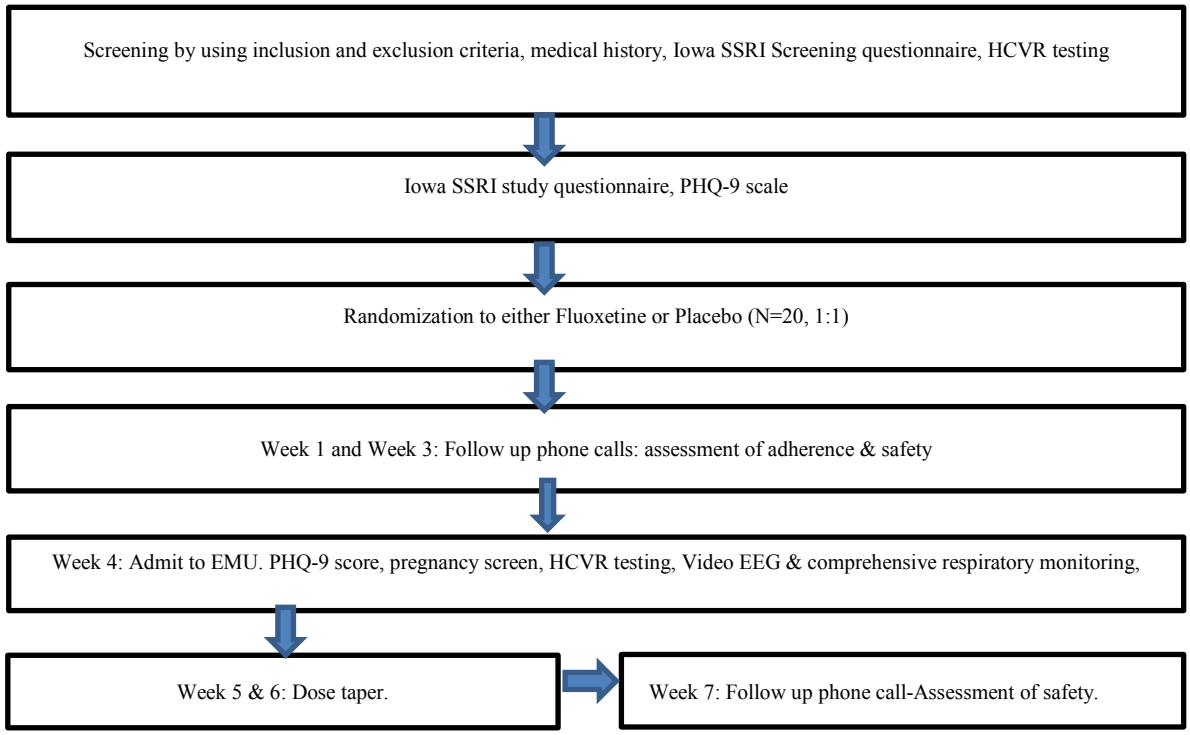
Endpoint	Primary End point: Study recruitment rate assessed every 3 months through the study completion Study retention rate assessed every 3 months through the study completion Secondary End point: Change in mood score using PHQ-9 score Change in minute ventilation during HCVR testing Change in slope of HCVR Difference in duration of abnormal breathing in periictal period Difference in oxygen saturation during periictal period Differrence in CO_2 level in periictal period Exploratory End points: Change in $VRTCO_2$ Change in time to $VRTCO_2$ Change in time to end of HCVR testing
Population:	Subjects age 18-75 years, both genders, drug resistant epilepsy with no prior severe depression or other psychiatry co-morbidities but otherwise healthy, mostly living in State of Iowa will be screened for this study.
Phase:	2
Number of Sites enrolling participants:	1
Description of Study Agent :	Fluoxetine, given by mouth with a standard titration over 6 weeks. 10 mg for 1 week, 20 mg for 1 week, 40 mg for 2 weeks, 20 mg for 1 week, 10 mg for 1 week then STOP
Study Duration:	2 years
Participant Duration:	6 weeks

SCHEMATIC OF STUDY DESIGN

A. Group 1: Enrollment in Epilepsy Monitoring Unit (EMU) or Neurology Clinic (patients not planned for Video EEG admission.)



B. Group 2: Enrollment in neurology clinic: Epilepsy patient planned for video EEG admission



1 KEY ROLES

Rup Sainju, MBBS
Role: Principal investigator
Institution: The University of Iowa,
Address: 200 Hawkins Drive, 2nd floor- RCP neurology clinic, Iowa City, Iowa, 52242, USA
Phone number: 319-353-7767
Email: *rup-sainju@uiowa.edu*

Brian Gehlbach, MD
Role: Co-Investigator
Institution: The University of Iowa,
Address: 200 Hawkins Drive, Iowa City, Iowa, 52242, USA
Phone number: 319-356-3601
Email: *brain-gehlbach@uiowa.edu*

Mark Granner, MD
Role: Co-Investigator
Institution: The University of Iowa,
Address: 200 Hawkins Drive, 2nd floor- RCP neurology clinic, Iowa City, Iowa, 52242, USA
Phone number: 319-356-7235
Email: *mark-granner@uiowa.edu*

George Richerson, MD PhD
Role: Co-Investigator
Institution: The University of Iowa,
Address: 200 Hawkins Drive, 2nd floor- RCP neurology clinic, Iowa City, Iowa, 52242, USA
Phone number: 319-356-4296
Email: *George-richerson@uiowa.edu*

Patrick Ten Eyck, PhD
Role: Biostatistician
Institution: The University of Iowa,
Address: 200 Hawkins Drive, Iowa City, Iowa, 52242, USA
Phone number: 319-384-5242
Email: *patrick-teneyck@uiowa.edu*

Gordon Buchanan, MD PhD
Role: Independent Medical Examiner
Institution: The University of Iowa,
Address: 200 Hawkins Drive, 2nd floor- RCP neurology clinic, Iowa City, Iowa, 52242, USA
Phone number: 319-335-9812
Email: *Gordon-buchanan@uiowa.edu*

Abnormal ventilatory response to carbon dioxide in epilepsy patients a potential biomarker for seizure induced respiratory depression & modification by SSRI- a pilot study

Version 01.2
Protocol:201607761 06/28/2017

Deidre Dragon
Role: Research Coordinator
Institution: The University of Iowa,
Address: 200 Hawkins Drive, 2nd floor- RCP neurology clinic, Iowa City, Iowa, 52242, USA
Phone number: 319-356-4337
Email: deidre-nitschke-dragon@uiowa.edu

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Sudden unexpected death in epilepsy patients (SUDEP) ranks second only to stroke among neurological diseases in years of potential life lost (YPLL). While the specific mechanisms of death in SUDEP are not well understood, per-ictal respiratory dysfunction likely plays an important role in many cases. Seizure related respiratory depression of variable severity is commonly observed, and on rare instances may be fatal. There is a gap in our knowledge regarding the mechanisms responsible for peri-ictal respiratory dysfunction. A better understanding of these mechanisms would open the door to interventions to decrease the risk of SUDEP.

Several lines of evidence support a critical role for the serotonergic system in central control of ventilation. Serotonin neurons in the raphe nuclei of the brainstem sense rising carbon dioxide and low pH, thereby stimulating breathing and arousal. These responses may serve as mechanisms that protect against asphyxia, particularly during sleep or the post-ictal state. In addition, data from animal and human studies also indicate that serotonin possesses independent anti-seizure properties. Finally, in mouse models of seizure-induced sudden death, pre-treatment with selective serotonin reuptake inhibitor (SSRI) agents prevents death following seizures. These observations led us to formulate our *central hypothesis:* that a subset of epilepsy patients has impaired central chemo-responsiveness as tested by the ventilatory response to inhalation of carbon dioxide (hypercapnic ventilatory response, HCVR), and this correlates with a greater degree of peri-ictal respiratory depression, thereby increasing the risk of terminal cardiorespiratory dysfunction and death. We further hypothesize that the administration of fluoxetine (an SSRI agent) to patients with a depressed HCVR will enhance CO_2 sensitivity, and reduce the severity of peri-ictal respiratory depression.

2.2 RATIONALE

Fluoxetine is an FDA approved drug commonly and safely used to treat depression, anxiety, obsessive compulsive disorder and eating disorders. Its toxicology has been well studied in animal and human. Fluoxetine has been used safely in human to modify HCVR in panic disorder population. However, it has been not studied yet in epilepsy population. We hypothesize that administration of fluoxetine (an SSRI agent) to patients with a depressed HCVR will enhance CO_2 sensitivity, and reduce the severity of peri-ictal respiratory depression.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

Fluoxetine is a FDA approved antidepressant medicine which is used very frequently and safely. Some patients may experience some side effects that include: Nausea, diarrhea, anorexia, dry mouth, dyspepsia, insomnia, anxiety, nervousness, somnolence, tremor, decreased libido, abnormal dreams, headache, asthenia, flu like syndrome, fever, weight loss, Flatulence, constipation, rash, pruritus, abnormal vision etc. However, rare and serious side effects are also possible which include; worsening depression, increase suicidal thoughts or actions, mood swings, acting aggressive or violent, serotonin syndrome, severe allergic reaction- trouble breathing, swelling of face, tongue, eyes or mouth, rash, hives, abnormal bleeding if you are taking blood thinner coumadin warfarin (Coumadin®), a non-steroidal anti-inflammatory drugs (NSAIDs, like Ibuprofen or naproxen), or aspirin, increase in seizure frequency and possible irregular heart rhythm called Torsades de pointes due prolonged QT.
Fluoxetine is classified by FDA as category "C" for use in pregnancy which means there is a known risk of birth defect animal fetus but there are no adequate data and well-controlled studies in human.
Stopping fluoxetine suddenly can cause serious symptoms including:

- Anxiety, irritability, high or low mood, feeling restless or changes in sleep habits
- Headache, sweating, nausea, dizziness
- Shaking, confusion etc

2.3.2 KNOWN POTENTIAL BENEFITS

Improvement in mood.

3 OBJECTIVES AND PURPOSE

Primary Object: To assess feasibility of successfully enrolling subjects

Secondary Obect: To evaluate effect of fluoxetine on HCVR and peri-ictal respiratory depression in drug resistant epilepsy patients.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

Double blind placebo-controlled randomized prospective trial

Two enrolling locations: EMU and Neurology Clinic

- 1) *To test effect of Fluoxetine on HCVR:* Subjects will be screened in the EMU and neurology clinic. Subjects eligible are those with patients with epilepsy who have a reduced baseline HCVR slope (<2.0 L/min/mmHg). Subjects will be enrolled, and randomized to either fluoxetine or placebo (1:1) until 20 subjects complete the study. Investigational Drug Services (IDS), at the University of Iowa Hospitals and Clinics (UIHC), will be responsible for randomizing and dispensing the

drugs. Both fluoxetine and placebo will be encapsulated in identical casing to mask their identity. Both the subjects and the investigators will be blinded to the randomization process. A standard titration plan will consist of fluoxetine or placebo 10 mg per day for a week, then 20 mg per day for a week and then 40 mg per day for 2 weeks, upon discharge. Subjects will return after 4 weeks for follow up HCVR testing in the Clinical Research Unit at UIHC. For each subject, the magnitude of post-treatment HCVR in terms of HCVR slope, minute ventilation, $VRTCO_2$, time to $VRTCO_2$ and time to target $ETCO_2$ at the end of the study will be compared with their baseline HCVR. The medication adherence rate, safey concerns and change in mood scale will be assessed at follow up. Reported adverse effects will be collected for all subjects. Enrolment rate and study completion rates will also be assessed every 6 months.

- **2)** *To test effect of fluoxetine on peri-ictal respiratory depression:* Subjects will be screened in neurlogy clinic from a group of previously diagnosed patients with epilepsy who are planned for VEEG study. Eligible subjects will undergo HCVR testing. Eligible subjects with a reduced baseline HCVR slope (<2.0 L/min/mmHg) will be enrolled and randomized to fluoxetine vs placebo (1:1) until a total of 20 subjects complete the study. Both the subjects and investigators will be blinded to the randomization process. A standard titration plan for fluoxetine & placebo will be as above, for 4 weeks prior to EMU admission. During EMU admission, the subjects will be monitored with VEEG, EKG, EMG, SpO_2, $tcCO_2/ETCO_2$, oral & nasal thermistor & pressure transducer to measure airway pressure, and RIP with abdominal & chest belts to measure change in circumference during respiration. Peri-ictal respiratory changes in the two groups will be compared to assess the effect of fluoxetine.

4.2.1 PRIMARY ENDPOINT

Study recruitment rate assessed every 3 months
Study completion rate assessed every 3 months

4.2.2 SECONDARY ENDPOINTS

Change in mood score using PHQ-9 score
Change in minute ventilation during HCVR testing
Change in slope of HCVR
Difference in duration of abnormal breathing in periictal period
Difference in oxygen saturation during periictal period
Differrence in CO_2 level in periictal period

4.2.3 EXPLORATORY ENDPOINTS

Change in $VRTCO_2$
Change in time to $VRTCO_2$
Change in time to end of HCVR testing

We will compare all the secondary outcome and exploratory measures using generalized linear models (GLMs), specifying an identity link for normally distributed data and the log link for right-skewed data.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

1. Adult patients aged 18 or older
2. Patients with epilepsy,
3. Native English speaker or adequate fluency in English to provide informed consent.
4. Female patients of child-bearing potential must be using two acceptable method of contraception, or willing to refrain from sexual intercourse during the study.

5.2 PARTICIPANT EXCLUSION CRITERIA

1. Progressive neurological disease.
2. Clinical diagnosis of bipolar disease, panic disorder, psychosis or severe depression, or PHQ-9 score > 20
3. Patients with prior hospitalization related to depression or Electroconvulsive therapy.
4. History of suicidal ideation or intent in past or present
5. Clinical history or laboratory evidence of hepatic or renal insufficiency.
6. Pregnant or lactating women.
7. Current heavy alcohol use (>14 drinks per week for men or >7 drinks per week for women) or) known medical disorder related to alcohol use or current illicit drug use.
8. Patients with recent use (<1 month) or already taking fluoxetine or other selective serotonin reuptake inhibitors (SSRIs).
9. Concurrent use of monoamine oxidase inhibitors, antipsychotic agents, antidepressant agents other than SSRIs or frequent use of triptan agents (>2/week).
10. History of a previous allergic reaction or adverse effects with fluoxetine, hypersensitive reaction-anaphylaxis; laryngeal edema; hives
11. History of serotonin syndrome.
12. History of uncontrolled pulmonary or cardiac illness.
13. Patients with normal hypercapnic ventilatory response, i.e. HCVR slope of $\geq$2.0
14. Patients with known prolong QT interval
15. Patients with family history of prolong QT interval
16. Patients with family history of sudden cardiac death.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Patients, 18-75 year age, with epilepsy being treated at EMU & neurology clinic at the University of Iowa Hospital & Clinics will be screened for their eligibility for this study. Subjects with lack of consent capacity including severe mentally ill, prisoners, cognitive impaired, pregnant, children, prisoner, non English speaking and employee vounteers will be excluded from the study.

Approximately, 100 subjects will undergo HCVR testing for screening; Eligible subjects will be randomized to fluoxetine vs placebo, until each group has 20 completed subjects.
Anticipated accrual rate: 2/month
Number of sites and participants in US site: 1
Source of participants and Recruitment venues: Patients, 18-75 year age, with epilepsy being treated at EMU & neurology clinic at the University of Iowa Hospital & Clinics
Types of advertisements planned: Emails with information regarding the study will be distributed among neurology physicians working at the University of Iowa Hospitals & Clinics.

Subjects will be contacted via telephone as a part of study protocol followup which may improve retention.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may terminate participation in the study if:

- Any clinical serious adverse events related or unrelated to the study.
- The participant meets an exclusion criteria (either newly developed or not previously recognized) that precludes further participation.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

If the participant withdraws or terminates participation in the study prior to randomization to one of the intervention arms, no attempt for follow up will be made.

If the participant withdraws or terminates participation in the study after randomization to one of the intervention, we will attempt to contact them via phone calls for safety follow up. No other outcome measures will be collected.

If the participant in the study develops serious adverse events (SAEs), they will be terminated from further participation in the study. They will be referred for appropriate evaluation and treatment (if already not being done). They will be followed up until resolution of the adverse events.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Poor recruitment and/or completion rates

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

The study drug (fluoxetine) and placebo are compounded by Belmar Research, Lakewood Colorado but will be dispensed by Investigational Drug Service, University of Iowa Hospitals and Clinics.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Blue opaque capsule, no lettering or embossing

6.1.3 PRODUCT STORAGE AND STABILITY

NA

6.1.4 PREPARATION

Belmar Research will use commericially available generic fluoxetine for the study but will prepare identical placebo.

6.1.5 DOSING AND ADMINISTRATION

Once a day by mouth

6.1.6 ROUTE OF ADMINISTRATION

Oral

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

10 mg once a day for one week, 20 mg once a day for one week, 40 mg once a day for two weeks, 20 mg once a day for one week and 10 mg once a day for one week. Then STOP.

6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

Subjects will be allowed to take 40 mg/day of drug vs placebo for maximum of an extra 1 week in case of delay in follow up appointment.

6.1.9 DURATION OF THERAPY

6 weeks

6.1.10 TRACKING OF DOSE

Phone follow up and counting pill in the pill container at follow up visit.

6.1.11 DEVICE SPECIFIC CONSIDERATIONS

NA

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

Belmar Research will be responsible for compounding. But, Investigational Drug Services, Department of pharmaceutical care at the University of Iowa Hospitals & Clinics will be responsible for storing, labelling and dispensing of the drugs.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

- Review medical history using electronic medical record, interviewing the potential subjects and use Iowa SSRI screening questionnaire.
- Medication history will be reviewed by direct interview with the subjects
- Screening for pregnancy will be done using questionnaire and urinary pregnancy test as applicable
- HCVR testing will be performed (as applicable) for baseline assessment prior to randomization and a follow up HCVR testing after randomized to an intervention arm.
- Eligible subjects will answer Iowa SSRI study questionnaire, and PHQ-9 scale
- Assessment of study drug adherence will be done by follow up phone calls and direct pill count at follow up visit
- Continuous comprehensive respiratory monitoring (respiratory inductance plethysmography abdominal and chest belts, nasal pressure, oro-nasal thermistry, pulse oximetry and transcutaneous carbon dioxide) will be done when the subjects are in EMU.
-

7.1.2 STANDARD OF CARE STUDY PROCEDURES

- Continuous video EEG study in EMU

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Urine pregnancy test (as applicable) will be done prior to HCVR.

HCVR testing in EMU:
Ultima PFX with gas chromatography machine will be used for HCVR testing. The machine will be operated by a well-trained respiratory therapist.

Subjects enrolled in EMU will have their baseline HCVR testing at the bedside either in a sitting up in their bed or in a chair. And those newly enrolled or follow up subjects in neurology clinic will have their

HCVR testing in CRU. Subject will wear a noise cancelling headphone and will be asked to look at a white/blank screen during the procedure.

The subjects will apply a nasal clip and then breathe through through a Y-valve that allows switching from room air to a 5-liter rebreathing bag filled with 50% oxygen, 6% carbon dioxide, and balance nitrogen.. The test will begin with measurement of baseline respiratory parameters- respiratory rate, minute ventilation, tidal volume, $ETCO_2$, end-tidal oxygen breathing in room air for 2 minutes. They will then asked to hyperventilate to bring their $ETCO_2$ to 30 mm Hg.Then, they will be switched to a rebreathing bag. The subjects will be instructed to take 2 deep breaths promote rapid equilibration of PCO2 between the rebreathing bag and the alveolar, arterial and mixed venous compartments and then however they feel to make themselves comfortable.

The test will be terminated for: (1) $ETCO_2$ > 55 mm Hg; (2) ETO_2< 160 mm Hg; (3) minute ventilation > 100 liters/minute; and/or (4) subject intolerance.

At the end of the test, the subjects will be asked to fill out questionnaire regarding their experience during the tests.

7.2.2 OTHER ASSAYS OR PROCEDURES

None

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

None

7.2.4 SPECIMEN SHIPMENT

NONE

7.3 STUDY SCHEDULE

7.3.1 SCREENING

Potential subjects for the study will be are referred by treating neurologists at neurology clinic & EMU at the University of Iowa Hospitals & Clinics.

- Referred subjects are screened based on review of medical history & Iowa SSRI screening questionnaire.
- Written informed consent will be obtained from the participants
- Subjects will undergo HCVR testing for determining eligibility for enrolling in intervention part of the study

7.3.2 ENROLLMENT/BASELINE

- Obtain informed consent of potential participant verified by signature on study informed consent form.
- Verify inclusion/exclusion criteria.
- Obtain urine pregnancy test when applicable.
- Obtain demographic information, medical history, medication history, alcohol and tobacco use history.

- Perform HCVR testing
- PHQ-9 score
- Randomize to one of the intervention arm
- Discharge randomized subjects with assigned treatment with one week of 10 mg, one week of 20 mg and 4 weeks of 40 mg pills.

7.3.3 FOLLOW-UP

Follow up phone call at end of week 1 and 3

Record for adverse events as reported by the participants.
If suspicious for mania, will perform full Altman self-report maniac scale
Record compliance to assigned treatment as reported by the participants.
Record current medication

Follow up visit 4 weeks (+/-1 week) after randomization

Record for adverse events as reported by the participants.
Record compliance to assigned treatment as reported by the participants and by counting pill from the pill container brought by the participants.
Record PHQ-9 score
Repeat HCVR testing
Record current medication
Video EEG study with comprehensive respiratory monitoring in EMU for paticipants enrolled at neurology clinic

7.3.4 FINAL STUDY VISIT

Follow up phone call at week 7:

Record for adverse events as reported by the participants.
Record current medication

7.3.5 EARLY TERMINATION VISIT

NA

7.3.7 SCHEDULE OF EVENTS TABLE

Procedures	Screening	Enrollment	Follow up phone call	Follow up phone call	Follow up visit at week 4	Final follow up phone call								
Informed consent	X													
Demographics	X													
Medical history	X													
Iowa SSRI Sscreening Questionnaire	X													

Procedures	Screening	Enrollment	Follow up phone call	Follow up phone call	Follow up visit at week 4	Final follow up phone call								
Pregnancy screening questionnaire & urinary pregnancy test	X				X									
Medication history	X		X	X	X	X								
Iowa SSRI study questionnaire		X												
HCVR testing	X													
PHQ-9 score		X			X									
Randomization		X												
Video EEG & respiratory monitoring in EMU	X[a]	X[a]			X[b]									
Adverse events evaluation			X	X	X	X								
Assessment of compliance			X	X	X									

a: Subjects enrolled from EMU
b: Subjects enrolled from neurology clinic

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

Titration schedule of the drug is designed to minimize adverse events as well as withdrawal symptoms.

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

7.5.1 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

Use of pain medicines like NSAIDs, and Aspirin. Use of blood thinner including Aspirin and warfarin.

7.6 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

Subjects will be asked to avoid starting any new medications that may be associated with interaction or lead to serotonin syndrome. These include antidepressants, antipsychotics, other SSRI agents, migraine medicine- particularly “Triptan family”, linezolid, methylene blue. These medication can lead to “serotonin syndrome”.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

NA

7.8 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

NA

7.9 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

NA

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

All serious and non serious adverse events will be recorded

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention, whether or not considered intervention-related.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant physical or mental incapacity including worsening depression, increase suicidal thoughts or actions, acting aggressive or violent , or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic reactions including angioedema of face, tongue, mouth, and bronchospasm requiring intensive treatment in an emergency room or at home, or increased seizure frequency from baseline that do not result in inpatient hospitalization.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

Unanticipated problems (UP) per OHRP, are any incident, experience, or outcome that meets all of the following criteria:

• Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

• Related or possibly related to participation in the research (“possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

• Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. This study will use the OHRP definition of UP

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 RELATIONSHIP TO STUDY AGENT

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or"definitely related," as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whosetemporal relationship to drug administration makes a causal relationship improbable (e.g., theevent did not occur within a reasonable time after administration of the trial medication) and inwhich other drugs or chemicals or underlying disease provides plausible explanations (e.g., theparticipant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study drug administration, and/or evidenceexists that the event is definitely related to another etiology. There must be an alternative,definitive etiology documented by the clinician.
-

8.2.3 EXPECTEDNESS

PI, will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate RF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.
Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 days after the last day of study participation. At each study visit/followup phone visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

Adverse events that are serious will reported to independent medical examiner and IRB.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

The study clinician will complete a SAE Form within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the SAE Form and submitted to the independent medical examiner within 24 hours of site awareness. See **Section 1, Key Roles** for contact information.
- Other SAEs regardless of relationship, will be submitted to independent medical examiner within 72 hours of site awareness.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the adherence to be stable. Other supporting documentation of the event may be requested by the independent medical examiner and should be provided as soon as possible. The principal investigator will be responsible for notifying IRB of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after site awareness.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. The investigator shall report UPs to the IRB and to the IME. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB projectnumber;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcomerepresents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken orare proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are lifethreatening or death, will be reported to the IRB and to the IME within 1 day of the investigator becoming aware of the event. But other SAEs, will be reported within 3 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IME only within 7 days of the investigator becoming aware of the problem.

8.4.4 REPORTING OF PREGNANCY

Women with potential for being pregnant will be screened to rule out active pregnancy using standard questionnaire and urinary pregnancy test. If pregnancy is reported by the participant while on study medicine, the medicine will be discontinued and she shall be referred for high risk obstetric clinic.

Each pregnancy must be reported by the Investigator to the IME on the initial pregnancy report form within 30 days after becoming aware of the pregnancy. The Investigator must follow-up and document the course and the outcome of all pregnancies even if the subject was withdrawn from the clinical study or if the clinical study has finished.

All outcomes of pregnancy must be reported by the Investigator to the Sponsor on the pregnancy outcome report form within 30 days after he/she has gained knowledge of the normal delivery or elective abortion.

8.5 STUDY HALTING RULES

Administration of study agent will be halted when three grade 3 (severe) AEs determined to be "probably related" are reported to the IME. The IME will notify the study sponsor and investigators immediately when the third grade 3 event is reported and enrollment screens will stop accepting new study participants. The study sponsor will provide the IME with AE listing reports within 24 hours. The IME will provide recommendations for proceeding with the study to the study sponsor. The study sponsor may inform the FDA of the temporary halt and the disposition of the study.

8.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of an independent medical examiner (IME). The IME will assess safety and efficacy data on each arm of the study. The IME will provide its input to IRB, sponsor and principal investigator

9 CLINICAL MONITORING

NA

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

This study is designed as a pilot to obtain estimates to be used in future studies. We will report the descriptive statistics for both treatment groups for each endpoint.

10.2 STATISTICAL HYPOTHESES

No statistical hypothesis

10.3 ANALYSIS DATASETS

Per protocol analysis

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

Categorical data descriptive statistics will be presented as percentages, while continuous data descriptive statistics will be presented with the mean and standard deviation. Due to our goal of establishing estimates of centrality and spread for various measures to be used in future studies, no inferential tests will take place in this study.

10.4.2 ANALYSIS OF THE PRIMARY ENDPOINT(S)

Enrollment rate:
This will be assessed every 3 months until the end of the enrollment period. It will be reported as number of participants enrolled per 3 months. It will also be reported as percentage of all the patients screened.

Retention rate:
This will be assessed every 3 months. It will be reported as number of participants completed the study per protocol per 3 months. It will also be reported as percentage of all the patients screened.

10.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Observations with missing data are dropped from those analyses involving data variable.

Change in mood score using PHQ-9 score
This will be analyzed as the difference in PHQ-9 scores from baseline to final measurement between drug and placebo. The mean and standard deviation estimates of the differences will be provided.

Change in minute ventilation during HCVR testing:
This will be analyzed as the difference in minute ventilation during HCVR testing from baseline to final measurement between drug and placebo. The mean and standard deviation estimates of the differences will be provided.

Change in slope of HCVR
This will be analyzed as the difference in slope of HCVR between drug and placebo. Using a linear mixed model, we will include a predictor that is the interaction between HCVR slope and the treatment group. The mean and standard deviation estimates of the differences will be provided.

Difference in duration of abnormal breathing during periictal period:
Mean duration of abnormal breathing during perictal periods between drug and placebo will be compared. The mean and standard deviation estimates of the differences will be provided.

Difference in oxygen saturation during periictal period
Mean drop in oxygen saturation during perictal period between drug and placebo will be compared. The mean and standard deviation estimates of the differences will be provided.

Difference in CO_2 level in periictal period
Mean change in CO_2 level in periictal period compared to pre seizure baseline will be compared between drug and placebo. The mean and standard deviation estimates of the differences will be provided.

All the secondary outcome measures will be estimated using generalized linear mixed models (GLMMs), specifying an identity link for normally distributed data and the log link for right-skewed data.

Since we are interested in obtaining estimates for future studies, we will not run non-parametric analyses, which only provide p-values.

Assessment of adverse events
All the adverse events will be recorded. Frequency of each adverse events will be compared between the two groups and tested using one tail t-test with type I error at 0.025

10.4.4 SAFETY ANALYSES

All the adverse events will be recorded. Severity and relationship to intervention will be determined as described in section 8. Frequency of each adverse events will be compared between the two groups and tested using one tail t-test with type I error at 0.025.

Adverse events leading to premature discontinuation from the study drug and serious treatment-emergent AEs should be presented either in a table.

10.4.5 ADHERENCE AND RETENTION ANALYSES

Adherence to the randomized intervention will be assessed by telephone interview and in person interview during follow up.

10.4.6 BASELINE DESCRIPTIVE STATISTICS

We plan to compare the baseline descriptive statistics between the treatment and control groups and establish if there is a significant difference of any variables. For continuous variables, a two sample t-test will be used to compare data that appear to be normally distributed (Wilcoxon Rank Sum test for skewed data). For categorical data, Pearson's Chi-square test of independence will be used.

10.4.7 PLANNED INTERIM ANALYSES
None

10.4.7.1 SAFETY REVIEW
NO statistical plan for safety review.

10.4.7.2 EFFICACY REVIEW
Not applicable

10.4.8 ADDITIONAL SUB-GROUP ANALYSES
No additional sub-group analyses are needed for this pilot study.

10.4.9 MULTIPLE COMPARISON/MULTIPLICITY
NA

10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

NA

10.4.11 EXPLORATORY ANALYSES

Change in $VRTCO_2$
Mean change $VRTCO_2$ between drug and placebo will be compared. The mean and standard deviation estimates of the differences will be provided.

Change in time to $VRTCO_2$
Mean change in time to $VRTCO_2$ between drug and placebo will be compared. The mean and standard deviation estimates of the differences will be provided.

Change in time to end of HCVR testing
Mean change in time to end of HCVR testing will be compared between drug and placebo. The mean and standard deviation estimates of the differences will be provided.

10.5 SAMPLE SIZE

No formal sample size calculation is performed because the primary puporse of the project is assess feasibility.

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Subjects will be screened and enrolled in two locations, neurology clinic and EMU. A total of 20 subject will be enrolled in each location and they will be randomized to drug vs placebo group equally (1:1) using computer generated random numbers. The assignment for each subject will be kept in a sealed envelope and will be provided to investigational drug pharmacy at the University of Iowa.

The participants as well as the investigators (except one) will be blinded to the drug the participant is receiving. Bottles of the study drug will be labelled with the name of study, drug A or drug B, and instruction of titration plan.

10.6.2 EVALUATION OF SUCCESS OF BLINDING

NA

10.6.3 BREAKING THE STUDY BLIND/PARTICIPANT CODE

The blinding would be broken in the circumstance of severe adverse events. The treating physicians will be unblinded. Both intentional and unintentional unblinding will be reported to IME.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, video EEG, study questionnaires, HCVR data, AE and concomitant medication reporting, raw

data collection forms, etc.) designed to record all observations and other pertinent data for each subject receiving investigational product.

The source documents will be maintained and stored in password protected network drives. The Investigator will allow Sponsor representatives, independent medical examiner (IME), and the IRB to have direct access to all documents pertaining to the study.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

12.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

12.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting any procedure/administering study product.

Once the participant has signed the consent form, he/she will be asked to fill out “Iowa SSRI screening questionnaire, Iowa SSRI study questionnaire and PHQ-9 questionnaire”, as approved by IRB.

12.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual’s agreeing to participate in the study and continues throughout the individual’s study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator and or a designee will explain the research study to the participant and answer any questions that may arise. All

participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A record of consent will be filled out and scanned on the electronic health record of the subject.

If a protocol amendment is required, the informed consent document and other study related questionnaires may need to be revised to reflect the changes to the protocol. If the informed consent & other study related questionnaires/documents is revised, it must be reviewed and approved by the responsible IRB, and signed by all subjects subsequently enrolled in the clinical study as well as those currently enrolled in the clinical study.

12.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

IRB or federal regulatory body may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by IRB.

13 DATA HANDLING AND RECORD KEEPING

13.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Clinical data will be entered directly from the source documents. Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered

into microsoft excel database and stored in a network drive. The network drive is secured with password protection.

13.2 STUDY RECORDS RETENTION

Study documents will be retained for a minimum of 2 years after last patient is enrolled. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

13.3 PROTOCOL DEVIATIONS

Any deviation will be informed to IRB and appropriate corrective actions will be implemented within 3 days of after becoming aware of the deviation.

13.4 PUBLICATION AND DATA SHARING POLICY

By signing the clinical study protocol, the Investigator agrees with the use of results of the clinical study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the competent authorities will be notified of the Investigator‟s name, address, qualifications and extent of involvement.

An Investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with the Sponsor in advance.