Section 4. PROTOCOL

Investigational New Drug (IND)

PDC-1421 Capsule

(*Polygala tenuifolia* Willd.)

EDITION NUMBER : Version 2.3
SPONSOR : BioLite, Inc.
RELEASE DATE : 2018/03/16

A Phase II Study of PDC-1421 Capsule to Evaluate the Safety and Efficacy in Patients with Major Depressive Disorder

Table of Contents

PROTOCOL SYNOPSIS

Name of sponsor	BioLite, Inc.
Name of finished product	PDC-1421 Capsule
Name of active ingredient	PDC-1421
Protocol title	A phase II study of PDC-1421 Capsule to evaluate the safety and efficacy in patients with major depressive disorder (MDD)
Clinical trial phase	Phase II
Study site	Multiple sites
Study period	2 years
Study population	Patients with MDD according to the Diagnosis and Statistical Manual of Mental Disorders-IV, text revision (DSM-IV-TR) criteria.
Primary study objective	To assess the efficacy profile of PDC-1421 Capsule in major depressive disorder with Montgomery-Å sberg Depression Rating Scale (MADRS).
Secondary study objective	To evaluate the efficacy and safety profile of PDC-1421 Capsule in major depressive disorder with other rating scales.
Study design	Part I: open label Part II: Randomized, double-blind, placebo-controlled, parallel groups
Sample size	72 subjects
Test product	PDC-1421 Capsule
Dose and regimen	1 and 2 capsules thrice daily, p.o., after meal
Duration of treatment	42 days
Study intervention	The screening phase is intended for diagnosing and assessing the patient for possible inclusion in the study and for providing an adequate washout period. The following study will be conducted in two parts. Part I is an open-label study, multiple center and dose escalation evaluation in twelve patients. Six subjects each will be evaluated for safety and efficacy assessments at 1 or 2 capsules TID dose for 28 days, sequentially. Each of them

	will be assessed twice in the first week after administration of PDC-1421 Capsules and once a week in the following treatment. Part II is a randomized, double-blind, placebo-controlled, parallel-group study. 60 subjects will be randomly assigned on a 1:1:1 basis to one of the three arms (1 PDC-1421 Capsule plus 1 placebo TID, 2 PDC-1421 Capsules TID, 2 placebo TID) for 6 weeks and evaluated the safety and efficacy every two weeks during the treatment period.
Primary endpoint	• Change in Montgomery-Å sbergDepression Rating Scale (MADRS) total score from baseline to week 6 compared to placebo.
Secondary endpoints	• Change in MADRS total score from baseline to week 2 and 7. • Change in Hamilton Rating Scale for Depression (HAM-D-17), Hamilton Rating Scale for Anxiety (HAM-A), Depression and Somatic Symptoms Scale (DSSS), Clinical Global Impression Scale (CGI) total score from baseline to week 2, 4, 6 and 7. • Percentage of partial responders (defined as a participant with a 25-50% decrease from baseline in total score) and responders (defined as a participant with ≧50% decrease from baseline in total score) in MADRS by week 2, 4, 6 and 7. • Evaluate Safety Assessments and Columbia-Suicide Severity Rating Scale (C-SSRS) from screening stage to the last visit • Difference in the mean changes of MADRS, HAM-D-17, HAM-A, DSSS, CGI and C-SSRS from the baseline to week 2, 4, 6 and 7 between the PDC-1421 Capsule and placebo groups.
Safety Evaluation	• Change from baseline in physical examination, vital sign, electrocardiogram (ECG), hematology and blood chemistry • Incidence of AE/SAE
Statistical method	Descriptive Statistics

LIST OF ABBREVIATIONS/DEFINITIONS

- AE
  Adverse Events, whether or not considered related to the investigational drug, must be recorded in CRF.
- CGI
  Clinical Global Impression Scale (see Attachment III)
- CHO
  Chinese Hamster Ovary
- Clinical monitor
  The designated CRA monitoring this study for the sponsor
- CRA
  Clinical Research Associate
- CRC
  Clinical Research Coordinator
- CRF
  Case Report Form
- CRO
  Contract Research Organization
- C-SSRS
  Columbia-Suicide Severity Rating Scale (see Attachment VI)
- DSSS
  Depression and Somatic Symptoms Scale (see Attachment V)
- ECG
  The Electrocardiogram (ECG) is a graphical recording of the cardiac cycle produced by an electrocardiograph.
- HAM-D-17
  Hamilton Depression Rating Scale (see Attachment I)
- HAM-A
  Hamilton Anxiety Rating Scale (see Attachment IV)
- HDL
  High-Density Lipoprotein
- HPLC
  High Performance Liquid Chromatography
- ICF
  Informed Consent Form

- ICH-GCP
  International Conference of Harmonization-Good Clinical Practice)
- IEC
  Independent Ethics Committee
- IRB
  Institutional Review Board
- LDH
  Lactate Dehydrogenase
- LDL
  Lower-Density Lipoprotein
- MADRS
  Montgomery and Å sbergDepression Rating Scale (see Attachment II)
- MOHW
  Minister of Health and Welfare
- NET
  Norepinephrine Transporter
- NLT
  Not Less Than
- NMT
  Not More Than
- NOAEL
  No Observable Adverse Effect Level
- NRI
  Norepinephrine Reuptake Inhibitor
- PITDC
  Medical and Pharmaceutical Industry Technology and Development Center
- SAEs
  Serious Adverse Event(s), whether or not considered as related to the investigational drug must be recorded and reported.
  The serious adverse event is defined as following:
  Death
  Life-threatening condition
  Inpatient hospitalization or prolongation of existing hospitalization
  Persistent or significant disability/incapacity
  Congenital anomaly/birth defect
  Required intervention to prevent permanent impairment/damage
- Severity Rating of AE

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL†.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

†Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

- SOP
  Standard Operation Procedure
- Study Cohort
  The Study Cohort is defined as a group or cohort of subjects who are assigned to take the same dose level of the study drug.
- TLC
  Thin Layer Chromatography
- TBZ
  Tetrabenazine

1 BACKGROUND INFORMATION

1.1 Description of PDC-1421 Capsule

PDC-1421 Capsule is a botanical investigational new drug containing the extract of Radix Polygalae (*Polygala tenuifolia* Willd.) as active ingredient. Radix Polygalae is a traditional herb commonly prescribed in China to induce sedation, benefit the mentality and to promote expectoration. It has also been used for insomnia, anxiety, and heart palpitations[1].

1.1.1 Preparation

Raw materials originated from *P. tenuifolia* were obtained from Shanxi, China. After having been cleaned, and residual stems removed, the root was sun-dried and become the botanical raw material for producing the study drug (PDC-1421 capsule). Raw materials shall comply with physical/chemical specifications, and fingerprint determination via HPLC and TLC shall show desired contents. The materials shall comply criteria for heavy metal, pesticide residues and aflatoxins to avoid contaminations. Acceptable raw materials were water extracted, partially purified by column chromatography, concentrated, and spray-dried to become the drug substance PDC-1421, a yellowish powder. The yield rate from raw materials to PDC-1421 is about 1.4 %. Formulation and encapsulation of drug substance PDC-1421 produced the final product of PDC-1421 Capsule. Specifications of PDC-1421 Capsule are tabulated in Table 1.

Table 1. Specifications of PDC-1421 Capsule

Test Items	Specifications
Physical Appearance	Yellowish Granules in brownish capsule.
Loss on Drying	NMT 6%
Water Extractives	NLT 80%
Dilute-Alcohol Extractives	NLT 70%
Total Ash	NMT 5%
Acid-Insoluble Ash	NMT 4%
Alcohol Extractives	NLT 38%

TLC	1. One grayish blue band has same R_f value as reference standard (3',6-disinapoylsucrose); 2. One watchet blue band has same R_f value as reference standard (Glomeratose A).
HPLC of markers	1. 7.0 mg/g ≤ Glomeratose A ≤ 17.5 mg/g. 2. 38 mg/g ≤ 3',6-Disinapoylsucrose ≤ 95 mg/g.
UV Spectrum	λ_{max}: 230 – 240 nm; 315 – 325 nm
IR Spectrum (cm^{-1}, %T)	Peak at: 3380±100 Broad, 2938±20 Sharp, 1605±10 Sharp, 1455±10 Sharp, 833±10 Sharp.
pH	Dissolved in distill deionized water at a concentration of 0.1 g/ml, 3.8 ≤ pH ≤5.8.
Uniformity	90-110%
Weight Variation	90-110%
Heavy Metal	Cu < 20 ppm, As < 1 ppm, Pb < 5 ppm, Cd < 0.2 ppm, Hg < 0.1 ppm
Microbial Purity	Total aerobic plate count: NMT 10^3 CFU/g Mold and yeast count: NMT 100 CFU/g *Echerichia coli*: Undetectable *Staphylococcus aureus*: Undetectable *Salmonella*: Undetectable

NMT: not more than; NLT: not less than

PDC-1421 used in phase I clinical trial was manufactured at the Medical and Pharmaceutical Industry Technology and Development Center (PITDC) in New Taipei City, Taiwan. In order to scale up the production and in compliant with GMP (Good Manufacturing Procedures), the producer has been changed to the Herbal Medicine GMP Plant of the Industrial Technology Research Institute (ITRI) in Hsinchu County, Taiwan. Products produced at ITRI Plant have been tested and meet defined specifications prior to be used in this study.

1.1.2 Composition

Each PDC-1421 Capsule consists of 380 mg PDC-1421 drug substance and 20 mg of excipients. The composition of PDC-1421 Capsule is shown in Table 2.

Table 2. Composition of PDC-1421 Capsule

Name of Ingredient	mg per Capsule	Function
PDC-1421	380 mg	Active ingredient
Silicon dioxide	10 mg	Excipient
Magnesium stearate	10 mg	Excipient

1.1.3 Storage and Handling

During stability test, physical examination, loss on drying and contents assays of the characteristic markers (3',6-disinapoylsucrose and Glomeratose A) were conducted. The results indicated that PDC-1421 Capsule is stable when stored in a well-closed brown bottle at room temperature (15 ~ 25℃) for at least 24 months.

1.2 Non-clinical Study of PDC-1421

Pharmacology studies were conducted at MDS Pharma Services, Taiwan. Non-clinical toxicity studies were conducted in accordance with Good Laboratory Practice at the Development Center for Biotechnology, Taiwan. Safety pharmacology studies were compliance with Good Laboratory Practice by Level Biotechnology, Inc. and Charles River Laboratory, Inc.

1.2.1 Efficacy Pharmacology

Two *in vitro* studies and one *in vivo* study have been completed to evaluate pharmacological activities of PDC-1421.

1.2.1.1 Radio-ligand Binding Assay

Radio-ligand binding assays were performed on 168 molecular targets, including receptors, ion channels and transporters, to discover the possible functional agonism and/or antagonism of PDC-1421 at concentration of 100 μg/ml. Table 3 showed the result in this study, indicating that PDC-1421 specifically inhibited norepinephrine transporter. Other molecules, such as histamine receptor or cholinergic receptor, were not significantly influenced. The results suggested that PDC-1421 shall not induce side effects such as hypersomnia or dry month. Subsequent studies applying different concentration showed that the inhibition of norepinephrine transporter by PDC-1421 is dose-dependent, within 3 to 100 μg/ml. Norepinephrine transporter inhibitors have developed as antidepressants, such as imipramine, venlafaxine and reboxetine[2]. Therefore, PDC-1421 has the potential to be used as an antidepressant drug. The

subsequent studies were performed to investigate the antidepressant activities of PDC-1421.

Table 3. Summary of Significant Activities in radio-ligand binding assay

Assay Name	IC_{50} (μg/ml)
Norepinephrine Transporter (NET) Binding	11.8

1.2.1.2 Neurotransmitter Uptake Assay

Many antidepressants, including selective serotonin reuptake inhibitor or serotonin/norepinephrine reuptake inhibitor, are related to the inhibition of neurotransmitter reuptake[3]. Uptake assays were conducted to study the effect of PDC-1421 on the reuptake of norepinephrine, serotonin and dopamine. The results of uptake assay are summarized in Table 4.

Table 4. Summary for neurotransmitter uptake assay

Assay Name	Test Substance	Dose	Inhibition %	IC_{50}
Norepinephrine Uptake	PDC-1421	1 μg/mL	52	0.70 μg/mL
	Desipramine			0.44 μg/mL (1.66 nM)
Dopamine Uptake	PDC-1421	300 μg/mL	55	246 μg/mL
	Nomifensine			1.62 μg/mL (6.83 nM)
Serotonin Uptake	PDC-1421	100 μg/mL	52	107 μg/mL
	Fluoxetine			0.87 μg/mL (2.83 nM)

PDC-1421 inhibited Norepinephrine reuptake at a 0.70 μg/mL, much lower than of the concentration to inhibit dopamine (246 μg/mL) or serotonin (107 μg/mL). The results indicated that primary pharmacological effect of PDC-1421 may be with its norepinephrine reuptake inhibition, rather than serotonin or dopamine reuptake inhibition. The results are consistent with radio-ligand binding assay, indicated that PDC-1421 is highly specific in the inhibition of norepinephrine transporter.

1.2.1.3 Tetrabenazine (TBZ)-induced Hypothermia Model

According to above assays, PDC-1421 is a selective norepinephrine reuptake inhibitor. A previous study has shown that the elevated concentration of norepinephrine produced by norepinephrine reuptake inhibitor (NRI) resulted in elevation of body temperature in reserpine-induced hypothermia animal model[4]. Reversed temperature in hypothermia model is an index to quantify the effect of NRI. Several studies have been conducted using this model to evaluate the antidepressant effects of investigational drugs[5, 6]and support the usefulness of the TBZ Hypothermia model.

PDC-1421 was tested by the TBZ-induced hypothermia model to study the possible effect on depression. Vehicle, PDC-1421 and imipramine (positive control) were orally administrated to individual groups of experimental mice one hour before TBZ treatment and body temperatures were recorded at appropriate time point. The results were summarized in Table 5.

Table 5. Summary for pharmacology study of antidepressant-like effect

Treatment	Dose (mg/kg)	% Inhibition in Body Temperature		
		60 min after TBZ	90 min after TBZ	120 min after TBZ
PDC-1421	10	5	0	0
PDC-1421	30	30	33	27
PDC-1421	100	55*	59*	63*
PDC-1421	300	77*	74*	80*
Imipramine	3	89*	84*	83*

* The inhibition of TBZ-induced hypothermic response by ≧50% is considered as significant response

The results suggest that PDC-1421 at 100 mg/kg p.o. may be associated with antidepressant activity as evidenced by effect on TBZ-induced hypothermia in mice.

1.2.2 Safety Pharmacology

In order to verify the possible effects other than antidepressant, five studies were conducted to evaluate the effects of PDC-1421 on central nervous system, autonomic system, cardiovascular function, respiratory function, gastrointestinal system, renal function, allergy, inflammation, metabolism and hERG tail current assay:

- EfficacyProfiling *in vivo*®

- Safety Pharmacology Testing Package
- AdverseEventProfiling *in vivo*®
- Safety Pharmacology test in Central Nervous System, Cardiovascular System and Respiratory System
- hERG tail current assay

The significant findings were summarized in Table 6.

Table 6. Summary of the finding in safety pharmacology

Study package	**Study condition and findings**
EfficacyProfiling	• Oral administration of PDC-1421 at 3000 mg/kg did not cause any significant change in vivo studies. • PDC-1421 at 300 μg/ml did not demonstrate any significant activity (≧50% change) in vitro studies.
Safety Pharmacology Testing Package	PDC-1421 concentration at 300 μg/mL: • Decrease in acetylcholine and $BaCl_2$-induced constriction in guinea pig ileum.
AdverseEventProfiling *in vivo*®	PDC-1421 oral dose at 3000 mg/kg: • Inhibition of gastric emptying in fasted rats (-45%) PDC-1421 oral dose at 3000 mg/kg for 7 days: • Increase in RBC on day 2 in blood chemistry • Increase in K^+ and decrease in glucose on day 2 in blood chemistry • Decrease in LDH (lactate dehydrogenase) on day 8 in blood chemistry
Safety Pharmacology test in Cardiovascular System	• RR interval elongation, heart rate decrease, diastolic arterial pressure decrease and increased sinoatrial arrest incidence were found in dogs in 2500mg/kg group. • Diastolic arterial pressure decrease was found in dogs in 2500mg/kg group.
hERG tail current assay	Below the dosage, 0.8mg/ml PDC-1421, should be viewed as the “no effect concentration” in this assay.

Few effects, including gastrointestinal effects, electrolytes equilibrium, blood sugar value, heart rate decrease and diastolic arterial pressure decrease were found in safety pharmacology. These findings provided scientific evidence in further clinical study to evaluate the influence of PDC-1421.

1.2.3 Cytochrome P450 Interaction with PDC-1421

Cytochrome P450 (CYP450) enzyme assay was conducted for PDC-1421 to evaluate existence of potential drug interaction. The IC_{50} values of each CYP450 isozymes are summarized in Table 7.

Table 7. Summary of IC_{50} values of CYP450 isozymes

CYP isozyme	Species	Concentration	Inhibition %	IC_{50}
CYP 1A2	Human	100 μg/mL	54	84 μg/mL
CYP 2C9	Human	100 μg/mL	57	74 μg/mL
CYP 2C19	Human	100 μg/mL	67	51 μg/mL
CYP 2D6	Human	100 μg/mL	69	53 μg/mL
CYP 3A4	Human	100 μg/mL	58	67 μg/mL

A lower potential of causing drug-drug interaction was verified with lower CYP450 inhibition in this study. IC_{50} of CYP inhibition is higher than IC_{50} of *in vitro* pharmacological study (11.8 and 0.7 μg/mL in NET radioligand binding assay and reuptake assay, respectively), indicating that taking PDC-1421 with substrate of these CYP enzymes, including imipramine, paroxetine and warfarin, may not influence the blood concentration of these prescribed drugs.

1.2.4 Toxicology

Toxic effects of investigational drug are important considerations for human use. Several toxicology studies have been conducted to evaluate the toxic effects of PDC-1421.

1.2.4.1 Single Dose Acute Toxicity Study in Rats

Acute toxicity of test drug may predict effect of over dose. Four doses of PDC-1421 at 0, 1250, 2500 and 5000 mg/kg p.o. were given to different groups of rats. General demeanor, clinical signs, mortality, body weight and gross necropsy findings were evaluated and recorded. There has been no treatment-related pharmacological effect

found with all 4 doses. Therefore, the "no observable adverse effect level" (NOAEL) is set at 5000 mg/kg in rats based on the results of this study.

1.2.4.2 28-day Repeated Dose Subacute Toxicity Study in Rats

The duration of repeated dose toxicity study will be extrapolated to human therapeutic duration. Thus, toxic effects related to test drug dose in long term administration and determination of NOAEL are very important in further clinical study. Four groups of rats were administered with 0, 750, 1500 and 3000 mg/kg /once daily p.o. for 28 days respectively. Moribundity/mortality, clinical signs, body weight, food consumption, ophthalmology, clinical pathology (including urinalysis, hematology and serum chemistry), gross necropsy, organ weight and histopathology, were evaluated on those experimental rats. No death nor severe side effects had been observed. However, some effects were considered treatment-related as shown in Table 8:

Table 8. Summary of 28-day repeated dose toxicity study in rats

Group	1	2	3	4
Dose (mg/kg/day)	0	750	1500	3000
Male				
Calcium (mg/dL)[1]	10.7 ± 0.7	10.8 ± 0.8	10.4 ± 0.9	9.6 ± 0.5*
Female				
Sodium (mEq/L)[2]	147.4 ± 1.8	148.1 ± 1.6	147.8 ± 1.8	150.5 ± 2.1*
Thymus weight (mg)	501.3 ± 122.9	449.4 ± 91.7	418.4 ± 66.7	377.2 ± 81.2*

Reference range: [1]: 10.5~12.2; [2]: 136~156
*: $p < 0.05$

Concluded by the investigating institute, those abnormal are mild and did not influence the physical condition of experimented rats. Therefore, NOAEL for PDC-1421 in rats under this study condition is 3000 mg/kg/day.

1.2.4.3 28-day Repeated Dose Subacute Toxicity Study in Dogs

In order to determine NOAEL in dogs, PDC-1421 was repeatedly administered to 4 groups of dogs at dose levels of 0, 300, 1000, 3000 mg/kg once daily for 28 days. Soft feces were found in male dogs at 3000 mg/kg. There are several differences of physical value, lower mean corpuscular volume and monocytes in males and lower cholesterol and triglyceride in females, between test groups and control group, but these effects are not treatment-related or dose-dependent and within DCB (Development Center for Biotechnology) historical data range. Therefore, NOAEL for PDC-1421 in dogs is set at 3000 mg/kg.

1.2.4.4 13-week Repeated Dose Subchronic Toxicity Study in Rats

To evaluate the subchronic toxicity of PDC-1421, four groups of rats were administered with 0, 750, 1500 and 2500 mg/kg/once daily p.o. for 13 weeks (90 days). There are no treatment-related mortality, clinical signs of toxicity and ophthalmologic abnormality in the study. And no significant toxicity evidences in mean body weights, mean body weight gains and food consumption in all treatment groups were noted.

In clinical pathology, no toxicity evidences were noted for all parameters (hematology, serum chemistry and urinalysis), no treatment-related gross abnormality was found at necropsy, and no significant toxicity evidences in organ weights evaluation. And the histopathological findings showed that no treatment-related changes in vehicle control and PDC-1421 treated groups.

It was estimated that the NOAEL of PDC-1421 of the 13-week repeated dose toxicity study is 2500 mg/kg/day for both males and females.

1.2.4.5 Genotoxicity

Three studies, including Ames test, micronucleus test and chromosome aberration assay, were conducted to evaluate the genotoxicity of PDC-1421. In Ames test, five different strains of *Salmonella typhimurium* were used to evaluate the mutagenic potential when incubated at different concentration of PDC-1421. No mutation induced by PDC-1421 was observed. In micronucleus test, three doses of PDC-1421 at 500, 1000, 2000 mg/kg p.o. were administered to three different groups of BALB/c mice and the ability of PDC-1421 to induce micronucleated reticulocytes in mouse peripheral blood was measured. The results of this study indicated that PDC-1421 posed-negative response to clastogenic micronucleated reticulocytes in mice. In chromosome aberration assay, clastogenic activity was evaluated in Chinese hamster ovary (CHO) cells after adding the different concentration of PDC-1421. Based on the results of this study, chromosomes in CHO cells are not affected by PDC-1421. In conclusion, PDC-1421 does not pose any risk of genotoxicity.

1.3 Results of Phase I Clinical Trial Study of PDC-1421

In phase I trial, all total of 85 subjects were screened at study site and 30 subjects were enrolled. They were 9 subjects in cohort A with 7 administered PDC-1421 (380 mg) and 2 administered placebo, 1 of 7 PDC-1421 subjects (P01) had no laboratory

test data at baseline; 8 subjects in cohort B with 6 administered PDC-1421 (1140 mg) and 2 administered placebo; 4 subjects in cohort C with 3 administered PDC-1421 (2280 mg) and 1 administered placebo; 9 subjects in cohort D with 7 administered PDC-1421(3800 mg) and 2 administered placebo, 1 of 7 PDC-1421 subjects (P27) had abnormal laboratory data at screening visit.

Physical examination was determined to be "normal" on every Body System in each cohort and no subject had DLT and toxicity grade.

All of the changes of vital signs from baseline of PDC-1421 and placebo group were mild and did not exceed the limit of normal range. Furthermore, all of the toxicity grades of vital signs were the lowest, systolic blood pressure in grade 1, increase >20 mm/Hg than baseline at 4 hour. No medical intervention/therapy was required. There were no correlation of changes from baseline or changes in the toxicity grade of vital signs between doses of PDC-1421.

All changes of laboratory test data from baseline of PDC-1421 group were mild and no clinically significant deviation from the normal range. Furthermore, the toxicity grades of laboratory test data of PDC-1421 and placebo groups were the lowest, grade 1. No medical intervention/therapy was required. There were no correlation of change from baseline or change in the toxicity grade of laboratory test between doses of PDC-1421. Only two grade 2 toxicity (at 24 hour in glucose in cohort A and at 4 hour in glucose in cohort B) occurred in placebo group and no medical intervention/therapy were required for these cases.

ECG was determined to be "normal" in each time point and in each cohort. No subject had DLT and toxicity grade.

C-SSRS were all 0 point on Suicidal ideation, Intensity of ideation, Suicidal behavior in each cohort.

No subject had serious adverse event and no subject discontinued due to adverse event, no clinically significant finding in physical examinations, vital signs, electrocardiogram, laboratory measurements, and C-SSRS was observed throughout the treatment period, and the oral administration of PDC-1421 in healthy volunteers was safe and well-tolerated for the dose from 380 mg to 3800 mg. During the treatment period, 5 subjects reported to experience 8 mild adverse events shown as

Table 9. The severity of these 8 adverse events was all mild and no medical action required. There was no correlation between number, severity, relationship and outcome of adverse events found between doses of PDC-1421 and placebo. Further, there was no clinically significant finding in electrolyte level and gastrointestinal discomfort during monitoring in the clinical trial. There were two mild adverse events such as lower heart rate and higher systolic blood pressure. Lower heart rate was that coupled to the dog telemetry study but not higher systolic blood pressure.

Table 9. Frequencies of Adverse Events

Adverse events	**Frequency**				
BODY System	**Cohort A (380 mg) N=7**	**Cohort B (1140 mg) N=6**	**Cohort C (2280 mg) N=3**	**Cohort D (3800 mg) N=7**	**Placebo N=7**
Digest System	3/7	0	0	0	1/7
FLATULENCE	2/7	0	0	0	1/7
CONSTIPATION	1/7	0	0	0	0
Nervous System	0	0	2/3	0	2/7
SOMNOLENCE	0	0	1/3	0	2/7
STOMATITIS ULCER	0	0	1/3	0	0

1.4 Potential Risk and Benefit

1.4.1 Potential Risk

The toxicological profile of PDC-1421 was characterized in single and repeat oral dose studies of up to 13 weeks in rats and repeat oral dose studies of up to 28 days in dogs. Genetic toxicology and safety pharmacology have also been conducted. PDC-1421 was observed to be associated with some adverse effects in serum electrolytes and GI function, but none were clinically significant. Therefore, potential risks of PDC-1421 are fluctuated electrolyte level and gastrointestinal discomfort. We monitored these in the Phase I study and there was no deviation of electrolyte level and no significant gastrointestinal discomfort during monitoring in the clinical trial. However, there were non-clinically significant mild lower diastolic blood pressure and lower heart rate in dog telemetry study and it has been only with the 2500 mg/kg, which is around the NOAEL for dogs. The cardiovascular systems will be monitored in the clinical study.

1.4.2 Potential Benefit

According to nonclinical study results, the results of two *in vitro* assays support the fact that PDC-1421 is a selective norepinephrine reuptake inhibitor and its potency are not inferior to other antidepressants. A TBZ-induced hypothermia study has shown that PDC-1421 is associated with antidepressant activity. Furthermore, PDC-1421 will not pose the adverse effects caused by the molecules which possess anticholinergic or antihistamine activities due to its selectivity on norepinephrine transporter.

1.4.3 Risk Benefit Ratio

Potential risks in nonclinical results outlined above are minor and easy to monitor. Even though there were adverse events in phase I trial, they were no consistent findings in the direction of changes of potential risks in nonclinical studies. Therefore, the subsequent trial could be benefited from the information obtained from this study.

1.5 Dosage Regimen

Based on the FDA's guidance documents, NOAELs from toxicity studies were used to estimate the starting dose in our study. Table 10 summarizes the MRSD for 60 kg human/day calculated from the NOAEL of 28 day repeating dose studies in rats and in dogs using safety margin at 75-fold.

Table 10. Calculation of starting dose

Study	Species	Dosage	HED	Dose for 60 kg human/day
28-day repeated dose subacute oral toxicity study in rats	rat	3000 mg/kg (NOAEL)	483.9 mg/kg	387 mg/day (MRSD)
28-day repeated dose subacute oral toxicity study in dogs	dog	3000 mg/kg (NOAEL)	1666.7 mg/kg	1333 mg/day (MRSD)

Based on these calculations, we proposed the starting dose of PDC-1421 to be at 380 mg/day. In the phase I study, a single dose of PDC-1421 (oral administration) was used to evaluate the safety when taken by healthy subjects. The starting dose and dosage regimen are:

380 mg PDC-1421 (one capsule of PDC-1421 Capsule) once daily after meal.

The dose escalation as follows.
Cohort A (one capsule; 380 mg):

Cohort A enrolled 9 subjects. Seven subjects received PDC-1421 Capsule and two received placebo. There was only one of placebo subjects who met a DLT due to the glucose value at 24 hours after drug administration. We continued the study to the next higher dosage, cohort B.

Cohort B (three capsules; 1140 mg):

Cohort B enrolled 8 subjects. Six subjects received PDC-1421 Capsule and two received placebo. There was only one of placebo subjects who met a DLT due to the glucose value at 4 hours after drug administration. We continued the study to the next higher dosage, cohort C.

Cohort C (six capsules; 2280 mg):

Cohort C enrolled 4 subjects. Three subjects received PDC-1421 Capsule and one received placebo. There was no DLT in this cohort. We continued the study to the next higher dosage, cohort D.

Cohort D (ten capsules; 3800 mg):

Cohort D enrolled 9 subjects. Seven received PDC-1421 Capsule and two received placebo. There is no DLT in the final cohort.

Based on the results, the maximum tolerated dose (MTD) is ≧3800 mg of PDC-1421 Capsule so that the safe and well-tolerated for PDC1-421 Capsule is from 380 mg to 3800 mg. According to studies in mouse model system to evaluate efficacious dose, TBZ-induced hypothermia assay, PDC-1421 was indicated to be efficient at 100 mg/kg and 300 mg/kg doses which are equivalent to about 480 mg and 1400 mg in human subjects. We choose 1140 mg (3 capsules), between the efficient dosage, and 2280 mg (6 capsules), above the efficient dosage, which is based on the mice study as the dosage regimen of Phase II trial.

1.6 Compliance with Protocol, Good Clinical Practice (GCP) and Applicable Requirement

This study and all operating procedures will be conducted in accordance with GCP guidelines, protocol and all applicable regulatory requirements issued by the Minister of Health and Welfare (MOHW) in Taiwan and US FDA, to protect the rights, safety and well-being of the studying subjects.

1.7 Description of the Population to be Studied

We chose patients with major depressive disorder as our subjects in this phase II trial.

2 TRIAL OBJECTIVE

The primary objective of this trial was to determine the effective doses and treatment period of PDC-1421 Capsule in subjects with MDD. The secondary objective was to evaluate the safety of PDC-1421 Capsule in subjects receiving PDC-1421at various dose levels.

3 TRIAL DESIGN

3.1 General Design

Table 11. Summary of general design

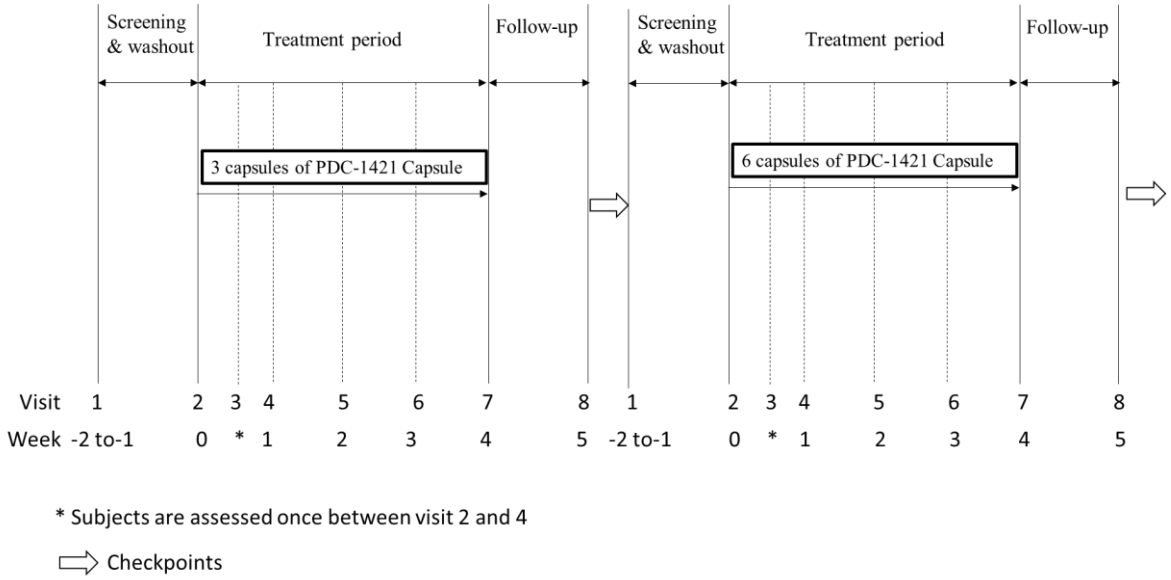
	Part I	Part II
Design	Open label, multiple center, dose escalation	Double-blind, randomized, parallel group, placebo-controlled
MDD patients	12	60
Treatment	(1) 1 capsule TID, 6 MDD patients, 28 days (2) Checkpoints (3) If passing the checkpoint: 2 capsules TID, another 6 MDD patients, 28 days (4) Checkpoints	Randomly assigned on a 1:1:1 basis to one of the three arms: (1) 1 PDC-1421 Capsule plus 1 placebo TID (2) 2 PDC-1421 Capsule TID (3) 2 Placebo TID for 42 days
Assessment interval	First week: twice, 3-4 days interval; Once a week during the following second to fourth week	Biweekly
Efficacy and safety evaluation	• Safety evaluation • Efficacy evaluation	• Primary endpoint: efficacy measurement (MADRS) • Secondary endpoints: other efficacy and safety measurements (MADRS, DSSS, HAM-D-17,

		HAM-A, CGI, C-SSRS, ECG, vital sign, physical examination, hematology, blood chemistry and concomitant medication evaluation)

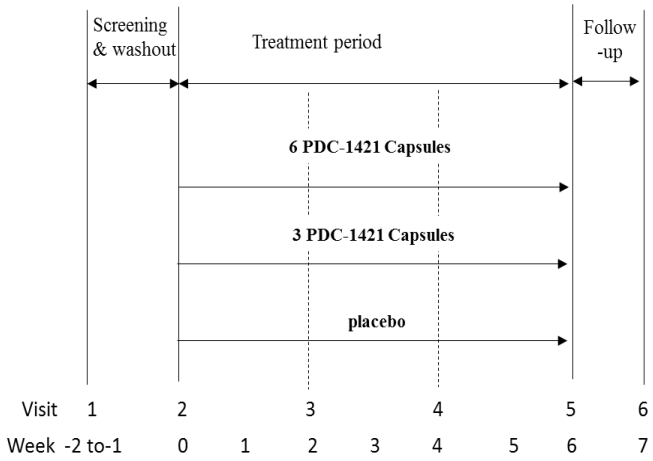
The screening phase is intended for diagnosing and assessing the patient for possible inclusion in the study and for providing an adequate washout period. The following study will be conducted in two parts. The targeted population of this study is 72 subjects who met the intent-to-treat (ITT) basis, in which 12 are assigned to part I, 60 are assigned to part II. Part I is an open-label study, multiple center and dose escalation evaluation in twelve patients. Six subjects each will be evaluated for any safety concerns at 1 or 2 capsules TID dose for 28 days, sequentially. Each of them will be assessed twice in the first week after administration of PDC-1421 Capsules and once a week in the following treatment. Part II is a randomized, double-blind, placebo-controlled, parallel-group study. 60 subjects will be randomly assigned on a 1:1:1 basis to one of the three treatment arms (1 PDC-1421 Capsule plus 1 placebo TID, 2 PDC-1421 Capsule TID, 2 placebo TID) for 6 weeks (42 days) and evaluated the safety and efficacy every two weeks during the treatment period. The performance of PDC-1421 Capsule on MDD subjects will provide us with valuable information for our next phase of the study.

Figure 1. General design of procedure

Part I.



Part II.



3.2 Primary Endpoint

In part II, the primary endpoint is the change of Montgomery-Å sberg Depression Rating Scale (MADRS) total score from baseline to Week 6 compared to placebo.

3.3 Secondary Endpoint

Table 12 The secondary efficacy endpoints in part II

Number	Items
1	MADRS total score－change from baseline to Visit 3 and 6 (Week 2 and 7).
2	HAM-D-17, CGI, DSSS and HAM-A－change from baseline to Visit 3, 4, 5 and 6 (Week 2, 4, 6 and 7)
3	Percentage of partial responders and responders (defined as participants with a 25-50% decrease and a ≧50% decrease from baseline in total score, respectively) in MADRS total score by Week 2, 4, 6 and 7.
4	Safety evaluation－measurements of physical examination, vital signs, laboratory data, ECG, Columbia-Suicide Severity Scale (C-SSRS) and adverse events reports from screening stage to the last visit.
5	Difference in the mean changes of MADRS, HAM-D-17, HAM-A, DSSS, CGI and C-SSRS from baseline to week 2, 4, 6 and 7 between PDC-1421 Capsule and placebo groups.

3.4 Randomization and Blinding

Table 13. Summary of randomization and blinding

	Part I	Part II
Randomization	None (open label)	Randomized; 1 capsule TID: 2 capsules TID: placebo = 1:1:1
Blinding	None (open label)	Double-blind; (1) Unblinding at the end of trial (2) Emergency unblinding at a serious adverse event.

The randomization scheme generated by computer will be prepared by the CRO prior to the start of the study. In part II, subjects will be randomly allocated to the group of one of PDC-1421 Capsule (3 or 6 capsules) or placebo in a ratio of 1:1:1. All subjects and medical staff will be blinded to the treatment assigned till the end of the trial. All emergency unblinding (e.g., due to a serious adverse event) should be reported immediately to the BioLite, Inc. Codes linking randomization number to actual

treatment for each subject will be secured in a sealed, opaque envelop and maintained in the study site and research department of BioLite, Inc.

3.5 Investigational Drug

Investigational drug (PDC-1421 Capsule or placebo) will be maintained and dispensed by the Department of Pharmacy of all clinical sites. No one but the Investigator and/or his/her designee is permitted to access the investigational drug.

3.5.1 Dosage Form and Strength

PDC-1421 Capsule: 380 mg PDC-1421/capsule
Placebo: 380 mg corn starch/capsule

3.5.2 Manufacturer

ITRI (Industrial Technology Research Institute), Hsinchu County, Taiwan

3.5.3 Route of Administration

Oral administration

3.5.4 Dosing Regimen, packing and labeling

Table 14. Summary of dosing regimen

	Part I	Part II
Visits	Once a week (twice in the first week) for 28 days	Biweekly for 42 days
Study drug bags	21 packs for the following week, total 84 packs	42 packs for the following two weeks, total 126 packs
Dosing regimen	Each pack including (1) or (2): (1) 1 capsule of PDC-1421 (380 mg), TID (2) After passing the checkpoint, 2 capsules of PDC-1421 (380 mg), TID	Each pack including (1), (2) or (3): (1) 2 capsules, TID: 1 capsule of PDC-1421 (380 mg) and 1 capsule of placebo TID (2) 2 capsules, TID: 2 capsules of PDC-1421 (380 mg) and 0 capsule of placebo TID (3) Placebo:

		0 capsule of PDC-1421 (380 mg) and 2 capsules of placebo TID

In part I, six subjects each will take 1 or 2 capsules thrice daily for 28 days, sequentially. Subjects are assessed twice in the first week after drug administration and they will receive the study drug bag for the following 7 days every week. In part II, eligible subjects are randomized into one of three treatment arms in a 1:1:1 ratio (one capsule of PDC-1421 Capsule and placebo thrice daily; two capsules of PDC-1421 Capsule thrice daily; two capsules of placebo thrice daily) for 42 days. After the two weeks of drug administration, subjects will return to the center for assessments then get another study drug bag for the following 14 days. The biweekly clinic visits are three times and one week later after the last dose for follow-up.

Investigational drug (PDC-1421 Capsule or placebo) are provided by BioLite, Inc. and stored in Department of Pharmacy of each clinical site. In part I, the first six subjects will take 21 packs with 21 capsules of PDC-1421 Capsule and another six take 21 packs with 42 capsules of PDC-1421 Capsule in every week of trial. In part II, subjects will be dispensed with study drug per 2 weeks which are packaged in a drug bag. A drug bag will contain 42 packs. Depending on the study arms, each pack may contain 2 PDC-1421 Capsules, 2 placebos or 1 PDC-1421 Capsule plus 1 placebo. Each subject will be administered 126 packs in the end of trial. The drug bag of investigational drug is clearly labeled as following:

For Part I-low dose (380 mg TID):

Test Drug: PDC-1421 Capsule Amount: capsules (reserved capsules included) Subject Number: Drug Pack Number: Study Protocol: BLI-1005-002 Drug Appearance: Brown capsule containing yellow powder Major Component: Each capsule contains 380 mg of PDC-1421 Dosing: 1 capsule thrice daily after meal for OO days and with extra OO days for reserved Storage Condition: Store at a dry place at room temperature (15~25℃) Lot Number: Manufacturer: Industrial Technology Research Institute Date of Manufacture: Date of Expiration: Sponsor: BioLite, Inc. Clinical Site: Taipei Veterans General Hospital, Linkou Chang Gung Memorial Hospital and Taipei City Hospital, Songde Branch. "Caution: New drug - Limited by Federal Law to investigational use". 注意：試驗新藥僅限特定臨床試驗使用
試驗藥物: PDC-1421 膠囊 數量: 顆膠囊 (含 顆備用膠囊) 藥物編號: 受試者編號: 回診次別: Visit 計畫書編號: BLI-1005-002 藥物外觀: 棕色膠囊內含黃色粉末 主成份: 每顆膠囊含有 380 mg PDC-1421 劑量: 三餐飯後服用 1 顆膠囊，OO 日份 (外加 OO 日備用藥) 儲存條件: 儲存於乾燥常溫之環境 (15~25℃) 批號: 製造商: 工業技術研究院 製造日期: 有效日期: 廠商: 萊特先進生醫股份有限公司 試驗機構: 臺北榮民總醫院，林口長庚紀念醫院，臺北市立聯合醫院松德院區 "Caution: New drug - Limited by Federal Law to investigational use". 注意：試驗新藥僅限特定臨床試驗使用

For Part I-high dose (760 mg TID):

Test Drug: PDC-1421 Capsule Amount: capsules (reserved capsules included) Subject Number: Drug Pack Number: Study Protocol: BLI-1005-002 Drug Appearance: Brown capsule containing yellow powder Major Component: Each capsule contains 380 mg of PDC-1421 Dosing: 2 capsules thrice daily after meal for OO days and with extra OO days for reserved Storage Condition: Store at a dry place at room temperature (15~25℃) Lot Number: Manufacturer: Industrial Technology Research Institute Date of Manufacture: Date of Expiration: Sponsor: BioLite, Inc. Clinical Site: Taipei Veterans General Hospital, Linkou Chang Gung Memorial Hospital and Taipei City Hospital, Songde Branch. "Caution: New drug - Limited by Federal Law to investigational use". 注意：試驗新藥僅限特定臨床試驗使用
試驗藥物: PDC-1421 膠囊 數量: 顆膠囊 (含 顆備用膠囊) 藥物編號: 受試者編號: 回診次別: Visit 計畫書編號: BLI-1005-002 藥物外觀: 棕色膠囊內含黃色粉末 主成份: 每顆膠囊含有 380 mg PDC-1421 劑量: 三餐飯後服用 2 顆膠囊，OO 日份 (外加 OO 日備用藥) 儲存條件: 儲存於乾燥常溫之環境 (15~25℃) 批號: 製造商: 工業技術研究院 製造日期: 有效日期: 廠商: 萊特先進生醫股份有限公司 試驗機構: 臺北榮民總醫院，林口長庚紀念醫院，臺北市立聯合醫院松德院區 "Caution: New drug - Limited by Federal Law to investigational use". 注意：試驗新藥僅限特定臨床試驗使用

For Part II:

Test Drug: PDC-1421 Capsule/placebo Amount: capsules (reserved capsules included) Subject Number: Drug Pack Number: Visit Number: Study Protocol: BLI-1005-002 Drug Appearance: Brown capsule containing yellow powder Major Component: Each capsule contains 380 mg of PDC-1421/placebo Dosing: 2 capsules thrice daily after meal for OO days and with extra OO day for reserved Storage Condition: Store at a dry place at room temperature (15~25℃) Lot Number: Manufacturer: Industrial Technology Research Institute Date of Manufacture: Date of Expiration: Sponsor: BioLite, Inc. Clinical Site: "Caution: New drug - Limited by Federal Law to investigational use". 注意：試驗新藥僅限特定臨床試驗使用
試驗藥物: PDC-1421 膠囊/安慰劑 數量: 顆膠囊 (含 顆備用膠囊) 藥包編號: 受試者編號: 回診次別: Visit 計畫書編號: BLI-1005-002 藥物外觀: 棕色膠囊內含黃色粉末 主成份: 每顆膠囊含有 380 mg PDC-1421 或安慰劑 劑量: 三餐飯後服用 2 顆膠囊，OO 日份 (外加 OO 日備用藥) 儲存條件: 儲存於乾燥常溫之環境 (15~25℃) 批號: 製造商: 工業技術研究院 製造日期: 有效日期: 廠商: 萊特先進生醫股份有限公司 試驗機構: "Caution: New drug - Limited by Federal Law to investigational use". 注意：試驗新藥僅限特定臨床試驗使用

3.5.5 Study Drug Storage

Investigational drugs will be stored in a locked cabinet at room temperature (15~25℃)

in Department of Pharmacy of each clinical site.

3.6 Premature Termination or Suspension of the Study

The Investigator and/or the Sponsor may decide to stop the trial if:

- Safety assessment clearly indicates that one study arm is associated with more severe or serious adverse experiences.
- The Sponsor decides to terminate the trial if necessary.

4 SUBJECTS SELECTION AND WITHDRAWAL

Inclusion and exclusion criteria in part I and part II are as follows.

4.1 Inclusion Criteria

1. Outpatients aged 20-65 years.
2. Subjects must be able to understand and willing to sign informed consent.
3. Female subjects of child-bearing potential must test negative to pregnancy and use appropriate birth control method from the beginning of study to the 15 days later after ending of study.
4. Met criteria for MDD without psychotic features as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision® (DSM-IV-TR) and confirmed by use of the Mini International Neuropsychiatric Interview (MINI).
5. 17-item HAM-D (Hamilton Rating Scale for Depression) total score ≧20 and CGI (Clinical Global Impression) total score ≧4.

4.2 Exclusion Criteria

1. Have a current or previous major psychiatric disorders which be defined to be per the DSM-IV-TR, including obsessive-compulsive disorder, posttraumatic stress disorder, bipolar I or II, manic or hypomanic episode, schizophrenia, major Axis II disorders which might compromise the study, and major depression with psychotic symptoms, mental retardation.
2. Use of any treatment for MDD in the last 2 weeks before visit 1 (4 weeks for fluoxetine).
3. Use of psychoactive drugs within the last 2 weeks before visit 1 other than that subjects had insomnia who need the treatment as determined by the Investigator.
4. Subjects who were non-responsive to two or more courses of antidepressant medications given an adequate dosage* for symptom treatment within four weeks, or by the judgment of the investigator considered to have treatment resistant depression (TRD), or a history of electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS) or psychosurgery within the last year.
5. Have a history of any seizure disorder.
6. Any clinically significant abnormal vital sign, ECG, laboratory values as determined by the investigator which might interfere with the study.

7. Any organic disorder caused medical related depression which cannot be under well-controlled such as clinically significant in neurological, gastrointestinal, renal, hepatic, cardiovascular, respiratory, metabolic, endocrine, hematological or other major disorders.
8. Have a high suicidal risk as measured by MINI.
9. Have a history of substance dependence/abuse** within the past 6 months or any positive drug screen result is determined to be caused by subject's drug abuse at visit 1.
10. Have a history of severe allergies to more than 1 class of medication or multiple adverse drug reactions.

* The adequate dosage of the antidepressant medication is defined as the average of the usual dose (mg/day) recommended in American Psychiatric Association (APA) Practice Guideline for the Treatment of Patients with Major Depressive Disorder, Third Edition. E.g. the usual dose of Citalopram is 20-60 mg/day, the adequate dosage is 40 mg/day.

** Tobacco is excluded here, and alcohol abuse is defined as average pure alcohol intake is more than 112 g (for male) or 56 g (for female) per week and/or with Alcohol withdrawal syndrome. Pure alcohol intake =% (Concentration or alcohol content) x c.c. (volume)x 0.79 (density of alcohol). Result of serum ethanol test should be equal to or lower than 10.0 mg/dL to be determined as eligible for the trial. If test result is between 10.1 to 29.9 mg/dL, only one re-test is allowed per subject to meet the criterion. Subject with test result equal to or higher than 30.0 mg/dL is to be excluded.

4.3 Subject Recruitment and Screening

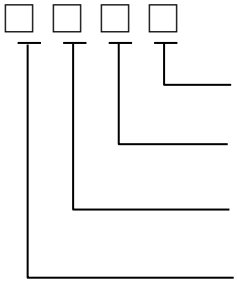
At Visit 1, Investigator will introduce the proposed clinical study to subjects who are interesting in this study. If a subject is willing to undergo an informed consent process based on GCP and participate in our study, the subject will be examined as described in section 5.2. At Visit 2, based on Visit 1 measurements, subject who meets the recruitment criteria will be enrolled in this study.

4.4 Subject Identification

In order to protect each subject's privacy, only subjects' initials will be used as part of subjects' identifications. In addition, a subject number will be assigned to each subject who signed the ICF and each subject who is eligible to enroll in the study.

4.4.1 Subject Initial Allocation

A subject's initial will be assigned to each subject by the clinical research coordinator (CRC). Remain blank in the 3rd and 4th space if there have no more initial(s) to be filled in.



English initial of the fourth character in the name

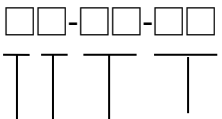
English initial of the third character in the name

English initial of the second character in the name

English initial of the first character in the name

4.4.2 Subject Number Allocation

Each subject will be assigned a number by the CRC. All subject numbers will have six digits.



The fifth and sixth digits: the randomized number will be consecutive numbers commencing with "01". At screening visit, these should be blank.

The third and fourth digits: the screening number will be consecutive numbers commencing with "01"

The second digit: the site number.

The first digit: the part number; Part I will be assigned to "1", Part II will be assigned to "2".

4.5 Early Withdrawal of Subjects

Subjects are not obligated to give reasons for discontinuation from this study. Investigator can ask a subject to be withdrawn from study in the best interest of the subject. Subjects withdrawn from the study due to adverse event(s) must be followed until the events are resolved or stabilized. The discontinuation will result from the following reasons:

- Subjects wish to withdraw.
- Subjects can't obey the regulation of the study.
- Investigator consider that withdrawal from study is the best interest of a subject.
- The subject is pregnant during the trial.

5 TREATMENT OF SUBJECTS

5.1 Description of Study Drug

PDC-1421 Capsule is a botanical investigational new drug. Each PDC-1421 Capsule contains 380 mg PDC-1421 drug substance extracted from dry root of *P. tenuifolia.* The detailed information of PDC-1421 Capsule is described in section 1.1. Placebo contains 380 mg corn starch and 20 mg excipient (10 mg silicon dioxide and 10 mg magnesium stearate); the appearance of placebo capsule and composition of excipient are the same as PDC-1421 Capsule.

5.2 Treatment Schedule

Potential subjects will be introduced by Investigator of the study design at Screening. If the subject agrees to participate and signs the consent form, the screening process will begin. Suitability of the subject will be evaluated by inclusion/exclusion criteria. In part I, sequentially, six eligible subjects each take 1 or 2 capsules of PDC-1421 Capsule thrice daily and will be assessed twice in the first week (Visit 3 and 4), every week (Visit 5, 6 and 7) for the following treatment and one week later after the last dose for follow-up (Visit 8). In part II, at the beginning of randomization (Visit 2), subjects who met eligibility criteria will be enrolled into this trial and take the study drug in the assigned dose. Subjects will be assessed biweekly (Visit 3, 4 and 5) and a follow-up (Visit 6). Screening, safety and efficacy assessments will be performed as Table 15 and 16.

Table 15. Part I-schedule of assessments

*On ±2 days.

*V3 (Week 1/2) only can be conducted in Day 3 or Day 4.

	Screening	Treatment Period						Follow-up
Visit	1	2	3	4	5	6	7	8
***Week**	-2 to -1 ~0	0	1/2	1	2	3	4	5
Informed consent	•							
Subject information	•							
Medical history	•	•						
DSM-IV-TR	•							
HAM-D-17	•	•		•	•	•	•	•
CGI	•	•		•	•	•	•	•
MADRS	•	•		•	•	•	•	•
DSSS	•	•		•	•	•	•	•
HAM-A	•	•		•	•	•	•	•
C-SSRS	•	•	•	•	•	•	•	•
Concomitant medication	•	•	•	•	•	•	•	•
Physical examination	•	•	•	•	•	•	•	•
Vital sign	•	•	•	•	•	•	•	•
ECG	•	•	•	•	•	•	•	•
Laboratory examination								
Drug screen	•							
Pregnancy test	•							•
Hematology	•	•			•		•	•
Blood chemistry	•	•			•		•	•
Eligibility evaluation	•							
Drug accountability		•	•	•	•	•	•	
AE/SAE evaluation			•	•	•	•	•	•

Table 16. Part II-schedule of assessments

*On ±2 days.

** If the interval of visit 1 and visit 2 is within 7 days, Hematology and Blood chemistry test of visit 2 can be skipped.

	Screening	Treatment Period				Follow-up
Visit	1	2	3	4	5	6
***Week**	-2 to -1 ~ 0	0	2	4	6	7
Informed consent	•					
Subject information	•					
Medical history	•	•				
DSM-IV-TR	•					
HAM-D-17	•	•	•	•	•	•
CGI	•	•	•	•	•	•
MADRS	•	•	•	•	•	•
DSSS	•	•	•	•	•	•
HAM-A	•	•	•	•	•	•
C-SSRS	•	•	•	•	•	•
Concomitant medication	•	•	•	•	•	•
Physical examination	•	•	•	•	•	•
Vital sign	•	•	•	•	•	•
ECG	•	•	•	•	•	•
Laboratory examination						
Drug screen	•					
Pregnancy test	•					•
Hematology	•	• **	•	•	•	•
Blood chemistry	•	• **	•	•	•	•
Eligibility evaluation	•					
Drug accountability		•	•	•	•	
AE/SAE evaluation			•	•	•	•

5.2.1 Screening Phase (Visit 1)

The Screening phase will be done within 1-2 weeks period and intended for diagnosing and assessing the patient for possible inclusion in the study and for providing an adequate washout period. The items of physical, rating scales, laboratory examination and drug accountability at screening are outlined below:

- Informed consent
- Subject information, including date of birth, body height and weight
- Medical history, including any clinically significant psychiatric, neurological, gastrointestinal, renal, hepatic, cardiovascular, respiratory, metabolic, endocrine, hematological or other major disorders
- Drug screen, including alcohol, benzodiazepine, amphetamine/MDMA, opiates, cannabinoid, cocaine, phencyclidine, and barbiturate. If a positive result is determined to be caused by prescription of benzodiazepine instead of subject's drug abuse, a 10-day washout period of the prescribed benzodiazepine is required before being treated with investigational products.
- Pregnancy test
- Physical examination: skin, head, neck, eyes, ears, nose, throat, heart, lungs, abdomen (liver, spleen), neurological examination, lymph node and extremities.
- Vital sign: heart rate, blood pressure, body temperature
- ECG
- Hematology: RBC, WBC, platelets, hematocrit, hemoglobin, prothrombin time (PT), partial thromboplastin time (aPTT)
- Blood chemistry: AST, ALT, LDH, total bilirubin, BUN, serum creatinine, free thyroxine (FT4), TSH, sodium, calcium, potassium, HbA1c, LDL, HDL, triglyceride, cholesterol.
- DSM-IV-TR, HAM-D-17, CGI, MADRS, DSSS, HAM-A, C-SSRS and concomitant medication evaluation.

5.2.2. Part I. - Treatment Period (Visit 2~7) and Follow-up (Visit 8)

Laboratory data , other tests and evaluation results shall be obtained at Screening to determine the appropriateness of subject enrolled in this study. Subjects who meet the eligibility criteria will be enrolled and take test drug at this period. First six eligible subjects will take 1 capsule of PDC-1421 Capsule thrice daily in the first week and be assessed twice in the first week after drug administration. They will receive 21 packs of PDC-1421 Capsule for the following week and be evaluated every week till visit 8. After one week of the last dose administration, subjects are assessed for a follow-up. Then, a checkpoint will be conducted. If subjects have no serious adverse event, the trial can continue to next step. If subjects have serious adverse events, there should be a meeting to consider the possibility of continuing the next step of the study or not. Another six subjects will take 2 capsules of PDC-1421 Capsule and all the visits are the same as previous three subjects'. Then, another checkpoint will be conducted as mentioned above. If subjects have no serious adverse events, the schedule can be conducted to the part II. If subjects have serious adverse events, there should be a meeting to consider the possibility of continuing part II of the study or not. The items of safety and efficacy parameters at treatment period are outlined below:

- Physical examination: skin, head, neck, eyes, ears, nose, throat, heart, lungs, abdomen (liver, spleen), neurological examination, lymph node and extremities.
- Vital sign: heart rate, blood pressure, body temperature
- ECG
- Hematology: RBC, WBC, platelets, hematocrit, hemoglobin, prothrombin time (PT), partial thromboplastin time (aPTT)
- Blood chemistry: AST, ALT, LDH, total bilirubin, BUN, serum creatinine, free thyroxine (FT4), TSH, sodium, calcium, potassium, LDL, HDL, triglyceride, cholesterol
- HAM-D-17, CGI, HAM-A, DSSS, MADRS, C-SSRS and concomitant medication evaluation. (At visit 3, subjects will not need to be assessed for above scales except C-SSRS and concomitant medication evaluation.)
- AE/SAE evaluation
- Drug accountability

Follow-up visit will be performed at visit 8 after one week of the last dose administration. The test items are outlined in the following:

- Physical examination: skin, head, neck, eyes, ears, nose, throat, heart, lungs, abdomen (liver, spleen), neurological examination, lymph node and extremities.
- Vital sign: heart rate, blood pressure, body temperature
- ECG
- Hematology: RBC, WBC, platelets, hematocrit, hemoglobin, prothrombin time (PT), partial thromboplastin time (aPTT)
- Blood chemistry: AST, ALT, LDH, total bilirubin, BUN, serum creatinine, free thyroxine (FT4), TSH, sodium, calcium, potassium, LDL, HDL, triglyceride, cholesterol.
- HAM-D-17, MADRS, DSSS, HAM-A, CGI and C-SSRS and concomitant medication evaluation.
- Pregnancy test
- AE/SAE evaluation

A Data and Safety Monitoring Plan (DSMP) will be provided in this study and approved by IRB. It describes the risk and benefit to subjects, the type of data or events to be captured under the monitoring plan, the frequency of reporting to IRB, etc.

5.2.3 Part II.- Treatment Period (Visit 2~5) and Follow-up (Visit 6)

Laboratory data and results of other tests and evaluation shall be obtained at screening to determine the appropriateness of subject enrolled in this study. Subjects who meet the eligibility criteria will be enrolled and take study drugs. Baseline data (Visit 2), including physical examination, vital sign, ECG, hematology, blood chemistry, HAM-D-17, CGI, HAM-A, MADRS, DSSS, C-SSRS and concomitant medication evaluation, will be collected before dosing study drug. At visits 2, 3 and 4 (weeks 0, 2 and 4), each subject will be dispensed a supply of study drug sufficient for 2-week treatment period. The subjects will be instructed to take one dose (i.e., two capsules) after a meal thrice daily for every week. Subjects will be assessed every two weeks and receive the study drug for next two weeks till the end of trial. At visits 2, 3, 4 and 5, the safety and efficacy parameters will be evaluated by the following items.

- Physical examination: skin, head, neck, eyes, ears, nose, throat, heart, lungs, abdomen (liver, spleen), neurological examination, lymph node and extremities.
- Vital sign: heart rate, blood pressure, body temperature
- ECG
- Hematology: RBC, WBC, platelets, hematocrit, hemoglobin, prothrombin time (PT), partial thromboplastin time (aPTT). If the interval of visit 1 and visit 2 is within 7 days, Hematology test of visit 2 can be skipped.
- Blood chemistry: AST, ALT, LDH, total bilirubin, BUN, serum creatinine, free thyroxine (FT4), TSH, sodium, calcium, potassium, LDL, HDL, triglyceride, cholesterol. If the interval of visit 1 and visit 2 is within 7 days, Blood chemistry test of visit 2 can be skipped.
- HAM-D-17, CGI, HAM-A, DSSS, MADRS, C-SSRS and concomitant medication evaluation.
- AE/SAE evaluation
- Drug accountability

Follow-up visit will be performed at visit 6 after one week of the last dose administration. The test items are outlined in the following:

- Physical examination: skin, head, neck, eyes, ears, nose, throat, heart, lungs, abdomen (liver, spleen), neurological examination, lymph node and extremities.
- Vital sign: heart rate, blood pressure, body temperature
- ECG
- Hematology: RBC, WBC, platelets, hematocrit, hemoglobin, prothrombin time (PT), partial thromboplastin time (aPTT)
- Blood chemistry: AST, ALT, LDH, total bilirubin, BUN, serum creatinine, free thyroxine (FT4), TSH, sodium, calcium, potassium, LDL, HDL, triglyceride, cholesterol.
- HAM-D-17, MADRS, DSSS, CGI, HAM-A and C-SSRS and concomitant medication evaluation.
- Pregnancy test
- AE/SAE evaluation

5.3 Other Medication During the Study

To avoid conflict or unknown effects with the study drug, subjects are prohibited from taking any antidepressants and psychoactive drugs, as per the exclusion criteria, including MAOIs (monoamine oxidase inhibitor), NRIs (norepinephrine reuptake inhibitor) and norepinephrine receptor agonist/antagonist. Subjects can't combine the newly-initiated psychotherapy. If subjects have severe insomnia, Zolpidem, Zopiclone or Zaleplon can be used in four nights per week from V1 to the first week after V2. (The maximum daily dose in Zolpidem, Zopiclone and Zaleplon are 10 mg, 7.5 mg and 10 mg respectively.)

6 EFFICACY ASSESSMENTS

6.1 Specification of the efficacy parameters

Efficacy parameters are outlined as follows:

- MADRS evaluation
- CGI evaluation
- HAM-D-17 evaluation
- HAM-A evaluation
- DSSS evaluation

6.2 Methods and Timing for Assessing, Recording, and Analyzing of Efficacy Parameters.

The data obtained from eligible subjects before study drug administration in Visit 2 are set as baseline. After the beginning of treatment, the collection of data of efficacy parameters are performed at planned treatment schedule as section 5.2. CRC shall record these data in CRF in detail after each visit during the treatment period. In part I, efficacy assessments are conducted from screening stage to the last visit. In part II, data

comparison between group of 1 capsule TID, 2 capsules TID of PDC-1421 Capsule and placebo will be conducted in final report of this study to evaluate which group(s) of PDC-1421 Capsule are efficient. The rating scales are as follows:

- **MADRS (Montgomery- Å sbergDepression Rating Scale)**
  The MADRS is a 10-item checklist including 1) depression [apparent]; 2) depression [reported]; 3) loss of interest; 4) suicidal ideation; 5) tension; 6) reduced appetite; 7) insomnia; 8) difficulty in activities; 9) concentration; and 10) pessimism. Each Item is rated on a scale of 0-6, with anchors at 2-point intervals; higher scores indicating more severity (i.e., ranging from 0 [no sadness] to 6 [extremely despondent]). Percentage of partial responders (defined as a participant with a 25-50%decrease from baseline in total score) and responders (defined as a participant with ≧50%decrease from baseline in total score.). The following are as an interpretation of scores:
  Recovered, 7
  Mild, 15
  Moderate, 25
  Severe, 31
  Very severe, 44

- **CGI (Clinical Global Impression)**
  The CGI actually comprises two companion one-item measures evaluating the following: (a) CGI-Severity (CGI-S) which rates illness severity of psychopathology from 1 to 7 and (b) CGI-Improvement (CGI-I) which rates change from the initiation (baseline) of treatment.

- **HAM-D-17 (Hamilton Rating Scale for Depression)**
  This is one of the earliest scales to be developed for depression, and is a clinician rated scale aimed at assessing depression severity among patients for treatment. There are four specific HAM-D symptom groups: psychomotor retardation, anxiety, cognitive disturbance and insomnia. The depression score ranges from 0 to 52. The following are as an interpretation of scores:

0-7: Normal
8-16: Mild
17-23: Moderate
≧24: Severe

- **HAM-A (Hamilton Rating Scale for anxiety)**

  HAM-A is a series of questions related to symptoms of anxiety. It rates the individual on a five-point scale (0~4) for each of the 14 items. Seven of the items specifically address psychic anxiety and the remaining seven items address somatic anxiety. The anxiety score ranges from 0 to 56, lower scores are better. The following are as an interpretation of scores:
  0-13: Normal
  14-17: Mild
  18-24: Moderate
  ≧25: Severe

- **DSSS (Depression and Somatic Symptoms Scale)**

  The scale includes a simultaneous measure of depression and somatic symptoms that two issues frequently co-occur. Though many preexisting questionnaires for depression contain items relating to somatic complaints, the DSSS is specifically concerned with the relationship between the two. Consisting of 22 items, the DSSS includes 12 depression-related items and 10 somatic items. Evaluation of the severity of the symptoms are the following:
  Absent: no symptoms
  Mild: symptoms caused slight discomfort or disturbance
  Moderate: symptoms caused significant discomfort or disturbance
  Severe: symptoms caused very significant discomfort or disturbance

7 SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

Safety parameters are outlined as follows:

- Data collected from the physical examinations, vital sign, ECG and laboratory test in scheduled visit
- Adverse events reported
- Serious adverse events reported
- C-SSRS evaluation

7.2 Methods and Timing for Assessing, Recording, and Analyzing of Safety Parameters

The data obtained before study drug administration in Visit 2 are set as baseline. After the beginning of treatment, the collection of data of safety parameters are performed at planned treatment schedule as section 5.2. AE or SAE will be closely monitored during the study period. CRC shall record these data in CRF in detail. In part I and part II, data are recorded from screening stage to the last visit.

7.3 Adverse Events (AEs)

Adverse event is any unfavorable and unintended symptom, syndrome, medical condition or experience that develops or worsens within the study period. In this Phase II trial, any clinically significant abnormal findings, including causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, are considered to be AEs and will be monitored and recorded. AE may not be causal relationship with study medication or clinical study. Whether related to study medication or not, CRC shall record the information of AEs in CRF. The information of AEs contains characteristic, onset and duration, frequency, the Investigator's opinion of the relationship to the study drug (unrelated, unlikely, possibly, probably, definitely), outcome (recovered, recovered with residual effects, continuing,

death, lost to follow-up) and severity. Common Terminology Criteria for Adverse Events v4.03 (CTCAE Table.17) is a descriptive terminology and the grading (severity) scale is provided for each AE term.

Table 17. Common Terminology Criteria for Adverse Events

Grade	Description
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL†.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.‡

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

†Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

7.4 Serious Adverse Events (SAE)

SAE is defined as any significantly untoward medical occurrence, including:

- Death
- Life-threatening condition
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Required intervention to prevent permanent impairment/damage

7.5 Serious and Unexpected Suspected Adverse Reaction (SUSAR)

SUSAR is defined as any significantly untoward medical occurrence, the nature or severity of:

1. Which is not consistent with the applicable product information (e.g., Investigator's Brochure) for an unapproved investigational medicinal product.
2. Which must be causal relationship with study medication or clinical study.

7.6 SAE Report Procedure

7.6.1 Expedited Reporting of SAEs

When expedited SAEs meet the informed criteria according to the relevant regulations, they will be reported to the regulatory authorities (e.g. Minister of Health and Welfare; MOHW, Taiwan and the Food and Drug Administration; FDA, U.S.) by the Sponsor. Written reports should be made on the ADR Reporting Form of regulatory authorities (e.g. Taiwan MOHW and on Medwatch 3500A Form of US FDA), and submitted in a timely fashion according to all relevant regulatory requirements. A copy of this report will also be provided to the principal investigator's local institutional review board if necessary. All SAEs will be monitored until they are resolved or until the principal investigator assesses them as irreversible, chronic, or stable, or until the subject dies. During the clinical trial, if expedited SAEs occur, the investigator should inform the sponsor/CRO within 24 hours by fax and/or telephone.

7.6.2 IRB Notification

Reports of serious adverse events will be communicated to IRB:

- Fatal or life-threatening SUSAR: Should be reported to IRB in the 7 calendar days and detailed written documents should be provided within 15 days after being aware of the event.
- Non-Fatal or life-threatening SUSAR: Should be reported to IRB no later than 15

calendar days.

- Non-SUSAR SAE: Should be reported to IRB following each IRB regulations.

7.6.3 Record Retention

All telephone/fax reports must be followed with a written narrative summary of the adverse event and any sequel. These narratives, which confirm the information collected by telephone and may provide additional information not available at the time of the initial report, must be reviewed by the clinical monitor within 7 calendar days following the telephone/fax report to sponsor.

8 STATISTIC METHODS

8.1 Sample Size Determination

In part I, six subjects each will be evaluated for safety and efficacy assessments at 1 or 2 capsules TID dose for 28 days, sequentially.
In part II, this is a small proof-of-concept study to evaluate 2 doses of PDC-1421 and placebo in the treatment of major depressive disorder. Based on our study design, it is planned to enroll 60 subjects who met the intent-to-treat (ITT) basis.

8.2 Statistical Method

Simple descriptive statistics with 95% confidence interval will be performed with data collected in this study wherever applicable. Treatment effects are evaluated based on 2-sided test with a 0.05 significant level. All data shall be tabulated and presented in the study report. The descriptive statistics will be provided. The safety and efficacy data will be analyzed using the non-parametric method.

8.3 Analysis of Efficacy and Safety

The efficacy measures are conducted on the per-protocol (PP) and intent-to-treat (ITT) basis. The per protocol will be defined as (1) The drug compliance is at least 80% (2) Subjects have completed data to determine the primary endpoint. (3) Subjects cannot have protocol deviation. The protocol deviation will be defined as (1) Inclusion or exclusion criteria not satisfied. (2) Not permitted concomitant medications (3) Wrong randomized treatment prescribed. An intent-to-treat (ITT) basis will be defined as the data are analyzed by the treatment groups to which the randomized patients who take at least one dose of study medication and have any post-baseline safety data collected. The safety measures are conducted on the ITT basis. The data are analyzed utilizing the last observation carried forward (LOCF) technique to impute the missing data.

The efficacy and safety information recorded on CRF will be summarized by tables presented in frequency and percentage for categorical variables, in mean with SD as well as median with the minimum and maximum for continuous variables. The comparison between three groups will be analyzed by nonparametric method as appropriate. All adverse events will be summarized with coding term, severity, relationship to study drug by frequency tables with the counts and percentage. In addition, serious adverse events will be listed with event narration.

8.4 Premature Termination and Handling of Missing Data

Any premature termination of the study therapy will be recorded in CRF. Listing of the subjects with premature termination will be provided with the dates and reasons. Missing data caused by premature termination will be utilized the last observation carried forward (LOCF).

9 QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Study Monitoring

The clinical monitor from sponsor-designated contract research organization (CRO) will be responsible for overseeing the progress of a clinical trial to be conducted, recorded, and reported in accordance with the protocol, Standard Operating Procedures (SOPs), GCP, and the applicable regulatory requirements. All written informed consent and CRFs will be carefully reviewed and the accuracy of the data will be validated by clinical monitor.

9.2 Sponsor Auditing

Prior to locking the database of safety, the representative of Sponsor's quality assurance department will visit the Investigator's site to implement an audit of study. The audit will to determine the compliance of Investigator with protocol, GCP and applicable regulations. Besides, auditors will assess the accuracy of clinical data record. The Investigator and trial personnel need to cooperate with auditor to facilitate the process of audit.

9.3 Inspected by Regulatory Authorities

Local regulatory authority may visit the Investigator to conduct an inspection of this study and the site. The Investigator must make the trial-related records accessible to regulatory agency inspectors. In addition, the Investigator needs to notify the Sponsor immediately when contacted with regulatory authority for the inspection.

10 ETHICAL CONSIDERATIONS

10.1 Confidential

All information about study subjects will be kept confidential in accordance with the applicable regulatory requirement, to protect and respect the privacy of study subjects. Only the members performed study-related processes, including trial-related monitoring, audits, IRB/IEC review, and regulatory inspection, could directly access to source data/documents.

10.2 Protocol Amendment

Any amendment to the Protocol which deemed necessary as the study progresses will be fully discussed by Investigator and the Sponsor. A written amendment must be submitted to US FDA, MOHW and Chairman of the IRB, except when the change(s) involves only logistical or administrative aspects of the trial (e.g. change in clinical monitors or change of telephone numbers). Investigator must await for their approvals of the protocol amendment before implementing the changes. A protocol change intended to eliminate an apparent immediate hazard to trial subjects may be implemented immediately while simultaneously informing FDA/MOHW/IRB.

When, in the judgment of Chairmen of the IRB, Investigator and/or the Sponsor, the amendment to the Protocol substantially alters the study design and/or increases the potential risk to the subjects, the currently approved written informed consent form will be modified according to the modification. A second informed consent will be obtained from subjects enrolled in the study before further participation.

10.3 Informed Consent

Written informed consent must be reviewed and approved by IRB. Informed consent will be designed to provide detailed information, including explanation of the purpose of this

study, the risks and discomforts involved, potential benefits and all basic elements required by regulatory agencies, to participants. Investigator (or his or her qualified designees) will be responsible for explaining the contents of the informed consent to participants as clear as possible. Once it is felt that the subject understands the implications of participating, the written informed consent form shall be signed by the subject and a witness. The written informed consent will be obtained from each subject before any study related procedures (including any pre-treatment procedures) are performed.

10.4 Institutional Review Board (IRB)

This study is conducted to be consistent with the principles of the Declaration of Helsinki and GCP. This protocol and any supplementary documents, including written informed consent form, subject recruitment advertisements and Investigator's Brochure, must be reviewed and approved by IRB of all clinical sites qualified with local legal prescriptions prior to study initiation. The members of IRB are average thirty people, including medical doctors, professors of pharmacology and members whose primary area of interest is in the nonscientific area such as law and social welfare policy. One group of members of IRB meet once a month. Any changes in protocol must be received the IRB approval/favorable opinion in advance of use except for the immediate hazards to trial subjects. Investigator shall submit a status report to IRB at the end of the study. IRB must be notified by the Investigators promptly for all unanticipated problems involving risk to human subjects or others. Investigator is required to maintain an accurate and complete record of all written submissions made to IRB and must agree to share all such documents and reports with the Sponsor and any regulatory agency, for example, MOHW in Taiwan.

11 DATA HANDLING AND RECORD KEEPING

An EDC (electronic data capture) system may be used in this study to handle data in an electronic CRF. Follow the EDC system guideline when using it.

11.1 Data Handling and Collection

11.1.1 Study Specific Binders

Four types of binder will be utilized in this study:

Subject Binder: Containing informed consent, all case report forms and certain source documents collected for each subject entered the treatment period of the study. Each Subject Binder will have tab dividers for the laboratory printouts. Subject Binders will be kept at each study center.

Screening Binder: Containing all case report forms of subjects participated in the screening period but found ineligible for entering the treatment period of the study. Screening Binders will be kept at each study center

Generic Forms Binder: Containing enough blank generic case report forms for all subjects participating in the study.

Study Center File: Documents pertinent to the conduct of this study will be filed in this binder.

11.1.2 Responsibilities of Investigators

The Investigator shall process, prepare and maintain complete and accurate study procedure and documentation in compliance with GCP standards and local laws, rules and regulations. It is the Investigators' responsibility to ensure that the designated CRC for this study at the hospital fully understands his/her responsibilities and has the ability to fulfill all the relevant tasks on behalf of the Investigator in many occasions. For each

subject enrolled, a CRF must be completed and signed by the Investigator, no matter if this subject has completed the study or not. The Investigator agree to fully cooperate and assist the clinical monitor in performing their duties, including enabling direct access to the relevant hospital and clinic records. Study documentation will be promptly and fully disclosed to the Sponsor by the Investigator and also shall be made available at the Investigators' site for inspection, copying, review and audit for a reasonable number of times by representatives of the Sponsor or any regulatory agencies. The Investigator agrees to promptly take any reasonable steps that are requested by the Sponsor as a result of an audit to correct the known error in the study documentation and case report forms. The Investigator shall submit to the Sponsor all original case report forms and all reports required by the Protocol when the clinical study have been completed or terminated.

11.1.3 Responsibilities of Clinical Monitor

Clinical monitor shall frequently inspect the source documents (clinic and hospital subject records) and compare them with the CRF at regular time interval throughout the study, to verify the adherence of study execution to the Protocol and confirm the completeness, consistency and accuracy of the data entered. The monitor will perform source data verification by visually comparison between the entries of the CRF and subjects' records, including informed consent, inclusion/exclusion criteria, key variables, drug administration, adverse experience records and safety variables. Additional monitoring will be required if the error rate is unacceptably high.

11.2 Records Retention

11.2.1 Source Document Retention

Source documents refer to any record pertained to a given subject in the study from which study data is, or could be, obtained or verified. This includes, but not limited to, records, charts, notes and laboratory reports. These records, maintained by the Investigator, shall comply with applicable regulations. At the aspect of ICF keeping, one of the original signed informed consent forms for each participant shall be filed with records kept by the Investigator and the other copy should be given to the subject. Besides, progress notes on each visit to the clinic will be required. The progress note of

each visit shall at least contain the date of the visit, a general reference to the procedures completed, general subject status remarks including any significant medical findings, and the signature of the clinician making the entry. In addition, any contact with the subject via telephone or other means that provides significant clinical information, such as adverse events, will also be documented in the progress notes. These will be filed with subject records kept by the Investigator and made available for inspection by authorized personnel.

11.2.2 CRF Retention

All information required by the Protocol will be collected on either CRF or source documents. Essential documents should be retained until the Sponsor informs the Investigator that documents are no longer needed to be kept.

11.2.3 Record Maintenance

Regulation requires all investigators participating in clinical trials to maintain detailed clinical data for the duration of one of the following periods, whichever is shortest:

(1) A period of at least two years following the approval date of an application for a marketing permit in support of which the results of the clinical investigation were submitted, or

(2) A period of two years after the Sponsor notifies the Investigator that no further application is to be filed with the regulatory authority.

The Investigator will not dispose of any record relevant to this study without informing the Sponsor and providing an opportunity for the Sponsor personnel to collect such record. The Investigator shall take full responsibility for maintaining all documentation related to the conduct of this study, including subject records, research data, and pertinent correspondence. These documents are subject to inspection by the Sponsor and appropriate regulatory authorities.

11.3 Study Center File Management

It will be the responsibility of the clinical monitors/CRC to maintain the study center file. The study center file for this study will contain, but not limited to, the information listed below:

(1) Investigator Brochure.
(2) Signed Protocol and Amendments approved by both Regulatory Authority and IRB.
(3) Copies of Regulatory Authority and IRB approval letter for this study.
(4) Curricula Vitae of Investigator and sub-investigator.
(5) Other documentation of IRB complied with regulations by Regulatory Authority.
(6) Copy of Informed Consent Form approved by both Regulatory Authority and IRB.
(7) All IRB correspondence (reports to IRB, including reports of all death and adverse experience, annual progress reports, final reports, acknowledgement of receipt of report and actions taken by IRB).
(8) All IND Efficacy and Safety Reports sent to the Investigator.
(9) Laboratory certification and/or license.
(10) All written correspondence with the Sponsor.
(11) Normal ranges of laboratory value for all laboratory tests required by Protocol.
(12) CRA monitoring logs.
(13) Drug Invoices and Drug Dispense/Return Records.

12 PUBLICATION PLAN

Sponsor and Investigator will determine authorship and govern other aspects of the publication process related to this trial. All publications must be approved by the Sponsor prior to publication.

13 REFERENCES

1. Robert Riesenberg, Joshua Rosenthal, Leslie Moldauer, Christine Peterson. Results of a proof-of-concept, dose-finding, double-blind, placebo-controlled study of RX-10100 (Serdaxin®) in subjects with major depressive disorder. Psychopharmacology 2012; 221 (4): 601-610.
2. Kari R Nations, Peter Dogterom, Larry Ereshefsky et al. Examination of Org 26576, an AMPA receptor positive allosteric modulator, in patients diagnosed with major depressive disorder: an exploratory, randomized, double-blind, placebo-controlled trial. Journal of Psychopharmacology 2012; 26(12): 1525-1539.
3. Enric Alvarez, Victor Perez, Francesc Artigas, et al. A double-blind, randomized, placebo-controlled, active reference study of Lu AA21004 in patients with major depressive disorder. International Journal of Neuropsychopharmacology. 2012; 15(5): 589-600
4. Sanjay Dube, Mary Anne Della, Prasad Rao, et al. A study of the effects of LY2216684, a selective norepinephrine reuptake inhibitor, in the treatment of major depression. Journal of Psychiatric Research 2010; 44(6): 356-363
5. Beth Pangallo, Mary Anne Della, Celine Goldberger, et al. A randomized, double-blind study comparing LY2216684 and placebo in the treatment of major depressive disorder. Journal of Psychiatric Research 2011; 45(6): 748-755
6. Cheng MC, Li CY, Ko HC, Ko FN, Lin YL, Wu TS. Antidepressant principles of the roots of Polygala tenuifolia. J Nat Prod. 2006; 69: 1305-1309.
7. Zhou J. Norepinephrine transporter inhibitors and their therapeutic potential. Drugs Future. 2004; 29(12): 1235-44.
8. Mann JJ. The medical management of depression. N Engl J Med. 2005; 353(17):

1819-34.

9. Englert LF, Ho BT, Taylor D. The effects of (-)-delta9-tetrahydrocannabinol on reserpine-induced hypothermia in rats. Br J Pharmacol. 1973; 49(2): 243-52.
10. Sanchez-Mateo CC, Bonkanka CX, Prado B, Rabanal RM. Antidepressant activity of some Hypericum reflexum L. fil. extracts in the forced swimming test in mice. J Ethnopharmacol. 2007; 112(1): 115-21.
11. Gylys JA, Muccia PM, Taylor MK. Pharmacological and toxicological properties of 2-methyl-3-piperidinopyrazine, a new antidepressant. Ann N Y Acad Sci. 1963; 107: 899-913.
12. Suehs BT, et al, Texas Medication Algorithm Project Procedural Manual: Major Depressive Disorder Algorithms. The Texas Department of State Health Services. 2008
13. Montgomery SA, Å sberg M. A New Depression Scale Designed to be Sensitive to Change. British Journal of Psychiatry 1979; 134: 382-389.
14. Mark. Zimmerman et al., Severity classification on the Hamilton depression rating scale. Journal of Affective Disorders. 2013; 150: 384–388
15. Hamilton, M. A rating scale for depression. Journal of Neurology, Neurosurgery and Psychiatry. 1960; 23: 56-62
16. Hamilton M. The assessment of anxiety states by rating. British Journal of Medical Psychology. 1959; 32:50–55.
17. Ching-I Hung et al., Preliminary study of a scale measuring depression and somatic symptoms. Psychological Reports. 2006; 99: 379-389
18. Ching-I Hung et al., The cut-off points of the Depression and Somatic Symptoms Scale and the Hospital Anxiety and Depression Scale in detecting non-full remission and a current major depressive episode. International Journal Psychiatry in Clinical Practice. 2011; 00: 1-8
19. Ching-I Hung et al., Depression and somatic symptoms scale: A new scale with both depression and somatic symptoms emphasized. Psychiatry and Clinical Neurosciences. 2006; 60: 700-708