Detoxification from the Lipid Tract by Cocktail Design

Study Protocol with Informed Consent Form

National Clinical Trial (NCT) Identified Number:

Principal Investigator: Yang I. Cao

Sponsor: Scientifique Global Limited

Protocol ID: SCI-CT-0001

Funded by: Scientifique Global Limited

Version Number: v.<1.0>

<07 March 2024>

Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale

Table of Contents

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- 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)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (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. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

INVESTIGATOR'S SIGNATURE

The signature below constitutes the approval of this protocol and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines, as described in the *Statement of Compliance* above.

Principal Investigator or Clinical Site Investigator:

Signed:  Date: March 7, 2024

Name: Yang Cao

Title: Principal Investigator (PI)

Investigator Contact Information
Affiliation: Scientifique Global Limited
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Email: *ypach@yangpachankis.us*

INFORMED CONSENT

The signature below constitutes the approval of this protocol and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines, as described in the *Statement of Compliance*.

Participant(s):

Signed: [redacted] Date: March 7, 2024

Name: [redacted]

Participant Contact Information

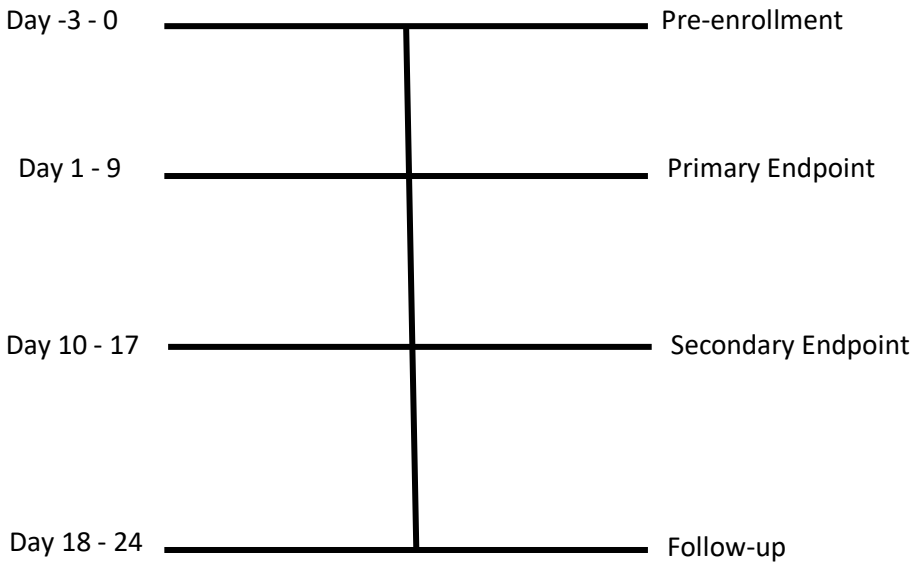
[redacted]

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Detoxification from the Lipid Tract by Cocktail Design
Study Description:	Apart from electroencephalogram biofeedback (EB) and electrical brain stimulation (EBS) adopted for maintenance treatment, the study utilizes infralow-frequency transcranial magnetic stimulation (ILF-TMS) for initial γ-aminobutyric acid (GABA) stimulation. The cocktail therapy starts after the primary efficacy endpoint, and concomitant therapy is adopted throughout the study.
Objectives:	Primary Objective: The primary objective is to detoxicate the substances leading to the adverse reactions after COVID-19 infection. Secondary Objectives: The secondary objective is to study the GABA's effects in autism spectrum disorder (ASD).
Endpoints:	Primary Endpoint: Mar. 6, 2024 Secondary Endpoints: Apr. 26, 2024
Study Population:	The study takes place in Chongqing, mainland China, without specification on gender or age. The population is mostly consisted of ethnic Chinese of the Han ethnicity. The individuals to be recruited are COVID-19-vaccinated. Sample size not specified, and recruitment on a rolling basis.
Phase:	IV.
Description of Sites/Facilities Enrolling Participants:	The site is located in Chongqing, mainland China in a residence place.
Description of Study Intervention:	The primary efficacy endpoint mainly adopts device interventions with EB, EBS, and ILF-TMS for GABA. Afterwards, the cocktail therapy adopts alprazolam, pravastatin sodium, and metoprolol.
Study Duration:	2 months.
Participant Duration:	1 month.
Intervention Duration:	2 months.

1.2 SCHEMA


Day -3 - 0
Pre-enrollment
Day 1 - 9
Primary Endpoint
Day 10 - 17
Secondary Endpoint
Day 18 - 24
Follow-up

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Pre-Enrollment Day -3 - 0	Primary Efficacy Day 1 - 9	Secondary Efficacy Day 10 - 17	Follow-up Day 18 - 24
Informed consent	X			
Demographics	X			
Medical history	X			
Administer study intervention		X	X	X
Administer device-based intervention		X		
Concomitant medication review		X		
Vital signs	X	X	X	X
Performance status	X	X	X	X
Hematology and other tests		X	X	
Adverse event review and evaluation	X	X	X	X

2 INTRODUCTION

2.1 STUDY BACKGROUND

Membrane fusion is established to be a critical mechanism of SARS-CoV viruses' entry into the lipid bilayers of the cells [1]. Not only correlative studies suggested that the changes in high-density lipoprotein – cholesterol (HDL-C) and low-density lipoprotein - cholesterol (HDL-C) influence on the severity of COVID-19 prognosis in patients[2], the evidence also exists in macropinocytosis and steroidogenesis pathways for neuronal infections to precede cytokine storms, bypassing the blood-brain barrier (BBB) by lipid circulation [3,4].

It was tested that GABA, in the joint action with topiramate, modulates macrophage activities by modulating cholesterol-metabolism associated molecules [5]. GABA A receptors exhibit highly dynamic trafficking and cell surface mobility and influence on post-endocytic effects [6]. Benzodiazepines (BZDs) exercise the mechanism of action by facilitating the binding of the inhibitory neurotransmitter GABA at various GABA receptors throughout the central nervous system (CNS) [7,8]. Alprazolam, a type of BZD, was tested by Al-Tubuly, Aburawi, Alghzewi, Gorash and Errwami [9] in joint action with water-soluble beta blocker atenolol, in comparison with the non-selective β-adrenoceptor antagonist propranolol on the pharmacological effects on depression. The study hypothesizes that by replacing the water-soluble beta blocker to lipid-soluble one metoprolol, the effect of detoxification from the lipid and sebaceous immunobiological pathways can be achieved by the clathrin-dependent endocytosis process [6,10,11].

2.2 SCIENTIFIC RATIONALE

Even though partial progress was made in the NCT05839236 trial by statin therapies, the therapeutic effects have not been lasting nor significant. The study develops from the previous protocol for a cocktail therapy by the joint mechanism of actions of alprazolam, metoprolol, and pravastatin sodium for the detoxification process.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The cocktail therapy may cause low heart rate, and consequentially induce sudden death during sleep by insufficient supply of blood and oxygen.

2.3.2 KNOWN POTENTIAL BENEFITS

The cocktail therapy may reduce the risks in atherosclerosis from post-COVID-vaccine complexities, and potentially cure the high blood pressure in the context. Preventive effects may be exercised on the SARS-CoV viruses from the lipid upstream infection paths, including steroidogenesis, which bypass the BBB, and from further proliferation in the neuronal infections.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The anti-depressant effects of alprazolam are mainly derived from its mechanism of action in releasing norephedrine, and in turn stimulate β2 receptors. The experiment trial of Al-Tubuly, Aburawi, Alghzewi, Gorash and Errwami [9] adopted water-soluble beta blocker that has anti-depressant effects to potentiate the anti-depressant effects of alprazolam. It differs from prazosin, which blocks or suppresses β1 receptors in order to stimulate β2 receptors, that produces depression but substantially potentiate the anti-depressant effects of alprazolam and imipramine [9].

In assessment of the purpose of the study alone, the main medicine of prazosin seems to have lower risks, higher risks arise from the potential side-effects of low blood pressure and heart failure. Moreover, with the participant's ASD, the inhibition of β1 receptors may suppress respiration reflex, substantially increase the risks of sudden death during sleep. With the overall assessments, the cocktail therapy is finalized with alprazolam, pravastatin sodium, and metoprolol.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Degenerate lipids via statin therapy and lipid-soluble beta blocker by the aid of alprazolam.	The pre-trial examinations suggest that brain excitation is insufficient and intercranial suppression is strong. GABA therapies with ILF-TMS is adopted before the cocktail therapy.	The excitation effects of clonazepam on the participant with ASD have been preparing the participant's neuronal excitability for the cocktail therapy [12].
Secondary		
Understand GABA therapy's mechanism of action in ASD persons.	Clonazepam is continuously used during the cocktail therapy, and the participant's mode changes during the secondary endpoint will lead to further understandings.	The correlative uses of benzodiazepines (alprazolam) and GABA-A receptor agonist (clonazepam) will reveal the mechanisms of action's potentials in detoxification with the qualitative study.
Tertiary/Exploratory		

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
If the detoxification were successful, can the cocktail therapy be generalizable to wider population regardless of neurodiversity? If not, what are the missing links in the study design and the experiment trial?	The exploratory objectives depend upon the result of the secondary efficacy endpoint.	Causal inference in the correlations between neuronal activities and metabolism will further our understandings in sebaceous immunobiology.

4 STUDY DESIGN

4.1 OVERALL DESIGN

The study is designed to utilize both the metabolic tract of lipid degeneration by statin therapy and the neuronal tract of lipid degeneration, where alprazolam increases level of oxidative degradation of lipids [13]. The study design framework is predicted to ameliorate oxidative stress in the metabolic symptoms, while inducing the SARS-CoV viral excretion from the lipid bilayers of cells [1].

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The phenyl rings and methyl groups are biochemically affined to GABA interactions, intermediated by the van der Waals radii [14]. It is then inferred that the van der Waals force's difference exists in autistic and neurotypical GABA receptor binding activities, explaining the mechanism of actions of clonazepam in excitation for ASD users [12] . The chemical structural similarities of clonazepam and alprazolam in the different effects exercised on ASD users can be exploratorily revealed by the lipid degenerative design in the study, i.e., intervention in the upstream steroidogenesis tract [15].

4.3 JUSTIFICATION FOR DOSE

All medicines start with the minimum dosage labelled and adjusted according to symptom amelioration and development. No exceeding dosage is intended unless the efficacy is proven, adverse event minimized, and necessities justified for intensive treatment.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), **Section 1.3**.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Above the age for consent
4. Have been SARS-CoV-2 vaccinated

5. Ability to take oral medication and device-enabled therapies, and be willing to adhere to the Detoxification from the Lipid Tract regimen
6. Agreement to adhere to Lifestyle Considerations (see **Section 5.3**) throughout study duration]

5.2 EXCLUSION CRITERIA

1. Below the age of 18 when entering the trial;
2. Healthy participants without COVID-19 vaccination.

5.3 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Abstain from alcohol for the whole interventional trial.
- Abstain from strenuous exercise for the whole interventional trial.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

With the systematic official denials on the post-vaccination conditions, only participants who have trust in the PI and have doubt in the vaccination methods in SARS-CoV-2 during initial contact are considered for recruitment.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Intervention	Method	Dosage	Time	Period	Concomitant
EB	Device	Twice	Per day	Primary	No
EBS	Device	Once	Per day		
ILF-TMS	Device	Once	Per day		
Alprazolam	Oral	0.4 mg	Per night	Starting at the end of primary	Yes
Clonazepam	Oral	1 mg	Per day		
Metoprolol	Oral	95 mg	Per night	Secondary	Yes
Pravastatin Sodium	Oral	20 mg	Per night		
Sertraline Hydrochloride	Oral	150 mg	Per day	Throughout	No
Olanzapine	Oral	10 mg	Per night		

6.1.2 DOSING AND ADMINISTRATION

See in **Section 6.1.1**.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Not applicable.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Not applicable.

6.2.3 PRODUCT STORAGE AND STABILITY

Not applicable.

6.2.4 PREPARATION

Not applicable.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Not applicable.

6.4 STUDY INTERVENTION COMPLIANCE

Daily records are kept by the nurses, investigator and / or participant.

6.5 CONCOMITANT THERAPY

Iodized lecithin 4.5 mg per day and augentropfen stulln mono three drops per day are adopted for concomitant therapy starting from the end of the primary efficacy endpoint.

6.5.1 RESCUE MEDICINE

The study site will supply sacubitril valsartan sodium for rescue medication.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from Medicine Induced Hemodialysis does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance

- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit(s) and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Diastolic Blood Pressure (DBP) is measured and monitored along with Systolic Blood Pressure (SBP) and heart rate. Efficacy for the primary objective is assessed by the three indicators from monitoring data. From participant interview, DBP is considered the etiological symptom for SBP sensitivity in elevation, and heart rate is considered both an effect from SBP sensitivity and immune response sensitivity indicator. Heart rate frequency shifts, therefore, have dual implication in time series and cardiac enzymes, myocardiogram, and Color Doppler Ultrasound are tested for further determinations.

Hematological indicators are reorganized for assessments of secondary and exploratory objectives. From the baseline characteristics, platelet distribution width (PDW) and mean platelet volume (MPV) have been anchored for the main assessment in pharmacokinetic efficacy, along with eosinophil absolute number [16,17]. The apoptotic pathway is closely monitored for efficacy assessment with neutrophil and monocyte indicators [18-20]. The determination on each period's changes' indications is assisted by lymphocyte and basophil indicators, apart from leukocyte regeneration healthiness and differentiation trends.

8.2 SAFETY AND OTHER ASSESSMENTS

COVID-19 specific tests are conducted, in combination with the clinical assessments. The efficacy of interventional medicines is retrospectively assessed with efficacy contribution plausibility from concomitant therapy. Safety assessments are conducted between each efficacy endpoint with relation to adverse events.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, 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 incapacity or substantial disruption of the ability to conduct normal life functions, 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 participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

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. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **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 study intervention administration and cannot be

explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (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 study intervention, 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.
- **Potentially 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, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 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 intervention.

8.3.4 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 CRF. 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.

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 research team will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study 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.

8.3.5 ADVERSE EVENT REPORTING

According to 21 CFR 312.64(b), "…The investigator must record nonserious adverse events and report them to the sponsor according to the timetable for reporting specified in the protocol". In addition, conditions such as coughing, bodily sores, tiredness, etc. common and expected in the study will not be reported per the standard process for reporting. These events will be notified to medical monitor and assigned with concomitant therapy.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The study clinician will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center / study sponsor and should be provided as soon as possible.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Events with long-term implications to the participants' health will be discussed to the participants by the PI.

8.3.8 EVENTS OF SPECIAL INTEREST

The participant's neurodiverse condition has been reported to the PI. The event leads to the adjustments of the study design and plan, just as the SAE that started the study.

8.3.9 REPORTING OF PREGNANCY

Not applicable.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing IRB and to the PI. The UP report will include the following information:

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

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

- UPs that are SAEs will be reported to the IRB and to the study sponsor within 6 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the study sponsor within 24 hours of the investigator becoming aware of the problem.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Ups will be discussed with participants by the PI.

9 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

9.1.1 INFORMED CONSENT PROCESS

9.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The consent materials have been aggregated with the protocol.

9.1.1.2 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. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's 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 family or 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. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. 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.

9.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the study purpose has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

9.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. 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.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product 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 the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored by the PI's facilities. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by PI's research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived by the PI with scientific data repository.

9.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored by the PI. After the study is completed, the de-identified, archived data will be transmitted to and stored on *Zenodo*, for use by other researchers including those outside of the study. Permission to transmit data to *Zenodo* is included in the informed consent.

With the participant's approval and as approved by local IRBs, de-identified biological samples will be processed by the local hospital. The hospital reports will be maintained with the blinding of the identity of the participant.

When the study is completed, access to study data and/or samples will be provided through *Zenodo*.

9.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Medical Monitor**
Rev. Yang I. Cao, Ph.D.	
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9.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertis. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the PI.

9.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Daily blood pressure monitoring will be conducted by the research team and according to the medical necessities of the participant. The participant can do self-monitoring in the tertiary phase if choosing to monitor at home. Baseline and endpoint hematology will be conducted by the local hospital. Targeted and random review of certain data at endpoints will be conducted by the PI.
- Independent audits will not be conducted.

9.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.]

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

9.1.9 DATA HANDLING AND RECORD KEEPING

9.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

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

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into iPhone and MacBook. The data system includes password

protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

9.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harminosation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. 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.

9.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 10 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to the PI. Protocol deviations must be sent to the IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

9.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the publicly available data sharing policies and regulations for open science practice.

9.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

9.2 ADDITIONAL CONSIDERATIONS

It is not certain if complete cure and excretion of S2 proteins from the human host are possible, even after the amount being untestable by HIV chemiluminescence test [21,22].

9.3 ABBREVIATIONS

AE	Adverse Event
ASD	Autism spectrum disorder
BBB	Blood-Brain Barrier
CFR	Code of Federal Regulations
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
EB	Electroencephalogram Biofeedback
EBS	Electrical Brain Stimulation
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GABA	γ-aminobutyric acid
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
ILF-TMS	Infralow-frequency transcranial magnetic stimulation
IND	Investigational New Drug Application
IRB	Institutional Review Board
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
PRC	People's Republic of China
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SARS-CoV	Severe Acute Respiratory Syndrome associated Coronavirus
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
USA	United States of America

9.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
1.0	2024-03-07	Initial draft.	Interim plans.

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