STUDY PROTOCOL

PROTOCOL TITLE:
A randomized phase I study on the changes in immune responses with Reduxium in healthy adults.

PROTOCOL NUMBER:
1.0

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STUDY PROTOCOL

1. BACKGROUND AND RATIONALE

Briefly sketch the background to the current proposal, critically evaluating the existing knowledge and specifically identify the gaps that the project is intended to fill.

Reduxium is a dietary supplement that provides immune support. This natural compound is orally-ingested in the form of droplets in water to boost the immune system and control inflammation. There is not enough data on the mechanism associated with the action of Reduxium or the extent of the immune response increase it produces. In this study, we propose treating a group of healthy volunteers with Reduxium and investigate the utility of this approach in boosting the native and adaptive immune responses that correlate with immune protection. This may form the basis for a future study employing the product in infectious disease patients.

1.1. General Introduction

Give a brief description of the drug/device to be studied. Their mechanism of action, whether currently in use and approved for use.

With the global population increasingly exposed to pandemic crises, permanent and expedient solutions are needed at an affordable cost. Virus outbreaks affect most severely less developed communities. Reduxium, a dietary supplement that provides immune support, is a low cost candidate for the treatment of infectious diseases.

Reduxium is a natural compound commercialised in the USA that helps restore homeostasis and controls inflammation. As no toxins or allergens are used, but purely food grade compounds, it is classified as a dietary supplement. Its current purpose is not to treat, diagnose, prevent or cure any disease, but it has immunomodulatory properties.

Reduxium is manufactured using a proprietary reactor - a “biochemical cavitation mixer” that allows to create a “smart small molecule”. The principal device belongs to the cavitation technology family and is used for the intensification of technological processes in liquid media (liquid processing, splitting of complex molecules, "cold" pasteurization, destruction of solid inclusions). The usage of this process technology enables to compress a set of 12 molecules into the size of 1.

The complex molecules generated scan at the cellular level for the presence of pathogenic (bacterial, viral, fungal) etiologies by reading the characteristics of the electron-proton (KNa) pump on the membrane. If these characteristics are violated, the drug “enters” the cell. At the intra-cellular level, the drugs scan the cell in search of pathology; this "scanning process" is made on the basis of selectivity (healthy – do not touch/ill - induce apoptosis) through the mechanism of mitochondria activity. Specifically, the complex molecules start a cascade of biochemical processes (switching to mitochondria aerobic oxidation, restarting the methyl group with the “epigenetic” effects on DNA, apoptosis). It is unclear how and to which extent this mechanism contributes to innate immune activation following cellular damage and stress, or how it contributes to the adaptive immune response of T and B cells.

1.2. Rationale and justification for the Study

Include a description and justification for the route of administration, dosage, dosage regimen, intervention periods, and selection of study population. Include a statement of hypothesis.

a. Rationale for the Study Purpose

Briefly sketch the background to the current proposal, critically evaluate existing knowledge and specifically identify the gaps that the project is intended to fill.

Therapeutic strategies that boost the activity of the immune system should be considered in the context of management/prevention of infectious diseases. Strategies for boosting B and T cell immunity and antibody secretion should include an analysis of the immunological status of the subject prior to treatment and a thorough investigation of the type of antibody response that will likely be engendered/influenced by the specific therapeutic approach.

b. Rationale for Doses Selected

Briefly sketch the rationale for selection of the study dosage

Subjects will receive Reduxium doses that have been proven to show effect on patients of infectious diseases.

c. Rationale for Study Population

Justify selection of target population.

We will test Reduxium's capacity to boost the immune response in healthy volunteers. If there is an observable boost in protective mechanisms, this will form the basis for a future study employing Reduxium in infectious disease patients as a way of augmenting their protective immune responses to a point where they can successfully fight-off the infection.

d. Rationale for Study Design

State the rationale behind the proposed study design (e.g. two period cross over, case control etc.)

This is a prospective, one-arm, interventional, phase I study where 20 healthy subjects will be treated with Reduxium. Two groups of subjects (one group of 10 healthy subjects, a second group of 10 healthy subjects who were vaccinated for influenza within the last 3 months) will be treated in parallel with Reduxium for 14 days and followed for six weeks. For the 20 subjects, immune response will be analysed and compared to their baseline values. The results of this pilot study will aid to decide whether to proceed with a future randomised controlled trial of Reduxium on infectious disease patients.

2. HYPOTHESIS AND OBJECTIVES

Study objectives are concise statements of the primary and secondary clinical and statistical questions that the study is designed to answer. Study hypothesis must relate to the hypothesis

present in the rationale and should be consistent with the objectives described. Number objectives in order of priority.

2.1. Hypothesis

State concisely what hypothesis is to be tested

Reduxium consumption will alter host immune system against acute infection.

2.2. Primary Objectives

State primary protocol objective. This should always address a specific hypothesis

The primary objective is to analyse the changes in the immunoresponses after six weeks of Reduxium intake.

2.3. Secondary Objectives

State secondary protocol objective if pertinent. This may or may not be hypothesis driven, may include secondary outcomes, and may include more general non-experimental objectives e.g. to develop a registry etc.

The secondary objective is to analyse the safety and tolerability of the product.

2.4. Potential Risks and benefits:

a. End Points - Efficacy

Include a discussion of anticipated benefits

- Pre/post-treatment T cell subsets and phenotypes utilising the following groups of labelled antibodies:

Marker	Phenotype
CD3-BUV-395	T cells
CD4-BUV496	T cells
CD14-APC-Cy7	monocytes
CD19-APC-Cy7	B cells
CXCR5-PE-Cy7	T follicular helper (Tfh) cell marker

ICOS-BUV737	Tfh costimulatory molecule
PD-1-BV711	Exhaustion marker/Tfh differentiation
CD40L-PE-CF594	Ig isotype switching and B-cell survival
IL-21R-BV786	Tfh cell differentiation, GC B cells activation
CD25-BV421	IL-2R, Regulatory T-cell (Treg) marker
CD127-BB515	IL—7R, Treg marker

- Pre/post-treatment B cell subsets and phenotypes utilising the following groups of labelled antibodies:

Marker	Phenotype
CD19-BV510	B cells
CD3-APC-Cy7	T cells
CD14-APC-Cy7	monocytes
CD10-PE-Dazzle594	Immature B cells
CD21-APC	Memory B cells marker
CD27-BV650	Memory B cells marker
CD38-PE-Cy7	Plasma cells / plasmablast marker
CD40-PE	GC formation, B cell maturation
ICOSL-BV605	Tfh cell development, antibody response, GC reactions

PD-1-BV711	Exhaustion marker

- Innate immune subsets (monocytes –CD14PE), NK cells (CD56 APC) and neutrophils (CD15PE and CD16 APC). Antibody germline repertoires will be analysed by massive parallel sequencing of VH/VL genes and T cell proliferative capacities determined by 3H thymidine incorporation following stimulation by mitogen (PHA).

b. End Points - Safety

Include a discussion of anticipated risks

Safety will be assessed on the basis of the frequency and severity of adverse events. Other safety assessments are clinical laboratory tests (haematologic testing and serum chemical testing), vital signs and physical examinations.

3. STUDY POPULATION

3.1. List the number of subjects to be enrolled.

Give a breakdown by institution for multi centre studies within Singapore. Indicate from where the study population will be drawn. State if there are any subject restrictions based on race of the subject. Justify the exclusion of women, children or minorities if the study tends to exclude them in context of the study design.

We will recruit 20 adult healthy subjects, age 21-50 years, consisting of 10 subjects without prior history of influenza vaccination and 10 subjects who have received the influenza vaccine within the last 3 months at recruitment. There are no restrictions based on sex or ethnicity.

3.2. Criteria for Recruitment

Discuss evaluations/procedures necessary to assess or confirm whether a subject meets the eligibility criteria and may be enrolled. Discuss the sequence of events that should occur during recruitment.

3.3. Inclusion Criteria

Provide a statement that subject must meet all of the inclusion criteria to participate in this study and list each criterion.

a. The disease or disorder under study, and how it is to be documented i.e. diagnostic methods, criteria for classification etc.
b. For populations with cancer or pre cancer please include requirements for histological confirmation of diagnosis, time for diagnosis and disease status at entry.
c. Demographic characteristics (e.g. gender, age). Please explain age restrictions if any
d. Ability to provide informed consent
e. If men and women of reproducible age are enrolled, provide details of allowable contraception methods for the trial.

- Subjects of 21 – 50 years of age
- Normal blood pressure (BP <140/90 nnHg)
- Normal fasting glucose (<6 mmol/L)
- Subjects must stop all supplement for 1 month prior to enrolment

3.4. Exclusion Criteria

Provide a statement that all subjects meeting any of the exclusion criteria at baseline will be excluded from participation and then list the criterion.
Examples include the following: medical condition or laboratory finding that precludes participation, recent (with time frame) illness that precludes or delays participation, pregnancy or lactation, characteristics of household or close contacts (e.g. household contacts who are immunocompromised), known allergic reactions to components of study product(s), treatment with another investigational drug (with time frame), history of drug/alcohol abuse, disallowed concomitant medications etc.

- Subjects with known history of lungs or cardiovascular disease
- History of previous pancreatitis;
- Past or current history of malignancy
- Subjects with type 2 diabetes with complications, having trophic ulcers, black foot;
- Past or current history of peptic ulcer disease
- Pregnant women

3.5. Withdrawal Criteria

List possible reasons for discontinuation of study intervention/product in this section, e.g. development of laboratory toxicities, study closure due to DSMB review etc.

A subject may be withdrawn from the study for any of the following reasons:

- Lost to follow-up
- Withdrawal by subject

The consequence of study withdrawal is that no new information will be collected from the withdrawn subject and added to the existing data or any database; however, every effort will be made to follow up all subjects for safety.

3.6. Subject Replacement

State whether subjects who drop out will be replaced.

Candidates who drop out will be replaced by new candidates who fulfil the inclusion and exclusion criteria to achieve 20 completed subjects.

4. TRIAL SCHEDULE

Information outlined in this section should be consistent with the information in the schedule of study visits and procedures.

All subjects will be consented. Eligible subjects will be given 1 oral drop (0.05ml) of

Reduxium per 10kg of body weight (max 8 drops), every 8 hours (3 times a day) for 14 days. Subjects will subsequently visit the research site 6 times (6 weeks, once per week) for blood drawing (one 10 ml draw per week). Six B/T-cell antibody panels will be measured per subject, one per week. Blood samples will be analysed to assess the product's safety, tolerability and efficacy.

5. STUDY DESIGN

Discuss in detail the experimental design (e.g. two period crossover, case control, placebo control, blinding, randomization, number of study arms, phase of trial, approximate time to complete study recruitment, expected duration of subject participation, sequence and duration of all trial periods, including follow up, changes in scheduling, single or multi centre, healthy or sick population, in or outpatient etc.) to accomplish the specific aims of the project. Use diagrams to explain design complexities.

This is a single-centre, one-arm, interventional, prospective phase I study of 20 healthy subjects who will be treated with Reduxium. Recruitment will start upon DRSB approval and end in one year. The expected duration of subject participation is 8 weeks including follow-up. After consenting to the study and having their baseline information collected, subjects will be treated with Reduxium for 2 weeks. Eligible subjects will be given 1 oral drop (0.05ml) of Reduxium per 10kg of body weight (max 8 drops), every 8 hours (3 times a day) for 14 days. Subjects will subsequently visit the research site 6 times (6 weeks, once per week) for blood drawing (one 10 ml draw per week). Blood samples will be analysed for serial B/T-cell antibody panels (6 per subject, once weekly) to assess the product's safety, tolerability and efficacy.

5.1. Summary of Study Design

Briefly describe the study design and indicate, in general terms, how the design will fulfil the intent of the study.

This is a single-centre, one-arm, interventional, prospective phase I study of 20 healthy subjects who will be treated with the oral supplement Reduxium for 2 weeks prior to 6 weekly blood draws for analysing immune response. The design will allow to detect changes in immune response caused by Reduxium in healthy subjects and establish if it can further boost immune response to flu in recently vaccinated subjects.

6. METHODS AND ASSESSMENTS

Discuss the procedures to be used to accomplish the specific aims of the project. Will any of the procedures be placed on the audiotape, film / video, or other electronic medium? If yes, what is the medium? Explain how the recorded information will be used? How long will the tapes etc. Be retained and how will they be disposed off?

Participants who fulfil the inclusion and exclusion criteria will be prospectively recruited as study subjects. Baseline clinical and laboratory data will be collected prior to trial commencement. Subjects will be monitored for toxicity and treatment efficacy. These will be recorded in the case report forms.

6.1. Randomisation and Blinding

This section should describe randomisation and blinding procedures (if applicable to the study design). Include a description or a table that describes how study subjects will be assigned to the study groups. The timing and procedures for planned and unplanned breaking of randomization codes should be included. Include statement when unmasking may occur and who may unmask.

N/A

6.2. Contraception and Pregnancy Testing

For females of childbearing age included in the trial describe methods of pregnancy testing and contraception if pregnancy is to be avoided during the trial.

The effect of Reduxium on a baby's development is not known. Therefore, pregnant and breast-feeding women may not take part in this study. Women who have a chance of becoming pregnant must have a negative pregnancy test at study entry and use birth control during the study.

6.3. Study Visits and Procedures

Provide a brief outline of the all the study visits, procedures to be done during the study, follow up after the study and discontinuation visit.

a. Screening Visits and Procedures

Include only those evaluations necessary to assess whether a subject meets recruitment criteria. Discuss the sequence of events that should occur during screening and the decision points regarding eligibility. List the timeframe prior to recruitment within which screening tests and evaluations must be done (e.g. within 28 days prior to recruitment). Describe all procedures that must be completed before the study begins

Eligible patients will be asked for informed consent, and their baseline investigation and eligibility (inclusion and exclusion criteria) will be documented. The baseline investigation data to be collected include the following:

- Demographics
- Medical/treatment history
- Physical examination
- Vital signs including blood pressure and pulse rate
- Blood tests of immune cell subsets and phenotypes

Results from within 30 days of the consent can be used as baseline results.

After completing the procedures mentioned above, subjects will start the Reduxium treatment: they will receive a dosage of 1 oral drop (0.05ml) per 10kg of body weight (max 8 drops), every 8 hours (3 times a day) for 14 days.

b. Study Visits and Procedures

Describe all the visits and procedures that must be performed during the study intervention phase.

After completing 14 days of Reduxium treatment, subjects will visit the research site 6 times, once per week, for a 10 ml blood draw to analyse serial B/T-cell antibody panels and general immunophenotype as mentioned in section 2.4.a.

c. Final Study Visit:

Define when the final study visit should occur and any special procedures / evaluations or instructions to the subject.

N/A

d. Post Study Follow up and Procedures

Include discussion of evaluations/procedures required to assess or confirm study outcome measures and study evaluations. Discuss the sequence of events that should occur during the visit, if applicable. Include, as applicable, counselling, medications, assessment of adverse events etc.

N/A

e. Discontinuation Visit and Procedures

Specify which of the evaluations required for the final study visit should be done if withdrawal occurs. Subjects may withdraw voluntarily from participation in the study at any time. Subjects may also withdraw voluntarily from receiving the study intervention for any reason. Clearly differentiate between what evaluations are to be done in each of these circumstances.
If voluntary withdrawal occurs, the subject should be asked to continue scheduled evaluations, complete an end of study evaluation, and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or the subject's condition becomes stable. Describe efforts to continue follow - up, especially for safety outcome measures.

A patient may be withdrawn from the study for any of the following reasons:

- Lost to follow-up
- Withdrawal by subject
- Toxicity
- Death
- Other

The consequence of study withdrawal is that no new information will be collected from the withdrawn patient and added to the existing data or any database.

7. TRIAL MATERIALS

If multiple products are to be evaluated in the study, the following sections should be repeated for each product and the sections should be renumbered accordingly. Describe placebo or control product

7.1. Trial Product (s)

Please provide background information on the trial product, its safety issues and duration of exposure. For drugs also include information on dosage.
Information about the drugs could also be obtained from the I.B or the package insert. Please include I.B or package insert.

Reduxium is a proprietary formulation supermineral concentrate using a patented blending process. Its components are: Phosphoric Acid (58%), Zinc, Copper, Iron Pyrophosphate, Potassium, Calcium, Manganese, Glycyrrhizic Acid (8.4%), Silica (0.1%). The microelement is made up of a homogenized complex with special indication, pH = 0.0008-0.4, waterless in the final composition.

Properties of the trial product:
1. Microelement composition in metastable ionized state with specific gravity of 1.61819, liquid, transparent, odorless;
2. Small molecule size (5-10 nm);
3. Generated complex molecule that does not have any chemical invasive element in the composition;
4. Multi-composition in production: INPUT: 6 elements (Mg, Fe, Si, Ca), food phosphoric acid; modified (structured) water and other small elements (see below); OUTPUT: new molecule with a complex composition;
5. The molecule scans at the cellular level for the presence of pathogenic: bacterial, viral, fungal etiologies - through the mechanism of reading the characteristics of the electron-proton (KNa) pump on the membrane - and if these characteristics are violated, the drug “enters” the cell;
6. At the intra-cellular level, the drugs scan the cell in search of pathology. This scanning process is made on the basis of selectivity of a "healthy – do not touch/ill - induce apoptosis" through the mechanism of “mitochondria activity.
7. The size, composition, as well as pH allows the drug, after scanning and receiving positive response to controlling the presence of a "pathogenic factor" in the cell (one of the keys are the characteristics of electron - proton pump/potassium-sodium pump on the membrane of cell) to pass freely through the membrane into the cell and start a cascade of biochemical processes that lead to corresponding results (switching to mitochondria aerobic oxidation, restarting the methyl group with the epigenetic effects on DNA, apoptosis).

7.2. Storage and Drug Accountability

Describe product’s storage needs. Include storage requirements and stability (temperature, humidity, security and container).

The product used as part of the abovementioned regimens will be subjected to the same storage and accountability conditions as per institutional requirements utilised in standard

clinical settings.

8. TREATMENT

8.1. Rationale for Selection of Dose

Clearly explain the rationale for the dose used during the study.

Subjects will receive a therapeutic dosage of 1 drop (0.05ml) per 10kg of body weight (max 8 drops), every 8 hours (3 times a day) for up to 14 days. This is a higher dosage than that with a preventive purpose (as per the bottle label, 2 drops per day), and is a dosage that will allow to test the product safety.

8.2. Study Drug Formulations

Describe in what form the study drug will be dispensed to the subjects.

The product will be administered orally, dissolved in water.

8.3. Study Drug Administration

Describe the drug regimen to be used. State any special precautions or warnings relevant for the study drug administration.

The drug regimen to be tested is usually recommended to subjects with infectious disease symptoms. It is a therapeutic –rather than preventive- dosage that will allow to test the product safety.

8.4. Specific Restrictions / Requirements

Indicate any limitations on medications, herbs, vitamins and mineral supplements (other than study agents) while participating in the study. Include time periods if applicable.

Subjects must have stopped all supplements for 1 month prior to enrolment.

8.5. Blinding

If applicable describe the measures that will be undertaken to blind the study participants and/or study staff from participant treatment assignments. State when unblinding is expected and if/when participants will be told their assignments. Note plans to handle early unblinding to protect participant safety, if any.]

N/A.

8.6. Concomitant therapy

All medications (prescription and over the counter), vitamin and mineral supplements, and / or herbs taken by the participant should be documented.

All concomitant therapies will be documented accordingly.

9. SAFETY MEASUREMENTS

9.1. Definitions

Define terms e.g. what would be regarded as UPIRTSO events, Serious adverse events etc. Include details of the protocol specific reporting, procedures, including the individual responsible for each step (e.g. the Investigator, the medical monitor, etc.), how decisions will be made regarding determining relatedness and grading severity, how reports will be distributed and what follow up are required. Include specific details of reporting procedures for:

- Deaths and life threatening events
- other SAEs
- Other adverse events

Adverse Events (AE)

An AE is any untoward medical occurrence in a study participant administered an investigational product and that does not necessarily have a causal relationship with this treatment

An AE, therefore, can be any unfavourable and unintended sign (including laboratory finding), symptom or disease temporally associated with participation in an investigational study, whether or not considered drug-related. In addition to new events, any increase in the severity or frequency of a pre-existing condition that occurs after the participant starts treatment for participation is considered an AE. This includes any side effect, injury, toxicity, or sensitivity reaction.

A suspected adverse reaction means any AE for which there is reasonable possibility that the drug caused the AE. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the AE. An adverse reaction means any AE caused by a drug. This means there is reason to conclude that the study drug caused the event.

An AE or suspected adverse reaction is considered 'unexpected' if it is not listed in the current prescribing information (PI) for a marketed compound. Any condition, laboratory abnormality, or physical finding with an onset date prior to the participant signing the ICF is considered to be pre-existing in nature and part of the participant's medical history.

All AEs must be recorded on the participant's CRF from date of informed consent to 30 days following the last dose of study drugs or initiation of new anticancer therapy, whichever occurs first. All treatment-related AEs are followed until resolution of until initiation of new anti-cancer therapy, whichever occurs first. During the long-term follow-up period, only secondary malignancies will be captured as AE.

Laboratory findings should not be recorded as AEs unless corrective action is required or deemed clinically significant by the treating physician.

Using the following criteria, the relationship of the AE to the study treatment should be assessed as follows:

1) The event is suspected to be related if:
    a. There is a clinically plausible time sequence between onset of the AE and administration of study treatment; and/or
    b. There is a biologically plausible mechanism for the study treatment to cause or contribute to the AE; and/or
    c. The event responds to the withdrawal of the study treatment (dechallenge) and/or recurs with rechallenge (when clinically feasible); and/or
    d. The AE cannot be reasonably attributed to concurrent/underlying illness, other drugs, or procedures
2) The event is suspected to be not related if:
    a. The AE is more likely to be explained by the participant's clinical state, underlying disease, concomitant medication, study or non-study procedure; and/or
    b. The time of occurrence of the AE is not reasonably related to administration of study treatment; and/or
    c. The event is not related to the study treatment

In the event of a possible study treatment-related AE, the investigator should to the best of his/her ability assess its relationship to the study treatment.

Serious Adverse Events (SAE)

An SAE is one that meets one or more of the following criteria:

1) Death
2) Life threatening experience defined as any adverse experience that place the participant, in the view of the Principal Investigator, at immediate risk of death at the time of occurrence; i.e. it does not include a reaction that, had it occurred in a more severe form, might have caused death
3) Requires inpatient hospitalisation or prolongation of an existing hospitalisation (except scheduled hospitalisations for a non-acute, unrelated cause such as elective surgery)
4) Results in persistent or significant disability/incapacity (i.e. substantial disruption in a participant's ability to conduct normal activities of daily living)
5) Is a congenital anomaly/birth defect in the offspring of an exposed participant
6) Important medical events that may not result in death, be life threatening, or require hospitalisation, may be considered an SAE when, based upon appropriate medical judgement, it jeopardizes the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

For both AEs and SAEs, the investigator will provide a record of the start and stop dates of the event, the action taken with study treatment as a result of the event (e.g. discontinuation or reduction of study treatment), and outcome of the event. If an AE is considered serious, both the AE page/screen of the CRF and the SAE Report Form must be completed.

9.2. Collecting, Recording and Reporting of "Unanticipated Problems Involving Risk to Subjects or Others" – UPIRTSO events to the NHG Domain Specific Review Boards (DSRB)

UPIRTSO events refers to problems, in general, to include any incident, experience, or outcome (including adverse events) that meets ALL of the following criteria:

1. **Unexpected**
   In terms of nature, severity or frequency of the problem as described in the study documentation (eg: Protocol, Consent documents etc).

2. **Related or possibly related to participation in the research**
   Possibly related means there is a reasonable possibility that the problem may have been caused by the procedures involved in the research; and

3. **Risk of harm**
   Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Reporting Timeline for UPIRTSO Events to the NHG DSRB.

1. Urgent Reporting: All problems involving local deaths, whether related or not, should be reported immediately – within 24 hours after first knowledge by the NHG investigator.

2. Expedited Reporting: All other problems must be reported as soon as possible but not later than 7 calendar days after first knowledge by the NHG investigator.

9.3. Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Science Authority (HSA)

1. **For Industry sponsored Trials**
   All SAEs will be reported to HSA according to the HSA Guidance for Industry "Safety Reporting Requirements for Clinical Drug Trials."

2. **For Principal Investigator initiated Trials**
   All SAEs that are unexpected and related to the study drug must be reported to HSA.

"A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- Results in death.
- Is life-threatening (immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in congenital anomaly/birth defect.
- Is a Medically important event.

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event should be reported as serious."

All SAEs that are unexpected and related to the study drug will be reported. The investigator is responsible for informing HSA no later than 15 calendar days after first knowledge that the case qualifies for expedited reporting. Follow-information will be actively sought and submitted as it becomes available. For fatal or life-threatening cases, HSA will

be notified as soon as possible but no later than 7 calendar days after first knowledge that a case qualifies, followed by a complete report within 8 additional calendar days.

9.4. Safety Monitoring Plan

Please include details on the Data Safety Monitoring Plan (DSMP) for the research study. Please discuss the plans in place to ensure the safety and well being of subjects, and integrity of data collected.

The PI will oversight and monitor the conduct of this study to ensure the health and safety of participants and the validity and integrity of the data. Participants will be fully informed of the study requirements throughout the conduct of the study and should comply with the research protocol or be allowed to withdraw from participation. The PI will notify participants of any information relevant to their continued participation.

Specifically, the PI will review the research protocol, evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial site, and other factors that can affect study outcome. Scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study will be considered. The PI will make recommendations to the DSRB and trial site concerning continuation or conclusion of the trial. The PI will protect the confidentiality of the trial data and the results of monitoring.

9.5. Complaint Handling

Briefly discuss how complaints will be handled and how the data obtained will be managed.

Participants will be advised as per the informed consent form that they may contact the Principal Investigator or DSRB secretariat if they have any complaints.

10. DATA ANALYSIS

10.1. Data Quality Assurance

Discuss the measures undertaken to ensure that the data obtained from this research is accurate, complete and reliable.

Compliance of regulatory documents and study data accuracy and completeness will be maintained through an internal study team quality assurance process.
The PI will be subjected to external audits by regulatory authorities as applicable.

10.2. Data Entry and Storage

Briefly discuss where data will be entered (i.e. will these entries be on paper or electronically), stored and handled.

Each participant recruited into the study will be assigned a unique participant number (UPN), and the participant's biosamples will be labelled using the UPN, with no direct

reference to the participant 's other identifying information. Information from the source documents will be transcribed onto an electronic document that is password protected in a user designated and password protected computer in the Department. Personnel in the laboratory have no direct access to the clinical history documents or other participant information. Information pertaining to the participant that arises from the research will not become part of the participant 's medical record. All records will be kept for a minimum period of 6 years following the date of study closure according to ICH GCP guidelines, or longer as applicable per institution guidelines.

11. SAMPLE SIZE AND STATISTICAL METHODS

11.1. Determination of Sample Size

Details on sample size calculation and the means by which data will be analysed and interpreted. In particular, specify all of the following:

- Null and alternate hypothesis
- Type I error rate
- Type II error rate

20 participants will be recruited into the study. This is based on the number of participants that can be practically recruited, considering time and budget constraints, that will allow a reasonable signal to expand to a larger study.

11.2. Statistical and Analytical Plans

a. General Considerations

T and B cell subsets and phenotypes will be analysed before and after treatment utilising the groups of labelled antibodies mentioned in section 2.4.a.
Innate immune subsets (monocytes –CD14PE), NK cells (CD56 APC) and neutrophils (CD15PE and CD16 APC) will be analysed.
Antibody germline repertoires will be analysed by massive parallel sequencing of VH/VL genes and T cell proliferative capacities determined by 3H thymidine incorporation following stimulation by mitogen (PHA).

b. Safety Analyses

Safety analyses will assess frequency and severity of adverse events, clinical laboratory tests (haematologic testing and serum chemical testing), vital signs and physical status.

c. Interim Analyses

Interim analyses will assess accrual, data quality, safety, external information relevant to the study, medical advances, adherence to treatment, resources to continue the study and study integrity. Deficiencies in any area could be a reason to stop the trial.

d. Describe the types of statistical interim analyses and stopping guidelines (if any) that are proposed, including their timing.

Stopping guidelines for the research study will be based on efficacy, futility and safety criteria.

12. ETHICAL CONSIDERATIONS

12.1. Informed Consent

Describe the procedures for obtaining and documenting informed consent of study subjects. Make provision for special populations e.g. non English speakers, children, illiterate or non writing individuals, vulnerable populations. In obtaining and documenting informed consent, the investigator should comply with the SGGCP guidelines and to the ethical principals that have their origin in the Declaration of Helsinki. Please specify when consent will be taken and who will take consent.
Identify different consent forms that are needed for the study(e.g. screening, study participation, HIV screening, future use specimens, assent from minors)

No investigator may involve a human being in research unless the investigator has obtained the legally effective informed consent of the participant or the participant's legally authorised representative. An investigator shall seek such consent only under circumstances that provide the prospective participant or the participant's legally authorised representative sufficient opportunity to consider whether or not to participate, and minimise the possibility of coercion or undue influence. The information that is given to the participant or the representative shall be in a language understandable to the participant or representative.

Before implementing any study procedure, informed consent will be documented in the subject case histories and by the use of a written consent form approved by the DSRB and signed and dated by the participant or the participant's legally authorised representative at the time of consent. A copy of the signed informed consent will be given to the participant or participant's legally authorised representative. The original, signed consent will be maintained by the investigator and available for inspection by the regulatory authority at any time. In obtaining and documenting informed consent, the investigator will comply with the SGGCP guidelines and the ethical principles that have their origin in the Declaration of Helsinki.

The participants will be informed about the background and aims of the study. The participant will be told of their right to withdraw from the study at any time without any penalty with regards to the continuation of care at this institution and by the same physicians as they choose. The participant will be told that tissue and blood samples obtained will be assigned unique participant numbers (UPN) to ensure participant confidentiality.

12.2. IRB review

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by the IRB / NHG DSRB.

This protocol and the associated documents will be sent to review and approval by the NHG DSRB.

12.3. Confidentiality of Data and Participant Records

Include procedures for maintaining subject confidentiality, any special data security requirements, and record retention. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to the participating subjects.

Protection and privacy of the personal data of individuals are covered under the Personal Data Protection Act 2012. Participant medical information obtained as part of this study is confidential, and must not be disclosed to third parties, except as noted below. Only the PI, co-investigators and the research team will have access to the data.

The participant may request in writing that medical information be given to his/her personal physician. The investigator/institution will permit direct access to source data and document by regulatory authorities. The access may consist of study-related monitoring, audits, IRB reviews and regulatory authority inspection.

Each participant recruited into the study will be assigned a unique participant number (UPN), and the participant's biosamples will be labelled using the UPN, with no direct reference to the participant 's other identifying information. Information from the source documents will be transcribed onto an electronic document that is password protected in a user designated and password protected computer in the Department. Personnel in the laboratory have no direct access to the clinical history documents or other participant information. Information pertaining to the participant that arises from the research will not become part of the participant 's medical record. All records will be kept for a minimum period of 6 years following the date of study closure according to ICH GCP guidelines, or longer as applicable per institution guidelines.

Consent will be obtained from participants to use their data for future research.

13. PUBLICATIONS

State publication policy for study findings.

The research team intends to submit the study results for publication in peer-reviewed scientific journals. Publications would not include individually-identifiable information. The team's publication policy is aligned with the Consolidated Standards of Reporting Trials (CONSORT) group.

14. RETENTION OF TRIAL DOCUMENTS

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as IRB records and other regulatory documentation should be retained by the PI in a secure storage facility. The records should be accessible for inspection and copying by authorized authorities. Describe the retention plans for study documents.

The investigators will prepare and maintain adequate and accurate source documents (medical records, raw data collections forms, recorded data from automated instruments,

laboratory data) relevant to the clinical study. These documents are designed to record all observations and other pertinent data for each participant enrolled in this clinical study.

The investigators will retain records required to be maintained under this part for a period of ten years following the completion or discontinuation of the study. The investigators will retain protocols, amendments, IRB approvals, copies of the signed and dated consent forms, medical records, case report forms, all correspondence, and any other documents pertaining to the conduct of the study.

List of Attachments

Appendix 1 **Study Schedule**

Appendix 2 **Blood Sampling Summary**

Appendix 3 **Questionnaires used in the Trial**

Appendix 4 **Laboratory Tests**

Appendix 5 **Sample Participant Information Sheet and Informed Consent Form**