Document Number	Version	Effective Date (YYYY-MM-DD)
PRO-MAS-002	2.0	2024-01-16

Protocol Title:
Biomarker Discovery in Lung Cancer-Malaysia

Protocol Version:
Version 2.0

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Industry-sponsored research with grant given by :
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VERSION HISTORY

No.	Description & Reason of Changes	Version	Effective Date
1	Initial Version	1.0	31 Mar 2023
2	Updated investigator-initiated research to industry-sponsored research. Removal of pneumonia as part of the inclusion criteria. Blood sample requirements updated. AE/SAE definitions and reporting Updated list of study team members. Clarification on Visit 2 blood collection visit. Administrative changes to Inclusion/Exclusion Criteria Removal of Appendix B,C,D	2.0	16 Jan 2024

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Sponsor Name: MiRXES Lab Pte. Ltd.

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1 LIST OF ABBREVIATIONS

CA	Cancer Antigen
CEA	Carcinoembryonic Antigen
cfDNA	cell-free Deoxyribonucleic Acid
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CYFRA	Cytokeratin 19 Fragment Antigen
DELFI	DNA Evaluation of Fragments for early Interception
DNA	Deoxyribonucleic Acid
FFPE	Formalin-Fixed, Paraffin-Embedded
ICF	Informed Consent Form
IRB	Institutional Review Board
LDCT	Low-dose Computed Tomography
miRNA	micro–Ribonucleic Acid
NELSON	Dutch–Belgian Lung-Cancer Screening Trial (Nederlands–Leuvens Longkanker Screenings Onderzoek)
NGS	Next-Generation Sequencing
NLST	National Lung Screening Trial
NSCLC	Non-Small Cell Lung Cancer
PI	Principal Investigator
PIS	Participant Information Sheet
qPCR	Quantitative Polymerase Chain Reaction
SCLC	Small Cell Lung Cancer
SOP	Standard Operating Procedure
USPSTF	United States Preventive Services Task Force

2 BACKGROUND INFORMATION AND STUDY RATIONALE

There are two major types of lung cancer, namely non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), with the former occurring 80% of the time (American Cancer Society, 2023). In the year 2020 alone, almost 2.2 million new cases of lung cancer were diagnosed worldwide, equivalent to about 6000 cases being diagnosed in a day (World Health Organization, 2020). Despite having about the same number of diagnoses as breast cancer, lung cancer (1.8 million deaths) caused more than twice as many deaths in 2020, when compared to breast cancer (680,000 deaths). More alarmingly, the incidence and deaths have been increasing in certain countries with lower socio-demographic indexes, as well as in female non-smokers (Ebrahimi et al., 2019). Lung cancer is the third most common cancer in Malaysia, but the most common cancer in men, accounting for 17% of all cancer diagnosed in men in 2020 (World Health Organization, 2020). Similar to trends observed globally, the number of deaths recorded by lung cancer patients in Malaysia is more than that of breast cancer patients, despite having a lesser number of new diagnoses. Further breakdown according to the three major ethnic groups in Malaysia, the lifetime risk for lung cancer was the highest among Chinese (1 in 51), followed by Malays (1 in 62), and Indians (1 in 145) for the years 2012-2016 (Ministry of Health Malaysia, 2019).

The advancement of technology in health care systems has brought us conveniences in early lung cancer screening. Many technologies have been employed for lung cancer diagnosis, for instance, sputum cytology, X-ray, and low-dose computed tomography (LDCT) imaging (American Cancer Society, 2023). Of all the technologies, LDCT has been most frequently used for lung cancer screening. LDCT involves a thin radiation beam to create a series of pictures taken from different angles to create detailed images of the lungs (American Cancer Society, 2023). In their latest guidelines, the United States Preventive Services Task Force (USPSTF) recommends annual LDCT screening for adults aged 50 to 80 years old who have a 20 pack-year smoking history and currently smoke or have quit within the past 15 years (USPSTF et al., 2021). In Malaysia, there is no national lung cancer screening programme. However, several public hospitals and many private healthcare institutions provide LDCT lung cancer screening services (Ministry of Health Malaysia, 2017). Current recommendations for lung cancer screening in Malaysia by the Lung Cancer Network Malaysia are for current and former smokers with at least 20 pack years, and non-smokers who have a family history of lung cancer (Lung Cancer Network Malaysia and National Cancer Society Malaysia, 2021).

Despite its advancements, LDCT does come with its challenges at the patient, provider, and medical system levels (Patel et al., 2021). Out of fear of a cancer diagnosis or concerns about cost, patients often refuse to undergo an LDCT test (Patel et al., 2021). Unlike colon or breast cancer screening, which is based on age, the eligibility for a lung cancer screening test is often based on smoking habits. High-risk smoking habits are often correlated with lower socioeconomic status among patients. Some obstacles faced by these individuals include lesser financial resources, lesser awareness about the importance of cancer screening, more difficulties in taking time off from work, etc. (Patel et al., 2021). On a medical provider level, some of the challenges include the management of detected nodules, quality of screening, and radiologists' expertise in

interpreting the results of an LDCT (Martini et al., 2021). There are also some doubts in physicians regarding the effectiveness of LDCT in lung screening, as two large randomised trials, the National Lung Screening Trial (NLST) and the Dutch–Belgian lung-cancer screening trial (Nederlands–Leuvens Longkanker Screenings Onderzoek [NELSON]) reported false-positive rates per screening round of 23.3% and 10.4% respectively (National Cancer Institute, 2022). On the other hand, to implement widespread lung cancer screening programmes, medical systems must be able to cope. This includes having a robust infrastructure, implementing referral policies, follow-up recommendations, and so on (Patel et al., 2021). All the points mentioned above come with the need for extra resources, which would then create another set of challenges in developing and less developed countries, including Malaysia. In Malaysia, the introduction of a pilot study to evaluate the feasibility and outcome of single LDCT was proven challenging, when it was terminated prematurely due to low recruitment from the general public (Rajadurai et al., 2019). This further bolsters the argument that it is difficult to promote LDCT lung screening in Malaysia, despite having high lung cancer diagnoses and death rates.

Liquid biopsy is one of the recent medical advancements that can potentially overcome the challenges faced by LDCT in the early detection of lung cancer. A liquid biopsy involves the isolation of circulating tumour cells, cell-free DNA and RNA, extracellular vesicles, etc. from human bodily fluids, like blood, urine, or sputum, collected through minimally invasive methods (Lone et al., 2022). Some of the clinical applications of liquid biopsy include early cancer detection, disease progress monitoring, detection of therapeutic targets, and many more (Lone et al., 2022). In normal conditions, miRNAs mainly function in regulating the degradation or translational repression of mRNA targets. However, it has been found that miRNA functions have been dysregulated in cancer cells, providing a potential to discriminate between miRNAs in a healthy versus diseased person (Ying et al., 2020). Recently, our group has developed and validated a serum microRNA (miRNA) panel that can be utilized to detect early-stage NSCLC (Ying et. al., 2020). Circulating cell-free DNA (cfDNA) are extracellular fragments of nucleic acid that are excreted from cancer cells via apoptosis or necrosis (Gao et al., 2022). Similar to miRNA, cfDNA in cancer patients also exhibit alterations like mutations, hyper- or hypo-methylation, chromosomal rearrangements, etc., which can be harnessed to detect cancer earlier than traditional screening methods (Gao et al., 2022). A recent study by Kruusmaa et al. showed that by identifying changes in cfDNA methylation, they could detect early-stage lung cancer (Kruusmaa et al., 2021). In another study, they showed the ability to detect lung cancer early by evaluating fragmentation patterns of cfDNA in the genome, through an approach called DELFI (DNA evaluation of fragments for early interception) (Mathios et al., 2021).

In this study, there will be a focus on cfDNA and miRNA derived from blood. These biomarkers and/or signatures are expected to improve current blood-based protein markers such as CA15-3, CYFRA 21-1, CA125, and CEA. They may be deployed as adjunct tests to enhance the accuracy of imaging diagnostics like X-ray and LDCT.

3 STUDY OBJECTIVES AND OUTCOME

3.1 Study Objectives

This study aims to

a. Identify biomarkers and signatures to differentiate between high-risk and lung cancer patients.
b. Discover blood-based biomarkers that can be used as an adjunct test to imaging diagnosis to improve patient follow-up.

3.2 Study Hypotheses

With existing evidence showing the utility of using liquid biopsy for early cancer detection (Klein et al., 2021; Wan et al., 2019; Cohen et al., 2018), we hypothesized that cell-free DNA and/or RNA expression changes during disease progression, and biomarker signature can be derived from the changes in expression levels.

3.3 Study Outcome

To discover and validate RNA and methylated cell-free DNA cancer biomarkers that can discriminate between high-risk and lung cancer patients.

4 STUDY DESIGN

This is an observational study estimated to enrol approximately 400 participants. The study is designed to have 2 cohorts A and B, with the number of targeted participants as listed in Table 1. Because of possible attrition, the “Target Final Number” column illustrates the desired number of participants to have at the end of the study.

Table 1. Number of targeted participants

Cohort	Target Enrolment Number	Target Final Number
A	200	160
B	200	160

This study comprises mainly of prospective analysis of blood, with an examination of FFPE tissue specimens, if participants undergo biopsy. The estimated start and end date of the study is August 2023 through August 2026, with the start date dependent on the approval by the Research Ethics Review Committee. Recruitment of participants will be carried out in University Malaya Medical Centre (UMMC) in this setting (details in Appendix).

In this study, the cohorts are split into two based on whether they are high-risk or highly suspicious of having lung cancer (Fig. 1). With consideration to the patient journey across the healthcare institutes, there are specific time-points (Fig 1. teal-coloured box) that the subject will be enrolled and briefed on the participant informed sheet (PIS) which covers the details of the study, objectives, risks, and benefits in participating in this clinical study. An informed consent form (ICF) will be signed by the participant if he or she agrees to participate in this study.

Upon completion of the recruitment process, the subject will undergo a blood collection at a designated area during Visit 1. A second blood collection will be performed during the subject's next standard of care follow up visit. This will be Visit 2. Blood will be processed by trained personnel according to MiRXES's procedure. This is denoted in Figure 1 as the red-coloured box. This will be further elaborated in the study methodology below.

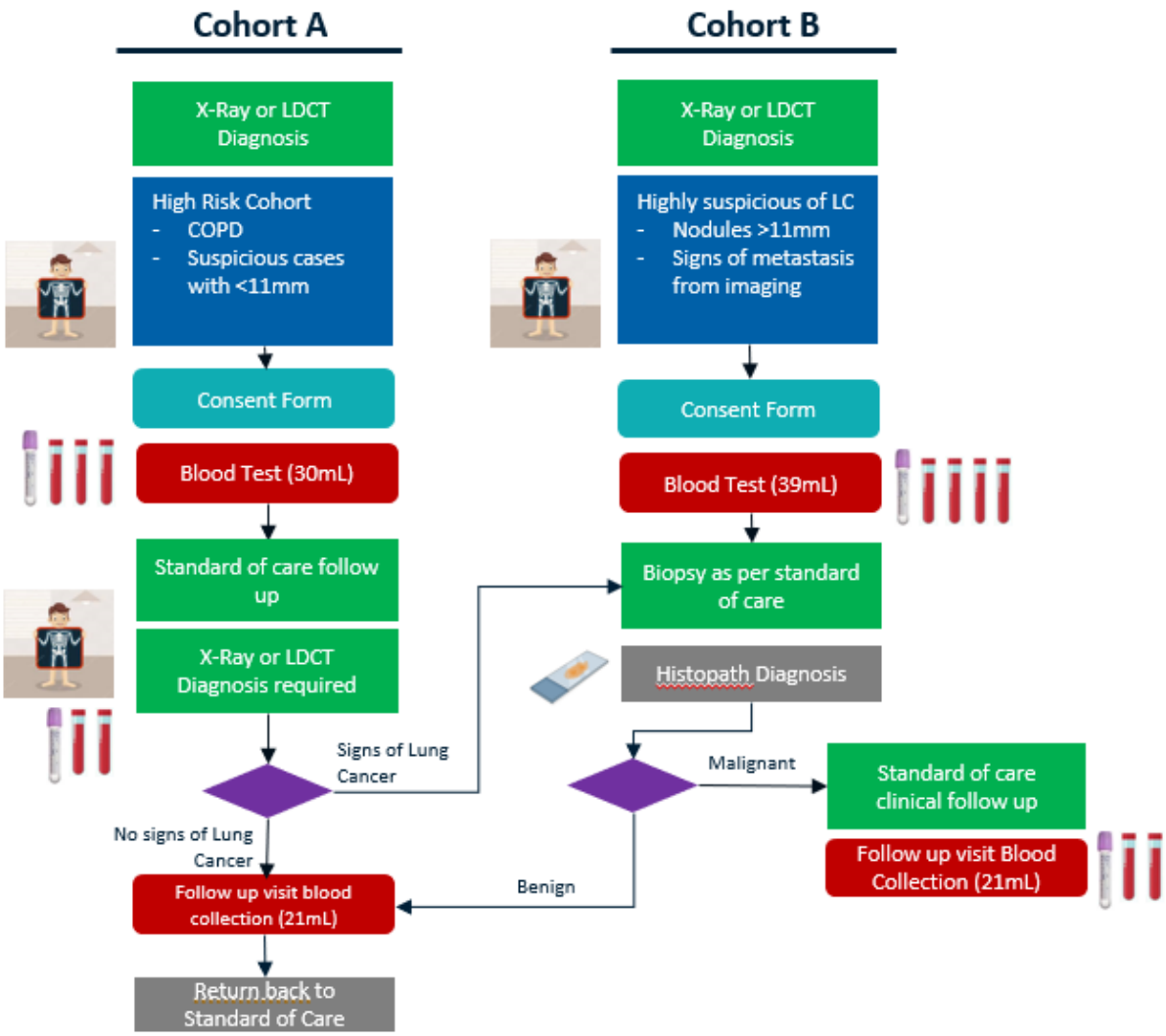

Figure 1. Recruitment cohorts and their patient workflow.

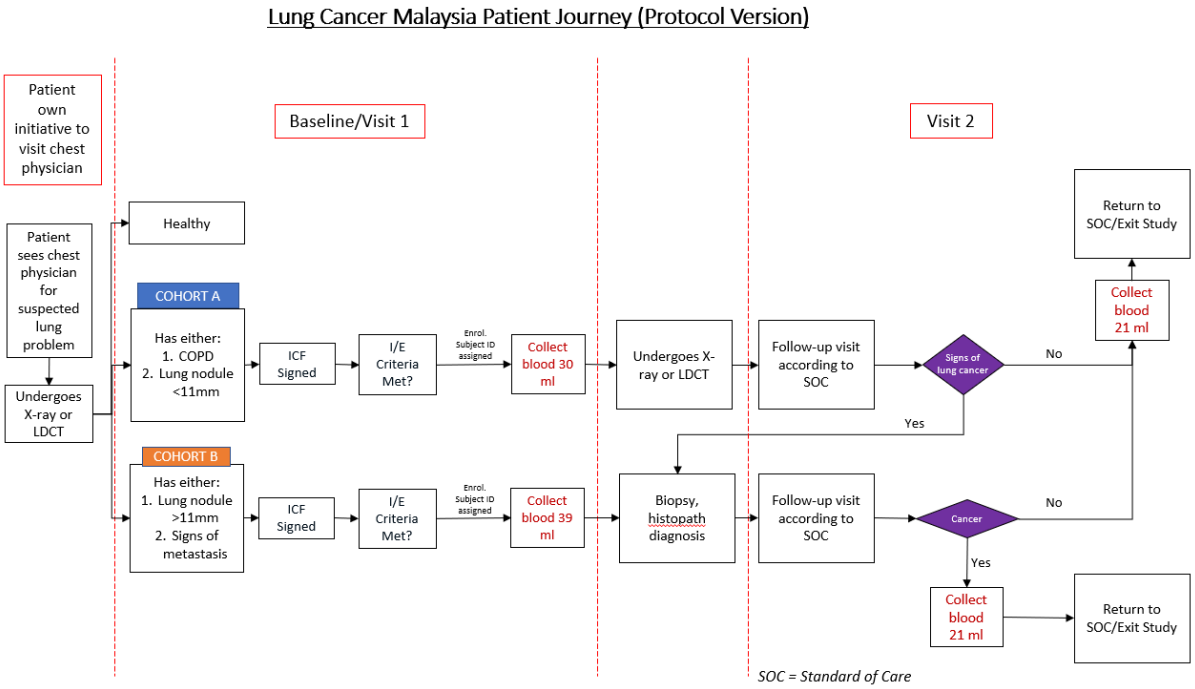

Figure 2. Detailed patient workflow.

5 STUDY POPULATION

5.1 Criteria for Recruitment

The description for Cohorts A and B are as follows:

5.1.1 Cohort A

Subjects in this cohort are recruited if they have either of the following: pre-existing chronic obstructive pulmonary disease (COPD) or are found to have lung nodules smaller than 11mm, or nodules that are deemed not for biopsy by the attending physician. Subjects in Cohort A will undergo a follow-up imaging according to standard of care (based on physician's choice). During follow-up, another blood collection will be carried out. If the follow-up X-ray or LDCT shows increase in the size of nodules or signs of cancer development, a biopsy may be performed for histopathology diagnosis. Subjects may remain in Cohort A, or, if lung cancer is diagnosed, they will be re-classified to Cohort B.

5.1.2 Cohort B

Subjects in this cohort are highly suspicious of having lung cancer. From the results of the X-ray or LDCT imaging, the subject would have nodules larger than 11mm, or show signs of lung cancer. After obtaining consent and collecting blood from the subject, he or she will proceed to a CT-guided lung biopsy as per standard of care. The biopsied lung tissue will be analysed by histopathology. In an event that lung cancer is not diagnosed based on histopathology results, the subjects will be re-classified to Cohort A. Subjects that were diagnosed with lung cancer will undergo another blood collection during their clinical follow up.

On average in a month, it is expected that 5 to 10 subjects will be recruited for each of Cohort A and B. Logistics and administrative matters to identify and enrol subjects will be largely driven by the lead PI's team.

5.2 Inclusion Criteria

5.2.1 Inclusion Criteria for Each Cohort

Table 2. Inclusion criteria specific for each cohort

Cohort	Inclusion Criteria
A	i. Male or female subjects aged 30 and above. ii. No previous history of any cancers. iii. Able to provide X-ray and/or LDCT results. iv. Have one of the following conditions: a. COPD b. Pulmonary nodules measuring less than 11mm, or deemed not for biopsy by attending physician v. Willing to go back for a follow-up X-ray or LDCT scan in the next clinical follow up as per standard of care. vi. Willing to provide up to 30mL of blood in month-0 (first visit) and up to 21mL in the next standard of care follow up visit (second visit).
B	i. Male or female subjects aged 30 and above. ii. No previous history of any cancers. iii. Able to provide X-ray and/or LDCT results. iv. Have one of the following conditions: a. Suspicious nodules measuring more than 11mm. b. Imaging diagnosis suggestive of lung cancer. v. Willing to enrol for biopsy for confirmation. vi. Willing to provide up to 39mL of blood in month-0 (first visit) and up to 21mL in the next standard of care follow up visit (second visit).

5.3 Exclusion Criteria

i. Subject has received chemotherapy or radiotherapy for cancer treatment, and any other cancer-related treatment.
ii. Subject is pregnant or lactating (self-declaration).
iii. Subject is unwilling or unable to provide signed informed consent.

5.4 Subject Withdrawal

Subjects' participation in this study is absolutely on a voluntary basis. Subjects are free to request to be withdrawn from the study at any time, without giving a reason, and this will not affect the benefits that they are entitled to. However, the site should make a reasonable effort to ascertain the reasons while fully respecting the subject's rights. There will also be instances where subjects are considered unsuitable to continue with the study, a decision made together by the research team and the Sponsor. These situations might include, but are not limited to, if subjects are physically unfit, subjects are unwilling to follow study procedures, subject was unable to provide sufficient blood for the research study or if subjects are found to be deliberately providing false information that is detrimental to the integrity of the study data.

5.5 Blood Sample Requirements

Blood samples meeting the following criteria will be rejected for blood samples collected during Visit 1 or Visit 2:

i. Blood samples collected from subjects within an hour after food.
ii. Blood samples collected from subjects who have been administered local or general anaesthetics/sedatives, or any agents used for medical imaging (e.g., contrast agents) within the last 24 hours before blood draw.
iii. Blood samples collected from subjects who have taken laxatives within the last 24 hours before blood draw.

Blood samples meeting the following additional criteria will be rejected for only Visit 1 blood collection:

i. Blood samples collected from subjects who have anti-cancer drug or anti-cancer treatment including chemotherapy, surgical removal of tumor, radiation therapy and any other cancer-related treatments.
ii. Blood samples collected from subjects who have received blood transfusion within the last 6 months before blood draw.

5.6 Possible Risks and Discomfort to Subjects

No experimental drugs will be administered in this clinical research study. Blood collection by venepuncture, which is a minimally invasive procedure performed in this study, carries minimal risk. Possible side effects of blood drawing include faintness, inflammation of the vein, pain, bruising, or transient bleeding at the site of venepuncture. There is also a slight possibility of infection. A separate third party insurance policy will be established to cover research related injury.

The processing of blood samples and molecular analysis will be performed in qualified laboratories non-accessible to the recruited subjects. The outcome of the molecular analysis will not be reported to the subjects. In addition, the study team and the sponsor will not give medical advice to the subjects. Therefore, subjects are not exposed to any medical risk.

5.7 Adverse Events

Adverse events (AEs) are any untoward medical occurrence (e.g. signs, symptoms, abnormal laboratory findings, unintended signs) that are temporally associated with the human biomedical research. There may or may not be any causal relationship with the study procedures performed as part of the research study.

Serious adverse event (SAE) in relation to human biomedical research, means any untoward medical occurrence as a result of any human biomedical research which:

- results in death
- is life-threatening
- requires in-patient hospitalization or prolongation of existing hospitalization
- results in or contributes to persistent or significant disability/incapacity
- results in or contributes to a congenital anomaly/birth defect

- is an important medical event that may be classified as serious based on medical judgement

AEs that are deemed related to the research study will be recorded.

SAEs deemed related (definitely/ probably /possibly) to the research study will be reported to **the IRB.** Related means there is a reasonable possibility that the event may have been caused by participation in the research. The investigator is responsible for informing **the IRB** after first knowledge that the case qualifies for reporting. Follow-up information will be actively sought and submitted as it becomes available.

5.8 Possible Benefits

Depending on the groups that subjects are assigned to, recruited subjects may benefit from health screening tests, medical tests, or consultations that may inform the underlying health risks that the subjects may not be aware of.

5.9 Costs and Payments if Participating in the Study

There will be no cost required from the participants. However, monetary and/or material compensation may be given to the participants and supporting healthcare professionals.

The research will require clinical information as indicated under Section 6.4 and also relevant imaging information for the analysis. This study will provide insights into the blood analytes of these cohorts and their concordance with the clinical information.

The investigator will be candid to the participants that the discovery work developed from this study may have financial value once approved by regulatory agencies for clinical use. However, the participants must be made to understand that the outcome of this study will not entitle them to any royalty fees. Further, it will be explained to the participants that the acquisition of the blood specimen will not affect their standard of care treatment.

6 STUDY PROCEDURES AND ASSESSMENT

6.1 Enrolment

Any subject will be considered for enrolment provided they meet all the inclusion criteria and none of the exclusion criteria. The PIS and ICF will be discussed with the subjects. Subjects will be given ample time to consider and ask questions as needed. If the subjects are agreeable to the terms of the ICF, it will be signed by the subject, the PI or the study team member conducting the informed consent discussion, and the witness. The subject will be assigned a subject code to de-identify the subject.

6.2 Data Collection

The demographics, lifestyle, personal health data, family history of diseases, clinical data, response to treatments and other medical records will be collected through

medical record review and interviews with participants. The scope of the data to be collected will be defined in the case report form completion guidelines for this study This de-identified information are then entered using the tablet/laptop available on-site with the study team member into the electronic data capture (EDC) system. There will be a review and collection of data from participants and the medical record database at multiple time points, namely the time of recruitment and subsequently at interim monitoring visits.

6.3 Blood Samples Collection

Up to 39mL of blood will be collected at the appointed blood collection point from each enrolled subject:

Visit / Cohort	Blood Volume	No. of Blood Tubes
Visit 1 (Cohort A)	30 mL	3 RNA Complete BCT™ tube (9 mL each) 1 BD Vacutainer® EDTA tube (3 mL)
Visit 1 (Cohort B)	39 mL	4 RNA Complete BCT™ tube (9 mL each) 1 BD Vacutainer® EDTA tube (3 mL)
Visit 2 (Cohort A & B)	21 mL	2 RNA Complete BCT™ tube (9 mL each) 1 BD Vacutainer® EDTA tube (3 mL)

The blood collection should be executed in the following blood tube order (Table 4):

Table 4. Sequence of blood collection according to blood tube

Sequence of Collection	Type of Blood Tube	Volume of Blood (ml)
First	RNA Complete BCT™	9
Second	BD Vacutainer® EDTA	3
Third	RNA Complete BCT™	9
Fourth (where applicable)	RNA Complete BCT™	9
Fifth (where applicable)	RNA Complete BCT™	9

The collected blood will be processed within 24 hours and stored at room temperature. Blood tubes will be labelled and processed according to MiRXES' Standard Operating Procedures (SOPs), available in the Appendix section.

Up to two attempts may be executed for the blood drawn and the definition of a successful blood drawn is at least 2 x RNA Complete BCT™ and 1 x BD Vacutainer® EDTA with no haemolysis. The definition of successful blood drawn requirement applies to only Visit 1. If the minimum volume is not collected in Visit 1, the patient will be withdrawn and excluded from the study.

6.4 Clinical Specimens and Key Data Requirements

The table of clinical information to be collected from each cohort is detailed under Appendix G. Out of which, critical clinical information listed under the table needs to be collected to be considered clinically evaluable.

6.5 Biomolecules Extraction and Sequencing

At least two types of biomolecules will be isolated from the samples used in this study.

6.5.1 miRNA

Total RNA from platelet-poor plasma will be isolated using a commercially available kit. RNA will be reverse transcribed using multiplex reverse transcription primer pools with ID3EAL cDNA synthesis reagents (MiRXES). cDNA will be pre-amplified using Augmentation Primer Pools (MiRXES). Singleplex qPCR will then be performed on the amplified cDNA samples using a miRNA-specific qPCR assay and ID3EAL miRNA qPCR Master Mix (MiRXES).

6.5.2 Cell-free DNA (cfDNA)

cfDNA will be extracted from platelet-poor plasma using commercially available kits. The methylation profile of the cfDNA samples will be assessed using hybridization-based targeted sequencing.

6.5.3 Others

Other biomolecules present in the buffy coat, red blood cells, and/or plasma may be isolated and analysed to discover additional biomarkers.

7 ANALYTICAL METHODS

For miRNA analysis, expression levels of the miRNAs profiled using MiRXES qPCR ID3EAL solution will be quantified based on an intra-run standard curve. A set of miRNAs with expression levels significantly altered between the comparison groups will be identified.

For methylated DNA analysis, the sequencing reads subjected to adapter and quality trimming will be mapped to the human genome. The methylation levels of the gDNA between the cancer cells and healthy cells will be compared. Similarly, the methylation levels of the cfDNA across the healthy control groups and the malignant group will be compared, and the differences will be subjected to statistical analysis to identify the DNA regions that are differentially methylated at a significant level between the groups.

8 DATA HANDLING AND RECORD KEEPING

8.1 Clinical Data Collection Forms

A case report form (CRF) is required and must be completed for each subject. The PI of the study team will be responsible for the completeness of the form and to review and approve all CRFs. CRFs must be signed by the PI or by an authorized staff member. These signatures serve to attest that the information contained in the CRF is true. At all times, the PI has final personal responsibility for the accuracy and authenticity of all clinical and laboratory data entered into the CRF.

Subject source documents are the physicians' subject records maintained at the study site (CIC UMMC). In this study, the source documents will include the patient hospital case notes, physician's charts, and the study source documents. The information collected on the CRFs must match these.

8.2 Data Entry and Storage

Any hardcopy documents of this study containing subjects' information will be stored in a designated locked cabinet(s) or room(s) that are accessible only to authorised study personnel. The hardcopy data will be transferred into an electronic database for record storage. These data will be stored in a secured computer protected with a password. The database will not contain subject identifiers. The data linking subject identifiers and the subject code will be stored separately.

9 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

All data obtained from the study will be kept confidential and be available to the PI, research staff, and study coordinators from the study team, including those on the delegation log. The study team will maintain the password-protected database linking the subject identifiers to unique subject codes, for as long as the samples are stored.

10 ETHICAL CONSIDERATIONS

10.1 Institutional Review Board (IRB)

The PI of each study team will obtain prospective approvals of the study protocol, protocol amendments, informed consent forms, and other relevant documents, if applicable, from the IRB for the recruitment of participants, collection of samples, and data.

Ethical approval for this study will be obtained from the University Malaya Medical Centre (UMMC) Medical Research Ethics Committee (MREC), a written informed consent will be obtained from volunteer participants prior to the collection of their biological samples, demographic data, and other relevant clinical information.

10.2 MiRXES' IRB

MiRXES will obtain the relevant approval from the assigned Institutional Review Board (IRB) for this study.

10.3 Ethical Conduct of the Trial

The study will be performed in accordance with International Conference on Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki (2000), and applicable local regulatory requirements and laws. All biomedical research principles that involve human subjects, such as beneficence, confidentiality, and respect for rights and justice, will be construed before the participants in the study.

10.4 Confidentiality of Data and Patient Records

Study participants will be asked for their written ICF, available in English, and will be assured of the confidentiality of their information and test results. The ICF process will have to take place on-site. The ICF will state the obligations, compensations, risks, and benefits to them as a participant. The clinical specimens collected will be used for research purposes.

To maintain the anonymity of the study, information of the participants is maintained in adherence to the Malaysian Personal Data Protection Act 2010 (PDPA) of Act 709. A unique code will be assigned to each participant to maintain anonymity. This unique code will be the only link to the participant's information, which will be stored in a password-secured database that can only be accessed by the data manager of the research team. It will be the only identifier that can be used by the other members of the research team when collecting and analysing their biological samples or when requesting information from the data manager. The data manager can share the participants' information with the other members of the research team using coded data. An electronic logbook will record who accessed the information and what information was obtained. Biological samples will be labelled with the same unique code assigned to the participant, and no other identifier information should be included in the said label. The biological samples and results of the analysis can neither be linked back to the participant nor be used to identify the participants.

To enable evaluations and/or audits from regulatory authorities, the PI will keep records, including the identities of all participating subjects (sufficient information to link records, e.g., ICFs and hospital records), all original signed informed consent forms, copies of all ICFs, source documents, and detailed records of treatment disposition. The records should be retained by the PI according to local regulations. The PI(s)/Institution(s) will permit study-related monitoring, audits, and/or Institutional Review Board review and regulatory inspection(s), for direct access to source data/documents.

The blood specimens collected from the study will be processed and shipped to Singapore for analysis. The remaining specimen from the analysis may be archived and stored for further analysis where required. The storage facility holds the responsibility for the clinical specimens and has to ensure that they are properly documented and tracked with the integrity of records of the consent and other information relating to the participant. A separate institutional review board (IRB) will be required, exercised in and by the storage facility. The storage facility will also bear the responsibility that the specimens are properly disposed of following recommended protocols by the local regulations, if necessary.

The results of this study will be presented to the public through different means, including but not limited to radio, newspaper, other periodicals, social media platforms, local and international conferences, conventions, and published in scientific or medical journals without revealing the participants' identities.

10.5 Addressing Vulnerability

To protect the vulnerable population from exploitation, the PIS and ICF are presented in English. The investigator will provide supplementary educational measures and even interpret and translate the materials for the participants. This is to ensure that the research subjects will receive and process the information accurately and express considered choices.

Prospective participants who do not give their consent will continue to be treated in the usual and customary fashion. Their biological specimens will not be included among the samples to be analysed.

Concerns and issues regarding the privacy and confidentiality of participants' information and results of molecular analyses will be addressed appropriately. Benefits and risks will be thoroughly and carefully assessed during the course of the clinical trial.

11 The estimated timeline for this study is shown below:

Milestone	Target date
Research Protocol consensus with all parties	1-Jun-23
Equipment and consumables check	15 Jul-23
Ethics approval	31-Jul-23
Lab personnel training	15-Aug-23
Site Initiation Visit – PPUM and Respiratory Clinic	30-Aug-23
Study start date	4-Sep-23
Send out 10 x Cohort A and 10 x Cohort B to Singapore for verification	29-Oct-23
50-subject pulse check	5-Nov-23
100-subject pulse check	4-Feb-24
200-subject pulse check	4-Aug-24
Post recruitment check Review on extension of study	19-Sep-24
Target 300 subjects	2-Feb-25
Data analysis	24-Oct-26
Study wrap up and report	28-Dec-26

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13 Appendix

13.1 Appendix A: Study teams and the participating site for this study

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