***Lignosus rhinocerus* TM02® as an immunomodulatory agent for the treatment of uncontrolled asthma: A prospective, open-label, single-arm, Phase II study.**

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Time Frame of study:
Expected start date : 01/07/2022
Expected end date : 31/05/2023

Research Background

Introduction

Lignosus rhinocerus, the Tiger Milk Mushroom, belongs to the *Polyporaceae* family and is one of the most important medicinal mushrooms used by natives in Southeast Asia and one of the most popular medicinal mushrooms used by indigenous communities of Peninsular Malaysia. It is known locally as “cendawan susu rimau” - literally “mushroom of tiger’s milk.” It has traditionally been used by the Malays, Chinese and indigenous communities in Malaysia to treat a variety of diseases, including cough, asthma, fever, chronic hepatitis, gastric ulcer, liver and breast cancer and as a general tonic. In 2002, our former prime minister, Tun Dr. Mahathir

Mohamad, mentioned that his chronic cough had been cured by this medicinal mushroom during his opening speech at BioMalaysia.

Since the inception of scientific research in 2002 into *Lignosus rhinocerus* TM02® (Tiger Milk mushroom) and its associated bioactivities by the Medicinal Mushroom Research Group (MMRG), [Faculty of Medicine, University of Malaya], they have revealed various relevant, solid scientific data that supports the translation of *L. rhinocerus* research into practical use. They have proven it to be safe for consumption (Lee et al, 2011, 2013). They have also identified the extract and fraction from *Lignosus rhinocerus* TM02® that play numerous roles in immunomodulation (Yap et al , 2020) , anti-inflammatory (Lee et al, 2014), bronchodilating (Lee et al, 2018), anti-proliferative (Yap et al, 2013, 2017), antioxidative (Yap et al, 2013) and antiglycation (Yap et al , 2018).

MMRG has also subjected *Lignosus rhinocerus* TM02® to corticosteroid screening by Toxicology Laboratory, National Poison Centre. The results confirmed the absence of corticosteroid (Dexamethasone, Betamethasone, Costisone, Hydrocortisone, Prednisone, Prednisolone, Methyl prednisolone, Triamcinolone) as part of its ingredient.

Lignosus rhinocerus TM02® is marketed as "Tigerus Tiger Milk Mushroom Sclerotia" with a registration number of MAL 13025025TC. (Please see supporting documents for NPRA documents).

Rationale of Study

Asthma and FeNO

Asthma is a disease with marked heterogeneity in its clinical course and in its response to treatment. Variability in the type of airway inflammation may underlie this heterogeneity. Despite treatment with inhaled glucocorticoids, many patients continue to have uncontrolled asthma that requires more intensive therapy. Based on the ethnobotanical uses of Tiger Milk mushroom in treating asthma, its significant anti-inflammatory activity and also the positive feedbacks received from asthmatic volunteers using LiGNO™ Tiger Milk Mushroom, it is worthwhile to explore the potential of Tiger Milk Mushroom to be used as an immunomodulatory treatment to support conventional therapy through a proper designed clinical study. This will allow patients with uncontrolled asthma to obtain access to a new complementary therapy which they may benefit from. The objective of the study is to investigate the efficacy of L. rhinocerus to be used as a complementary therapy in improving symptoms, preventing exacerbations and reducing underlying airway inflammation in asthma patients.

FE_{NO} (fractional exhaled nitric oxide) is the only available point of care test to assess type-2 inflammation in asthma. Raised FE_{NO} with blood eosinophilia should be regarded as treatable traits of type 2 inflammation. FE_{NO} maybe useful for providing

feedback to both physicians and patients taking asthma therapy (in particular inhaled corticosteroids, ICS) particularly when the spirometry is normal.

FE_{NO} values can be influenced by several non-disease-related factors, thus filling in a questionnaire for NO measurement is recommended. Confounding factors could be related to the patient, like genetics, sex, weight and height, diet (*i.e.*, coffee) or taking drugs such as anti-inflammatory medications; also current smoking and atopy seem to influence FE_{NO} levels. Allergen exposure is associated with higher levels of FE_{NO} but they could decrease during the early phase of allergic response. Smoking is an important determinant of FE_{NO} levels and current smokers exhibit lower levels of FE_{NO} in comparison to ex-smokers and never smokers. Both active and passive smoking have effects on lowering FE_{NO} as demonstrated in healthy adults and in both adults and children suffering from asthma, regardless their allergy status.

FE_{NO} could be influenced also by viral respiratory infections. Age seems to be important too, especially in children, but correlation between age and sex has still to be defined. Recently, also ethnicity seems to have a role in FE_{NO} results, impacting clinical management.

Moreover, there are also technical confounding factors in FE_{NO} measurement, like the NO analyzer used, measurement technique, exhalation flow rate or nasal NO contamination. Spirometry could also influence FE_{NO} results, thus it should not be performed first. FE_{NO} could increase due to bronchodilation and it could decrease due to bronchoconstriction.

Reference values have been described for FE_{NO}. In clinical practice, FE_{NO} <25 ppb in adults is considered the normal value. FE_{NO} levels between 25-50 ppb in adults (20-35 ppb in children) should be contextualized within the clinical context.

The eosinophilic asthma phenotype is characterized by sputum eosinophils ≥3% and identifies patients with a good response to corticosteroids and T2 immunomodulators. FE_{NO} values >50 ppb are likely connected with airway eosinophilic inflammation and this data may be used to predict a response to anti-inflammatory therapy, while low FE_{NO} <25 ppb correlates with less eosinophilic inflammation and responsiveness to corticosteroids. Diagnostic FE_{NO} cut point in well controlled asthma is usually indicated by normal values. FE_{NO} >30 ppb is usually associated with uncontrolled asthma.

According to GINA guidelines, following the diagnosis of severe asthma, FE_{NO}≥20 ppb is considered the cut-off characterizing Type 2 inflammation severe asthma and it is used to assess this asthma phenotype, together with other markers like blood and sputum eosinophils. It has been suggested a FE_{NO} cut point of 21 ppb best fits ≥3% sputum eosinophils in corticosteroid-naive patients.

Very few data from studies analyzing clinically important change of FE_{NO} in individual patients is available and different are the results depending on the considered outcome. Considering simply the within-subject coefficient of variation, in healthy subject is approximately 10% (corresponding to a raw change up to 4 ppb) while it

increases to about 20% in patients with asthma, therefore leading the ATS experts to recommend a change of at least 20% to indicate a significant rise or fall in FE_{NO} over time or following an intervention.

The ATS makes the following recommendation:

- We recommend that low FENO less than 25 ppb be used to indicate that eosinophilic inflammation and responsiveness to corticosteroids are less likely.
- We recommend that FENO greater than 50 ppb be used to indicate that eosinophilic inflammation and, in symptomatic patients, responsiveness to corticosteroids are likely.
- We recommend that FENO values between 25 ppb and 50 ppb should be interpreted cautiously and with reference to the clinical context.
- We suggest using a reduction of at least 20% in FENO for values over 50 ppb or a reduction of more than 10 ppb for values lower than 50 ppb as the cut point to indicate a significant response to anti-inflammatory therapy.

We hypothesize that *Lignosus rhinocerus* TM02® can be used as a complementary supplement to support conventional therapy in asthmatic patients through a properly designed clinical study.

Research Questions

1. Can *Lignosus rhinocerus* TM02® reduce airway inflammation, improve symptoms and prevent exacerbations in asthma patients.
2. Can *Lignosus rhinocerus* TM02® be used alongside conventional Western medications as per global guidelines to reduce airway inflammation, improve symptoms and prevent exacerbations in asthma patients.

Problem Statement

To date, there have not been any safe treatments that are void of side effects for the current respiratory related illnesses and/or pandemics. Based on the current handling procedures in health care centers, and as recently discussed in the Chinese Medicine Task Force Malaysia COVID19, it was revealed that natural products play an important role in alleviating and as adjunct therapy to present day Western medications. It is imperative that we thoroughly investigate the potential mode of action of these natural compounds, especially ones that has been proven safe for human consumption and with other known health benefits. In light of the current pandemic, *Lignosus rhinocerus* TM02®, which has been proven safe for consumption, contains synergictically acting biomolecules that will improve the quality of life of respiratory-related illnesses. This area of research is new, novel and has the potential to reveal valuable data for further pharmaceutical intervention studies.

Objectives

The primary objective is to assess the effect of *Lignosus rhinocerus* TM02® in reducing airway inflammation by measurement of exhaled nitric oxide (FE_{NO}) and to assess the effect of *Lignosus rhinocerus* TM02® in bronchodilation by measurement of trough forced exhaled volume in 1 second (FEV_1).

The secondary objective is to assess the effect of *Lignosus rhinocerus* TM02® to be used as complementary therapy to improve symptoms and prevent exacerbations in asthma patients as assessed by the asthma control questionnaire (ACQ-7).

The tertiary objective is to assess the safety profile of *Lignosus rhinocerus* TM02® as complementary therapy in asthma patients.

STUDY DESIGN AND SAMPLE SIZE

This is a prospective, open-label, Phase II study.
The study will consist of a single treatment phase including:

- Screening period
- 12 weeks (90 days) of treatment period.

The planned sample size for this study is 17 subjects. In order to evaluate primary and secondary outcomes, study assessments will be conducted at Day 0, 30, 60 and 90 of treatment.

The MCID for FEV_1 is 12% and 200 ml measured by spirometry obtained at baseline (immediately prior to the start of the treatment) with the results obtained 2 hour after administration of study medication (2-hour postdose FEV_1) after 12 weeks of treatment.

The MCID for FE_{NO} is a reduction of at least 20% in FE_{NO} for values over 50 ppb or more than 10 ppb for values lower than 50 ppb will be used as the cut point to indicate a significant response to treatment.

Endpoints
Primary Endpoints

- Change in mean trough FEV_1 expressed as mililitres after 90 days of treatment
- Change in mean ACQ-7 scores after 90 days of treatment

- The threshold for change in ACQ-7 scores that is considered clinically important (MCID) is 0.5.

Secondary Endpoints

- Changes in mean FeNO (ppb) after 90 days of treatment
- Change in mean eosinophil count (cells/dL) after 90 days of treatment
- Changes in mean serum immunoglobulin E level (IU/ml) after 90 days of treatment

Study Population

The target population for this study consists of 10 Patients with uncontrolled asthma aged 18 and above. Each subject will have to fulfil the inclusion criteria listed below. Subjects will not be included in the study if they meet any of the exclusion criteria listed below.

Inclusion Criteria

All adults aged 18 years and above with a prior documented diagnosis of atopic (Th2-driven) asthma by a physician based on Global initiative for Asthma (GINA) 2020 Guidelines whose asthma is uncontrolled on ICS/LABA combination therapy based on the following criteria:

a) A well documented requirement for regular treatment with a minimum dose of either fluticasone /salmeterol combination therapy or budeosnide/formoterol combination therapy in the 12 months prior to screening with or without maintenance of oral corticosteroids (OCS).

b) Forced expiratory volume (FEV1) > 50% to <80% predicted normal during the screening phase (3 attempts maximum) and on the randomization day prior to the first dose of IMP.

c) Reversibility of at least 12% and 200 ml in FEV1 after 200 ug to 400 ug (2 to 4 inhalations) of salbutamol during the screening phase (3 attempts maximum), or documented history of reversibility test that met these criteria within 12 months prior to screening.

d) **FE_{NO} of > 25 ppb measured at baseline screening.**

- Absence of a recent exacerbation 6 weeks prior to enrolment
- Non-smoker patient for at least six months and with a prior tobacco consumption < 10 packs/ year
- Patient with normal organ function
- Patient weight > 41 kg and body mass index (BMI) between 18 and 35 kg/m2

Exclusion Criteria

- Asthmatic patient still exposed to allergens or to triggering factors influencing asthma control
- Female subjects who are pregnant or lactating
- Chronic obstructive pulmonary disease (COPD) and/or other lung diseases imparing pulmonary function test
- Within 6 weeks prior to screening, patient who received oral corticosteroids for any other reason than to treat asthma (patients needing long term OCS to control asthma)
- Patient with history of acute infectious sinusitis or respiratory tract infection within 4 weeks prior to screening visit

- Patient presenting with cardiac disorders defined by at least one of the following conditions:

  a) Patient with recent cardiac history (within 6 months) of :
    - acute coronary syndrome
    - acute heart failure (Class III or IV of the NYHA classification)
    - significant ventricular arrhythmia (persistent ventricular tachycardia, ventricular fibrillation, resuscitated sudden death)

  b) Patient with cardiac failure class III or IV of the NYHA classification
  c) Patient with sever conduction disorders which are not prevented by permanent pacing (atrio-ventricular block 2 and 3, sino-atrial block)
  d) Syncope without known aetiology within 3 months
  e) Uncontrolled severe hypertension, or symptomatic hypertension

- Patient with active lung disease other than asthma (eg. Chronic bronchitis)
- Patient who had a major surgery within 4 weeks prior to screening visit
- Patient with a life expectancy of less than 6 months
- Patient with history of primary malignancy < 5 years, except treated basal cell skin cancer or cervical carcinoma in situ.
- Patient with any severe and/or uncontrolled medical condition aside from asthma
- Patient with a known diagnosis of human immunodeficiency virus (HIV) infection
- Patient with history of poor compliance or history of drug/alcohol abuse.
- Participation in a clinical study with exposure to any non-registered drug or botanical product within 30 days prior.
- Individual has a condition the investigator believes would interfere with his or her ability to provide informed consent, comply with the study protocol, which might confound the interpretation of the study results or put the person at undue risk

Early Withdrawal

Subject discontinuation should be considered at the discretion of the principal investigator. The circumstances of any discontinuation have to be documented in detail. If possible, the evaluations planned for the end of study will be carried out at the time when the subject is withdrawn from the study.

Criteria for removal of subjects from the study will include:

Personal reasons
As stated in the Informed Consent Form, a subject may withdraw from the study for any reason at any time.

Clinical judgment of physician
A subject may be withdrawn from the study if, in the opinion of the principal investigator, it is not in the subject's best interest to continue. This includes but is not limited to adverse events or serious adverse events related to the investigational product causing clinically significant illness; the need for prohibited concomitant medication; female subject who becomes pregnant during the course of the trial.

Protocol violation
Any subject found to have entered this study in violation of the protocol will be discontinued from the study at the discretion of the principal investigator. This will include any subject found to have been inappropriately enrolled (did not meet eligibility criteria). Subject non-compliance includes either not showing up for study visits, not taking investigational product as directed, or refusing to undergo study visit procedures. Subjects who are found to be less than 75% compliant with test article usage at any study visit will be withdrawn. Subjects who are found to be taking prohibited medications or supplements without the knowledge of the principal investigator will also be withdrawn. Any major protocol deviations (i.e., those that increase the risk to subjects and/or compromise the integrity of the study or its results) will result in subject discontinuation.

Study Procedures

Pre-study Visit (Screening)

At screening, a Subject Information and Consent Form will be given to the potential subject. The subject will read the information carefully and will be given the opportunity to seek more information if needed. The subject will also be provided with the option of taking the consent form home to review prior to making his or her decision. If agreeable, the subject will sign the consent form and receive a duplicate. After the subject has signed the informed consent, the screening number will be assigned sequentially and entered in the Screening and Enrolment Log. Screening numbers will be allocated in the chronological order of the subject's signing the informed consent.

Screening includes:

- Review of medical history and concomitant therapies.
- Symptoms examination
- Phyiscial examination
- Review of inclusion/exclusion criteria
- Exhaled nitric oxide (FE_{NO}) measurements

If FE_{NO} is > 25 ppb,

- Spirometry measurement (FEV_1 and FVC)
- Laboratory tests (basic hematology and chemistry)
- Urine pregnancy test for women of childbearing potential
- ECG

Baseline - (Visit 1 / Week 0/ Day 0)

Subjects will receive the treatment regimen of LiGNO™ TMM.

Eligible subjects will return to the clinic in a fasted condition (last food intake at least 12 hours before the visit; subjects will be allowed to drink water) for baseline assessments:

- vitals signs, weight and height measurement
- Asthma Control Questionnaire
- review of concomitant therapies
- physical examination
- Review of spirometry measurements (FEV_1 and FVC)
- Review of laboratory tests (basic hematology and chemistry)
- Review of urine pregnancy test for women of childbearing potential
- Review of baseline ECG

After all baseline assessments are reviewed, subjects will be dispensed the investigational product.

Subjects will receive LiGNO™ TMM. Subjects will be instructed in detail by site personnel about the dosing regimen. The first dose of study product is to be taken the day after the Baseline visit, treatment Day 1, consisting of :

- 2 capsules of 250 mg L. rhinocerus, once a day after breakfast and dinner daily for 4 weeks

Subjects will be given a diary card to record their symptoms every day. Subjects will be reminded to fast (12 hours) prior to their study visits. The next visit will be scheduled for Week 4/Day 30 .

Investigational Study Visit (Visit 2 / Week 4 / Day 30)

Subjects will return the clinic on Week 4 (Day 30). Any remaining investigational product or empty packaging will be returned and a new supply dispensed.

Study visit assessments will include:

- vitals signs, weight measurement
- review of concomitant therapies
- review of adverse events
- review of the diary card record
- symptoms examination
- compliance calculation
- Asthma Control Questionnaire
- Laboratory tests (basic hematology and chemistry)
- Exhaled nitric oxide (FE_{NO}) measurements
- ECG

Subjects will receive LiGNO™ TMM. Subjects will be instructed in detail by site personnel about the dosing regimen. Dosing will be escalated to

- 2 capsules of 250 mg L. rhinocerus, twice a day after breakfast and dinner daily for 8 weeks

Subjects will be reminded to fast (12 hours) prior to their study visits. The next visit will be scheduled for Day 60.

Investigational Study Visit (Visit 3 / Week 8 / Day 60)

Subjects will return the clinic on Week 8 (Day 60). Any remaining investigational product or empty packaging will be returned and a new supply dispensed.

Study visit assessments will include:

- vitals signs, weight measurement
- review of concomitant therapies
- review of adverse events
- review of the diary card record
- symptoms examination
- compliance calculation
- Asthma Control Questionnaire
- Laboratory tests (basic hematology and chemistry)
- Exhaled nitric oxide (FE_{NO}) measurements
- ECG

Subjects will be reminded to fast (12 hours) prior to their study visits. The next visit will be scheduled for Week 12/Day 90.

End of Treatment Visit (Visit 4 / Week 12/ Day 90) - CIC

Subjects will return the clinic on Day 90. Any remaining investigational product or empty packaging will be returned.

Study visit assessments will include:

- vitals signs, weight measurement
- review of concomitant therapies
- review of adverse events
- review of the diary card record
- symptoms examination
- compliance calculation
- Asthma Control Questionnaire
- Laboratory tests (basic hematology and chemistry)
- Exhaled nitric oxide (FE_{NO}) measurements, followed by
- Spirometry measurement (FEV1 and FVC)
- ECG

This visit will conclude the study.

Grant and Funding

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References

Preclinical toxicology and safety studies

1. S.S. Lee, N.H. Tan, S.Y. Fung, J. Pailoor, S.M. Sim (2011) Evaluation of the sub-acute toxicity of the sclerotium of Lignosus rhinocerus (Cooke), the Tiger Milk mushroom, Journal of Ethnopharmacology , 138:192-200
2. Sook Shien Lee, Francis Kanyan Enchang, Nget Hong Tan, Shin Yee Fung, Jayalakshmi Pailoor (2013) Preclinical toxicological evaluations of the sclerotium of

Lignosus rhinocerus (Cooke), the Tiger Milk mushroom, Journal of Ethnopharmacology,147 (157-163)

Therapeutic effects:

- **anti-acute inflammatory (Lee et al.2014) - inhibition of TNF-alpha production**

Sook Shien Lee, Nget Hong Tan, Shin Yee Fung, Si Mui Sim, Chon Seng Tan, Szu Ting Ng (2014) Anti- inflammatory effect of the sclerotium of Lignosus rhinocerotis (Cooke) Ryvarden, the Tiger Milk mushroom, BMC Complementary and Alternative Medicine 14:359 (8 pages)

- **strong antioxidant, free radical scavenging (Yap et al, 2013)**

Yeannie HY Yap, Nget Hong Tan,Shin Yee Fung, Azlina A Aziz, Chon Seng Tan and Szu Ting Ng (2013) Nutrient composition, antioxidant properties, and anti- proliferative activity of wild type and cultivated strain of Lignosus rhinocerus Cooke (Tiger milk mushroom) sclerotium, Journal of the Science of Food and Agriculture 93(12):2945-2952

- **modulates cytokines (Yap et al, 2020)**

Alvin Yap Chee Sum, Xiaojie Li, Yeannie Yap Hui Yeng, Muhammad Fazril Mohamad Razif, Amira Hajirah Abd Jamil, Ng Szu Ting, Tan Chon Seng, Peter Chi Keung Cheung, Shin-Yee Fung (2020) The Immunomodulating Properties of Lignosus rhinocerus TM02® Cultivar and Its Associated Carbohydrate Composition, International Journal of Medicinal Mushroom 10.1615/IntJMedMushrooms.2020035658

Supported by

- **Nutritional studies and amino acid compositions**

Yeannie HY Yap,Nget Hong Tan,Shin Yee Fung,Azlina A Aziz,Chon Seng Tan and Szu Ting Ng (2013) Nutrient composition, antioxidant properties, and anti- proliferative activity of wild type and cultivated strain of Lignosus rhinocerus Cooke (Tiger milk mushroom) sclerotium, Journal of the Science of Food and Agriculture 93(12):2945-2952

- **Genome, Transcriptome and Proteome Studies**

Hui-Yeng Y Yap, Yit-Heng Chooi, Mohd Firdaus-Raih, Shin- Yee Fung, Szu-Ting Ng, Chon-Seng Tan, Nget- Hong Tan (2014) The genome of the Tiger Milk mushroom, Lignosus rhinocerotis, provides insights into the genetic basis of its medicinal properties, BMC Genomics 15:635

Hui-Yeng Yeannie Yap, Shin-Yee Fung, Szu-Ting Ng, Chon-Seng Tan, Nget-Hong Tan (2015) Genome-based Proteomic Analysis of Lignosus rhinocerotis (Cooke) Ryvarden Sclerotium, International Journal of Medical Sciences 12(1):23-31

Hui-Yeng Y Yap, Yit-Heng Chooi, Shin-Yee Fung, Szu- Ting Ng, Chon-Seng Tan, Nget-Hong Tan (2015)Transcriptome Analysis Revealed Highly Expressed Genes Encoding Secondary Metabolite Pathways and Small Cysteine-Rich Proteins in the Sclerotium of Lignosus rhinocerotis, PLoS One http://dx.doi.org/10.1371/journal.pone.0143549

Hui-Yeng Y. Yap, Shin-Yee Fung, Szu-Ting Ng, Chon-Seng Tan, Nget-Hong Tan (2015) Shotgun proteomic analysis of tiger milk mushroom (Lignosus rhinocerotis) and the isolation of a cytotoxic fungal serine protease from its sclerotium, Journal of Ethnopharmacology 174(2015)437 -451

Other publication on human use using TM02® :

Chen CK, Hamdan NF, Ooi FK, Wan Abd Hamid WZ. Combined effects of lignosus rhinocerotis supplementation and resistance training on isokinetic muscular strength and power, anaerobic and aerobic fitness level, and immune parameters in young males. Int J Prev Med 2016;7:107.

FE_{NO}:

Dweik RA, Boggs PB, Erzurum SC, e tal. An official ATS clinical practice guidelineL: Interpretation of exhaled nitric oxide levels (FE_{NO}) for clinical applications. Am J Respir Crit Care Med 2011, 184 (5).

Heffler E, Carpagnano GE, Favero E, et al. Fractional Exhaled Nitric Oxide (FE_{NO}) in the management of asthma: a position paper of the Italian Respiratory Society (SIP/IRS) and Italian Society of Allergy, Asthma and Clinical Immunology (SIAAIC). Multidiscip Respir Med. 2020;15(1):36.