A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of TQ Formula in Treating Participants who have Tested Positive for Novel Coronavirus 2019 (BOSS-Covid-19)

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator will assure that no deviation from, or changes to the protocol, will take place without prior agreement from the Investigational New Drug (IND) sponsor, and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; in the event that a new consent form is developed and approved by IRB, a determination will be made regarding whether a new consent needs to be obtained from existing participants who already provided consent, using a previously approved consent form.

This document contains information that is privileged or confidential. As such, it may not be disclosed unless specific prior permission is granted in writing by the sponsor or such disclosure is required by federal or other laws or regulations. Persons to whom any of this information is to be disclosed must first be informed that the shared information is confidential. These restrictions on disclosure will apply equally to all future information supplied, which is indicated as privileged or confidential.

The investigator agrees to comply with the ICH-GCP, World Medical Association Declaration of Helsinki (and relevant updates) and applicable local regulations. The investigator agrees that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator:

Printed Name: _____

Signature: _____

Date: _____

Institution Name: _____

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of TQ Formula in Treating Participants who have Tested Positive for Novel Coronavirus 2019 (BOSS-Covid-19)
Study Description:	This is a randomized (1:1), double-blind, placebo-controlled phase 2 study to assess safety and efficacy of Total 3 g daily dose of Black Seed Oil ('TQ Formula ' this point forward) capsules versus placebo in treating patients who have tested positive for novel Coronavirus 2019 (Covid-19) in the outpatient setting. Patients will be treated at a dose of 500 mg, 3 capsules, two times a day for 14 days from date of randomization. Quantitative viral load as measured by RT-PCR will be evaluated at baseline and on days 7 and 14. Covid-19 symptoms will be measured throughout the study using Modified FLU-PRO Plus. A detailed schematic describing all visits and a schedule of assessments is included in the Schema and Schedule of Activities, Sections 1.2 and 1.3, respectively.
Objectives:	Primary Objective: To evaluate if treatment with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) given orally on outpatient basis can significantly reduce median time to sustained clinical response compared to placebo in participants with COVID-19 infection treated in the outpatient setting. Sustained clinical response is defined as a reduction of scores to </= 2 on all symptoms of the Modified FLUPRO Plus. Secondary Objectives: 1. To compare the viral load profile over time (from baseline through to Day 14) between treatment with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) given orally on outpatient basis and placebo in participants with COVID-19 infection treated in the outpatient setting 2. To compare the percentage of RT-PCR negative/undetectable (i.e., viral clearance) on Day 7 and Day 14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo 3. To compare the duration and severity of symptoms (measured by

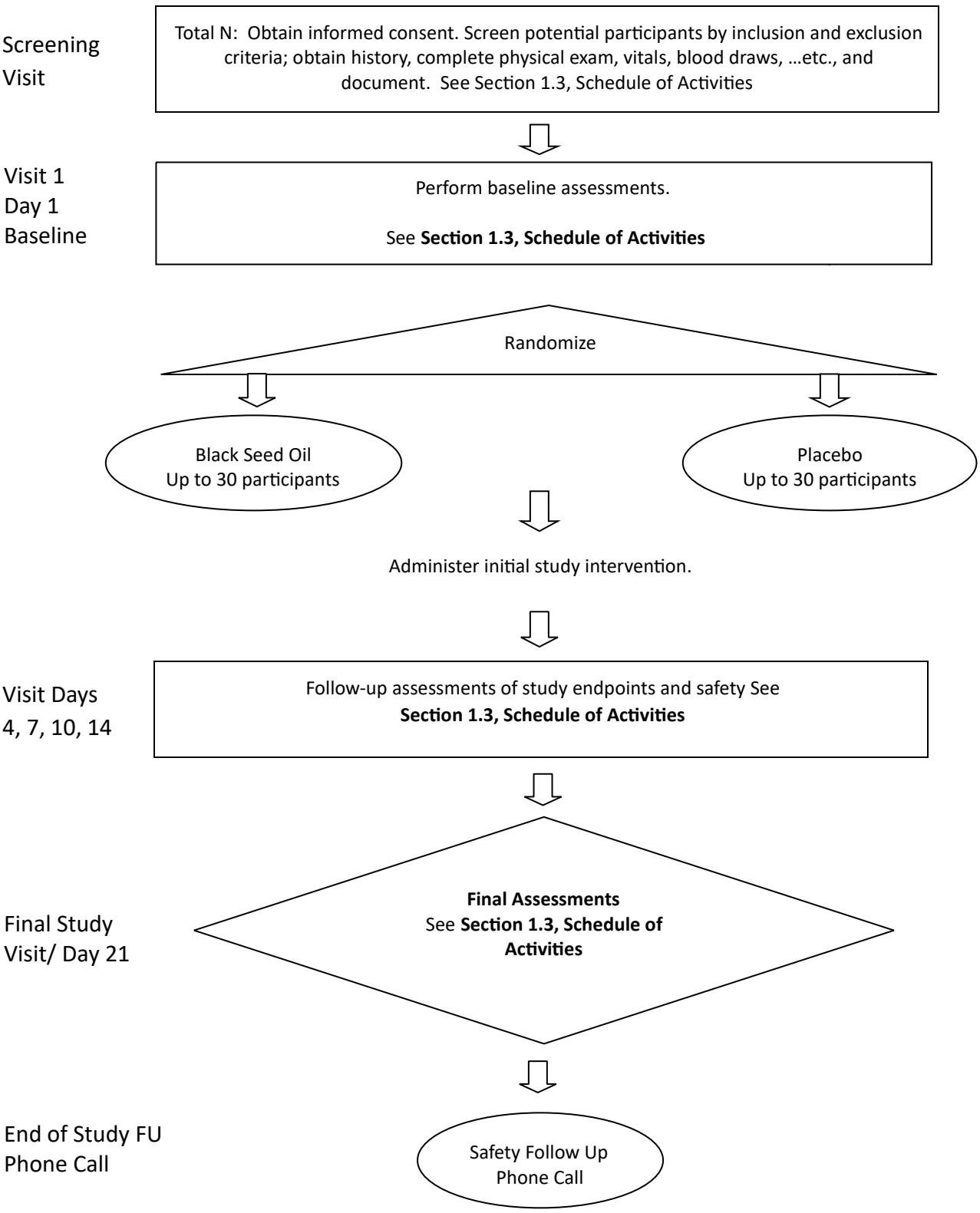
Modified FLU-PRO Plus) over time from Day 1 through Day 14 in

	total FLU-PRO Plus symptom severity score overall and in subdomain scores (namely, Nose, Throat, Eyes, Chest/Respiratory, Gastrointestinal, Body/Systemic, Taste/Smell), between treatment with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) given orally on outpatient basis and placebo in participants with COVID-19 infection 4. To investigate if there exists an association between viral load and symptom severity by study arm and if such associations change overtime 5. To evaluate the safety and tolerability of TQ Formula (500 mg oral capsule, 3 capsules BID) when given to participants with COVID-19 infection Exploratory Objectives: 1. To evaluate the basic pharmacokinetics of TQ Formula active ingredient (thymoquinone) at the same time points (Days 1, 7, and 14) in participants with COVID-19 infection. 2. To explore the effect of TQ Formula on inflammatory cytokines, coagulation factors and effector immune cells at same time points (Days 1, 7, and 14) in participants with COVID-19 infection.

Endpoints:	Primary Endpoint: Measurement of the difference in median time to sustained clinical response in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo. Sustained clinical response is defined as a reduction of scores to </= 2 on all symptoms of the Modified FLU-PRO Plus. Secondary Endpoints: 1. Measurement of change in quantitative viral load from baseline, Day 7, and Day 14 using RT-PCR in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo with COVID-19 infection. 2. Percentage of negative/undetectable RT-PCR (i.e., viral clearance) on Day-7 and Day-14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo 3. Measurement of severity of, and change in, Covid-19 symptoms per total score as well as sub-scores (Nose, Throat, Eyes, Chest/Respiratory, Gastrointestinal, Body/Systemic, Taste/Smell) measured through Modified FLU-PRO Plus from Day 1 through Day
	14 in participants with COVID-19 infection treated either with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) or placebo. 4. Correlation Coefficient of quantitative viral load and symptom severity at baseline, at Day 7, and Day 14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo 5. Number of overall adverse events, related adverse reactions, and hospitalizations reported in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo. All AEs/SAEs will be captured throughout the study as per schedule of assessments. Exploratory Endpoints: 1. Measurement of thymoquinone and metabolites' concentration in the plasma on day 1, Day 7 and 14 using HPLC in patients treated with TQ Formula. 2. Measurement of the inflammatory cytokine production, coagulation factors and the various effector immune cell subsets in the Peripheral Blood Mononuclear Cells (PBMC) of all patients on Day 1, Day 7 and Day 14 using FACS.

Study Population:	*Up to 60 participants will be randomized 1:1 to receive either TQ Formula Capsules + Standard of Care (SOC) or placebo +SOC* *There should be a good representation of senior population (65 years of age and above), as well as ethnic and racial variability*
Phase:	Phase II
Study Sites/Facilities Enrolling Participants:	*Approximately 2-4 centers in the United States*
Description of Study Intervention:	*TQ Formula (Nigella Sativa) 500 mg, 3 capsules, BID, taken orally*
Study Duration:	*6 months*
Participant Duration:	Up to 45 days

1.2 SCHEMA


Screening Visit
Total N: Obtain informed consent. Screen potential participants by inclusion and exclusion criteria; obtain history, complete physical exam, vitals, blood draws, …etc., and document. See Section 1.3, Schedule of Activities
Visit 1 Day 1 Baseline
Perform baseline assessments.
See Section 1.3, Schedule of Activities
Randomize
Black Seed Oil Up to 30 participants
Placebo Up to 30 participants
Administer initial study intervention.
Visit Days 4, 7, 10, 14
Follow-up assessments of study endpoints and safety See Section 1.3, Schedule of Activities
Final Study Visit/ Day 21
Final Assessments
See Section 1.3, Schedule of Activities
End of Study FU Phone Call
Safety Follow Up Phone Call

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	**Screen Visit**	**Baseline [d] Visit**	**Study Visit 2 [f]**	**Study Visit 3[g]**	**Study Visit 4 [f]**	**Study Visit 5[g]**	**Final Study Visit [f]**	**Safety Follow Up Phone Call**
	Day -3 to -1	**Visit 1, Day 1**	**Day 4 +/-1 day**	**Day 7 +/- 1 day**	**Day 10 +/-1 day**	**Day 14 +/1 day**	**Day 21 +/1 day**	**Day 45 +/- 1 day**
Informed consent	X							
Demographics	X							
Medical history	X							
Inclusion/Exclusion Criteria Evaluation	X	X						
Rapid Antigen Test for Covid-19	X[c]							
RT-PCR sample for quantitative viral load analysis		X[c]		X		X		
Serum Collection (Blood sample for immune monitoring)		X		X		X		
Physical exam	X			X		X		
Vital signs	X	X		X		X		
Pulse Oximetry	X							
Walking Oximetry	X							
Height	X							
Weight	X							
Hematology	X		X[e]	X	X[e]	X		
Serum chemistry [a]	X		X[e]	X	X[e]	X		
Pregnancy test [b]	X							
Randomization		X						
Administer study intervention		X.....X						
Concomitant medication review	X	X	X	X	X	X	X	
Adverse event review/evaluation	X	X	X	X	X	X	X	X
Patient Reported Outcomes (Modified FLU-PRO Plus)*	X[+]	X.....X						
Complete Case Report Forms (CRFs)	X	X	X	X	X	X	X	

a: Albumin, alkaline phosphatase, total bilirubin, direct bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, AST, ALT, sodium, CBC/diff, LDH, , CRP, prothrombin time, activated partial prothrombin time, , D-dimer b: Serum pregnancy test (women of childbearing potential).

c: Covid-19 Confirmatory test in addition to quantitative viral load

d: Screening and Baseline can be combined
e: If clinically indicated at the discretion of the investigator
f: Visits at Day 4 (visit 2), Day 10 (visit 4), and Day 21 (final visit) will be telehealth and can be changed to in-person at the discretion of the investigator g: In the event of a complete lockdown due to Covid, visits at Day 7(Visit 3) and Day 14 (Visit 5) could be replaced by home health visit for sample collection and telehealth visit for clinical evaluation.
*Administered everyday
+ Modified FLU-PRO Plus at Screen will be completed on site to determine if patient meets inclusion criterion

2 INTRODUCTION

2.1 BACKGROUND

In December of 2019, a series of acute atypical respiratory disease cases occurred in Wuhan, China which quickly spread to other areas. It was discovered that a distinctive coronavirus was responsible which was named the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, 2019-nCoV) as a result of its high similarity (~80%) to SARS-CoV, which caused acute respiratory distress syndrome (ARDS) and high mortality in the years 2002–2003 [91]. This virus has resulted in a global pandemic with an increasing death toll. It is easily transmittable from human to human. The outbreak of SARS-CoV-2 was considered to have originally started via a zoonotic transmission associated with the seafood market in Wuhan, China. It was later determined that human to human transmission played a major role in the subsequent outbreak [92]. The disease caused by this virus was called Coronavirus disease 19 (COVID-19) and a pandemic was declared by the World Health Organization (WHO). COVID-19 has infected a large number of people worldwide, being reported in roughly 200 countries [22,93]. The lack of a targeted therapy continues to be a problem.

Until today, six distinct strains of Human coronaviruses (HCoVs) had been described, in addition to the newly emerged COVID-19 [46,47,48,]. Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses of ~30 kb. They infect a broad array of host species [1]. They are essentially categorized into four genera; α, β, γ, and δ based on their genomic structure. α and β coronaviruses infect only mammals [2]. Human coronaviruses such as 229E and NL63 are responsible for the common cold and croup; they belong to the group of alpha coronaviruses. In contrast, SARS-CoV, OC43, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 are classified as β coronaviruses. SARS and MERS HCoV are the most aggressive strains of coronaviruses, leaving about 800 deaths each. SARS HCoV has a 10% mortality rate, while MERS HCoV has a 36% mortality rate, according to the WHO [47].

All coronaviruses contain specific genes in ORF1 (Open Reading Frames) downstream regions that encode proteins for viral replication, nucleocapsid and spikes formation [48]. The glycoprotein spikes on the outer surface of coronaviruses are responsible for the attachment and entry of the virus to host cells. MERS-coronavirus employs dipeptidyl peptidase 4 (DPP4), while HCoV-NL63 and SARS-coronavirus require angiotensin-converting enzyme 2 (ACE2) as a key receptor [49]. A number of host-cell receptors are reported to be recognized by the viral spike protein of SARS-Cov-2, among which is the cell-surface Heat Shock Protein A5 (HSPA5), also termed GRP78 or BiP [65]. In a fluorescent study, it was confirmed that the SARS-CoV-2 also uses the same ACE2 (angiotensin-converting enzyme 2) cell receptor and mechanism for the entry to host cell which is also used by the SARS-CoV [49,50,51,].
The life cycle of the virus within the host consists of the following 5 steps: attachment, penetration, biosynthesis, maturation and release. Once viruses bind to host receptors (attachment), they enter host

cells through endocytosis or membrane fusion (penetration). Once viral contents are released inside the host cells, viral RNA enters the nucleus for replication. Viral mRNA is used to make viral proteins (biosynthesis). Then, new viral particles are made (maturation) and released. Coronaviruses consist of four structural proteins; Spike (S), membrane (M), envelop (E) and nucleocapsid (N) [3]. Spike is composed of a transmembrane trimetric glycoprotein protruding from the viral surface, which determines the diversity of coronaviruses and host tropism. Spike comprises of two functional subunits; S1 subunit is responsible for binding to the host cell receptor and S2 subunit is for the fusion of the viral and cellular membranes. Angiotensin converting enzyme 2 (ACE2) has previously been identified as a functional receptor for SARS-CoV [4]. Structural and functional analysis shows that the spike for SARSCoV-2 also binds to ACE2 [[5], [6], [7]]. ACE2 expression is high in the lung, heart, ileum, kidney and bladder [8]. In the lung, ACE2 is highly expressed on its epithelial cells. Following the binding of SARS-CoV-2 to the host protein, the spike protein undergoes protease cleavage. After the cleavage at the S1/S2 cleavage site, S1 and S2 subunits remain non-covalently bound and the distal S1 subunit contributes to the stabilization of the membrane anchored S2 subunit at the prefusion state [6]. Subsequent cleavage at the S2 site presumably activates the spike for membrane fusion via irreversible, conformational changes.

The symptoms of patients infected with SARS-CoV-2 range from mild symptoms to severe respiratory failure with multiple organ failure. On Computerized tomography (CT) scan, characteristic pulmonary ground glass opacification can be seen even in asymptomatic patients [12]. Because ACE2 is highly expressed on the apical side of lung epithelial cells in the alveolar space [13,14], this virus can likely enter and destroy them. This matches with the fact that the early lung injury is often seen in the distal airway. Epithelial cells, alveolar macrophages and dendritic cells (DCs) are three main components for innate immunity in the airway [15]. DCs reside underneath the epithelium. Macrophages are located at the apical side of the epithelium. DCs and macrophages serve as innate immune cells to fight against viruses till adaptive immunity is involved.

T cell responses against coronaviruses are initiated by antigen presentation via DCs and macrophages. DCs and macrophages can phagocytize apoptotic cells infected by virus [16]. For example, virus-infected apoptotic epithelial cells can be phagocytized by DCs and macrophages, which leads to antigen presentation to T cells. SARS-CoV can also bind to dendritic-cell specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) and DC-SIGN-related protein (DC-SIGNR, L-SIGN) in addition to ACE2 [[17], [18], [19]]. DC-SIGN is highly expressed on dendritic cells and macrophages. These antigen presenting cells move to the draining lymph nodes to present viral antigens to T cells. CD4+ and CD8+ T cells play a critical role. CD4+ T cells activate B cells to promote the production of virus-specific antibody, while CD8+ T cells can kill viral infected cells.

Patients with severe diseases show lymphopenia, particularly the reduction in peripheral blood T cells [20,21]. Patients with severe diseases have been reported to have increased plasma concentrations of proinflammatory cytokines, including interleukin (IL)-6, IL-10, granulocyte-colony stimulating factor (GCSF), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein (MIP)1α, and tumor necrosis factor (TNF)-α [20,21]. The more severe conditions patients are in, the higher their IL-6 levels are. CD4+ and CD8+ T cells are activated in those patients as suggested by higher expression of CD69, CD38 and CD44. Higher percentage of checkpoint receptor Tm3+PD-1+ subsets in CD4+ and CD8+ T cells show that T cells are also exhausted. NK group 2 member A (NKG2A), another marker for exhaustion is elevated on CD8+ T cells [22]. Exhaustion of T cells could have led to the progression of the disease. Another interesting finding is that aberrant pathogenic CD4+ T cells with co-expressing interferon (IFN)-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) are seen in COVID19 patients with severe disease [20]. GM-CSF can help to differentiate innate immune cells and augment T

cell function, but it can initiate tissue damage at excess [23,24]. GM-CSF+IFN-γ+ CD4+ T cells were previously seen upon strong T cell receptor (TCR) responses in experimental autoimmune encephalomyelitis (EAE) models, where CD8+ T cells expressing GM-CSF were found at higher percentage and secreted IL-6.

The study of SARS-CoV shows that virus infected lung epithelial cells produced IL-8 in addition to IL-6 [15]. IL-8 is a well-known chemoattractant for neutrophils and T cells. Infiltration of a large number of inflammatory cells are observed in the lungs from severe COVID-19 patients [25,26], and these cells presumably consist of a constellation of innate immune cells and adaptive immune cells. Among innate immune cells, the majority would be expected to be neutrophils. Neutrophils can act as double-edged sword as neutrophils can induce lung injury [27,28,29]. The majority of the observed infiltrating adaptive immune cells are likely T cells. CD8+ T cells are primary cytotoxic T cells. Severe patients also show pathological cytotoxic T cells derived from CD4+ T cells [30]. These cytotoxic T cells can kill virus but also contribute to lung injury [31]. Circulating monocytes respond to GM-CSF released by these pathological T cells. CD14+CD16+ inflammatory monocyte subsets are also found at significantly higher percentage in COVID-19 patients. These inflammatory CD14+CD16+ monocytes have high expression of IL-6, which likely accelerate the progression of systemic inflammatory response.

An interesting note is that ACE2 is significantly expressed on innate lymphoid cells (ILC)2 and ILC3. NK cells are a member of ILC1, which constitute a large portion of ILCs in the lung (~95%). ILC2 and ILC3 work for mucous homeostasis.

In addition to respiratory symptoms, thrombosis and pulmonary embolism have been observed in severe diseases. This is in line with the finding that elevated d-dimer and fibrinogen levels are observed in severe diseases. The function of the endothelium includes promotion of vasodilation, fibrinolysis, and anti-aggregation. Because endothelium plays a significant role in thrombotic regulation [32], hypercoagulable profiles seen in severe diseases likely indicate significant endothelial injury. Endothelial cells also express ACE2 [33]. Of note, the endothelial cells represent the one third of lung cells [35]. Microvascular permeability as a result of the endothelial injury can facilitate viral invasion.

Interestingly, studies have shown correlation between viral load and disease severity. Liu et al [109] surmised that patients with severe COVID-19 tend to have a high viral load and a long virus-shedding period and as such, the viral load of SARS-CoV-2 might be a useful marker for assessing disease severity and prognosis. Tan et al. [110] in a 2020 study discovered that non-survivors of COVID-19 infection had obviously higher levels of viral load, indicated by ORF1ab Ct values, as compared to survivors.

There is currently no effective antiviral treatment for COVID-19. Existing drug alternatives as a result of clinical management of earlier discovered coronaviruses have been utilized in the treatment of COVID19 patients.

The growing interest in phytomedicine brings along the issue of their safety, and the requirements to meet health standards. It has been shown that the seeds and oil of NS plant are characterized by a very low degree of toxicity [39].

A detailed review of risk/benefits and findings from non-clinical studies and clinical studies can be found in sections 2.3.1 and 2.3.2.

2.2 RATIONALE AND RISK/BENEFIT ASSESSMENT

Black seed oil offers promising potential as a therapeutic option in management of SARS-CoV-2 as it has been shown in studies to possess beneficial properties like anti-viral effects (see sections 2.2.1 and 2.2.2).

A number of synthetic compounds initially thought to have shown promise in COVID-19 therapy, including hydroxychloroquine and chloroquine phosphate [54,55] (which act through several mechanisms, including alkalization of the host cell phagolysosomes), and newer antiviral medications such as lopinavir [56] have subsequently been shown to have little or no effect on hospitalized COVID19, as indicated by overall mortality, initiation of ventilation and duration of hospital stay [113]. Remdesivir [57,58] which also showed promise is the first FDA approved COVID antiviral therapy [114]. Currently (January 2021), two vaccines are authorized and recommended to prevent COVID-19 [116]:

- Pfizer-BioNTech COVID-19 vaccine
- Moderna's COVID-19 vaccine

Traditional herbal medicines and purified natural products may guide the development of novel antiviral drugs. In other words, more efficient drugs can often be designed based on the structure of natural compounds that exhibit the desired activity. Classic examples of this drug discovery pathway include emetine, an isoquinoline alkaloid isolated from Cephaelis ipecacuanha and used as an amoebicidal drug; quinine, derived from the bark of Cinchona trees; and numerous other drugs modified from natural compounds, such as aspirin, morphine and paclitaxel, an antineoplastic drug used for the treatment of cancer [59]. Indeed, half of all drugs approved between 1981 and 2014 were derived from or mimicked a natural compound [60].

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Among various medicinal plants, Nigella sativa (N. sativa) (Family Ranunculaceae) is emerging as an herb which many research studies have revealed its wide spectrum of pharmacological potential. N. sativa is commonly known as black seed. N. sativa is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia [36]. Nigella sativa is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of N. sativa have been widely used in the treatment of different diseases and ailments [42].

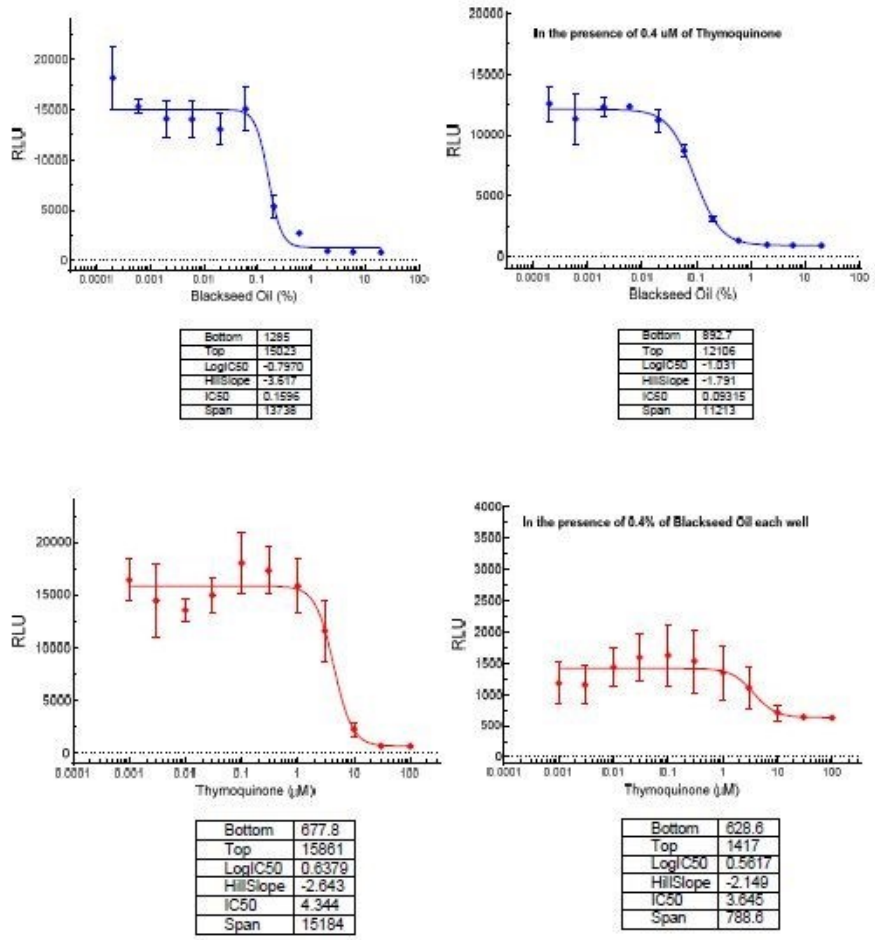
N. sativa has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities which include (among a host of others) antibacterial antifungal,

antiviral, antiparasitic, immunomodulatory, analgesic, antimicrobial, antiviral, anthelmintics, antiinflammatory, gastroprotective, hepatoprotective, renal protective [42]. Most of the therapeutic properties of this plant are due to the presence of thymoquinone which is major bioactive component of the essential oil [42]. Thymoquinone, the most prominent constituent of N. sativa seeds essential oil has been intensively investigated.

Thymoquinone may block the SARS-CoV-2 entry via ACE2 in pneumocytes. Abdel-Fattah et al. found that N. sativa oil and thymoquinone produce antinociceptive effects through indirect activation of supraspinal μ1- and κ-opioid receptor subtypes [103]. In addition, Takai et al. suggested that brain endogenous angiotensin II was involved in central nociceptive mechanisms by its antagonistic interaction with the endogenous opioid system [1104. Furthermore, Lantz et al. showed that opioid active peptides such as hemorphins have inhibitory effect on ACE [105]. The above line of evidence suggests that opioid receptors and ACE share similar inhibitory molecules and as such Rahman in a write up suggested that it is possible that thymoquinone might also block ACE2 [106].

Recently, and in collaboration with CODEX Bio Labs, Black Seed Oil and Thymoquinone were tested for their effect on viral entry and viral protein translation using Codex's Murine Leukemia Virus (MLV) particles pseudotyped (PP) with a SARS-CoV-2 Spike (unpublished data). In summary, various combinations/concentrations of black seed oil and thymoquinone were tested against SARS-CoV-2 MLV pseudovirus particles (pp). Luciferase activities were measured with Firefly Luciferase Assay Kit (CB80552-010, Codex BioSolutions Inc). It was observed that both Blackseed Oil and Thymoquinone seemed to block viral infection (see graphs below).

Results:



Bottom	1285
Top	15023
LogIC50	-0.7970
HillSlope	-3.517
IC50	0.1596
Span	13738

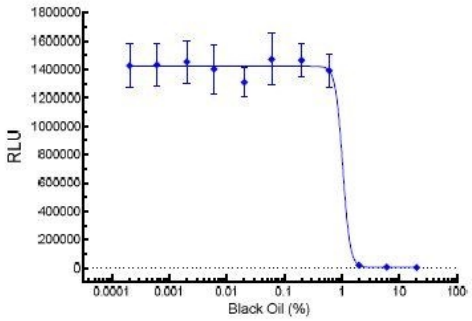
Bottom	892.7
Top	12106
LogIC50	-1.031
HillSlope	-1.791
IC50	0.09315
Span	11213

Bottom	677.8
Top	15861
LogIC50	0.6379
HillSlope	-2.643
IC50	4.344
Span	15184

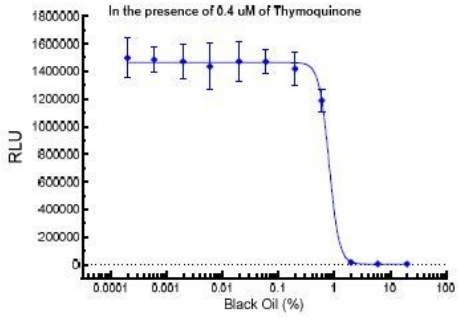
Bottom	628.6
Top	1417
LogIC50	0.5617
HillSlope	-2.149
IC50	3.645
Span	788.6

Fig. A Effect of Blackseed oil and Thymoquinone to block SARS-CoV-2-PP infecting the cells. X-axis: compound concentration. Y-axis, relative luminescence unit (RLU), reflecting the luciferase activity and the viral infectivity

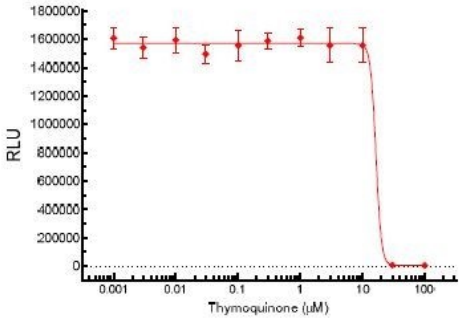
However, it was found that at high concentrations, Blackseed Oil and Thymoquinone caused cell death which indicates that both may have cell toxicity. To confirm it, cell growth assay was performed in the presence of Blackseed Oil or Thymoquinone with Codex's EnerCount cell growth assay kit which measures the ATP levels inside the cells



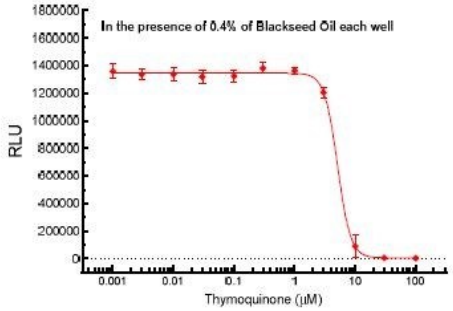
Bottom	5316
Top	1423711
LogIC50	0.01500
HillSlope	-6.938
IC50	1.035
Span	1418395



Bottom	1930
Top	1464904
LogIC50	-0.09240
HillSlope	-4.877
IC50	0.8084
Span	1462974



Bottom	3629
Top	1569394
LogIC50	1.215
HillSlope	-9.931
IC50	16.41
Span	1565765



Bottom	4427
Top	1345329
LogIC50	0.7086
HillSlope	-4.024
IC50	5.112
Span	1340901

Fig. B Cell toxicity of Blackseed oil and Thymoquinone on Expi-293F-ACE2 cells. X-axis: compound concentration. Y-axis, relative luminescence unit (RLU), reflecting the luciferase activity and the cell number

Conclusion: By comparing Fig. A and Fig. B, it was concluded that at certain concentrations, Blackseed oil (0.06%-0.6%) and Thymoquinone (1-10 μ M) can block the SARS-CoV-2 MLV-PP infection without any cell toxicity.

2.2.1 KNOWN POTENTIAL BENEFITS

Clinical Studies

Abdel-Moneim et al. in a study conducted on HCV patients were able to demonstrate that extracts of NS and Zingiber officinale, alone and together (500 mg of NS and/or Z. officinale twice daily for one month), improved liver function and decreased viral load in HCV patients [88]. Decreased viral load and improved liver function were similarly reported in another study by Barakat et al., where HCV patients received capsules of NS oil (450 mg) three times a day over a 3-month period [86].

Onifade et al. reported reduction of viral load to an undetectable level in 3 months, elevation of the CD4 count, alleviation of the symptoms, and a sustained sero-reversion in a sero-positive human immunodeficiency virus (HIV) infected man treated with NS (10 mL twice/day for six months) [87]. Similarly, another study conducted on a sero-positive HIV infected woman receiving NS and honey therapy (10 mL thrice/day for 1 year) demonstrated sustained sero-reversion which the author ascribed to the probable virucidal activity of NS [88].

A study by Salem et al. stated that, in a four-week course, the efficacy of NS powder (2 g/day) administered together with omeprazole to eradicate an H. pylori infection in non-ulcer dyspeptic patients was relatively the same as that of triple therapy [82].

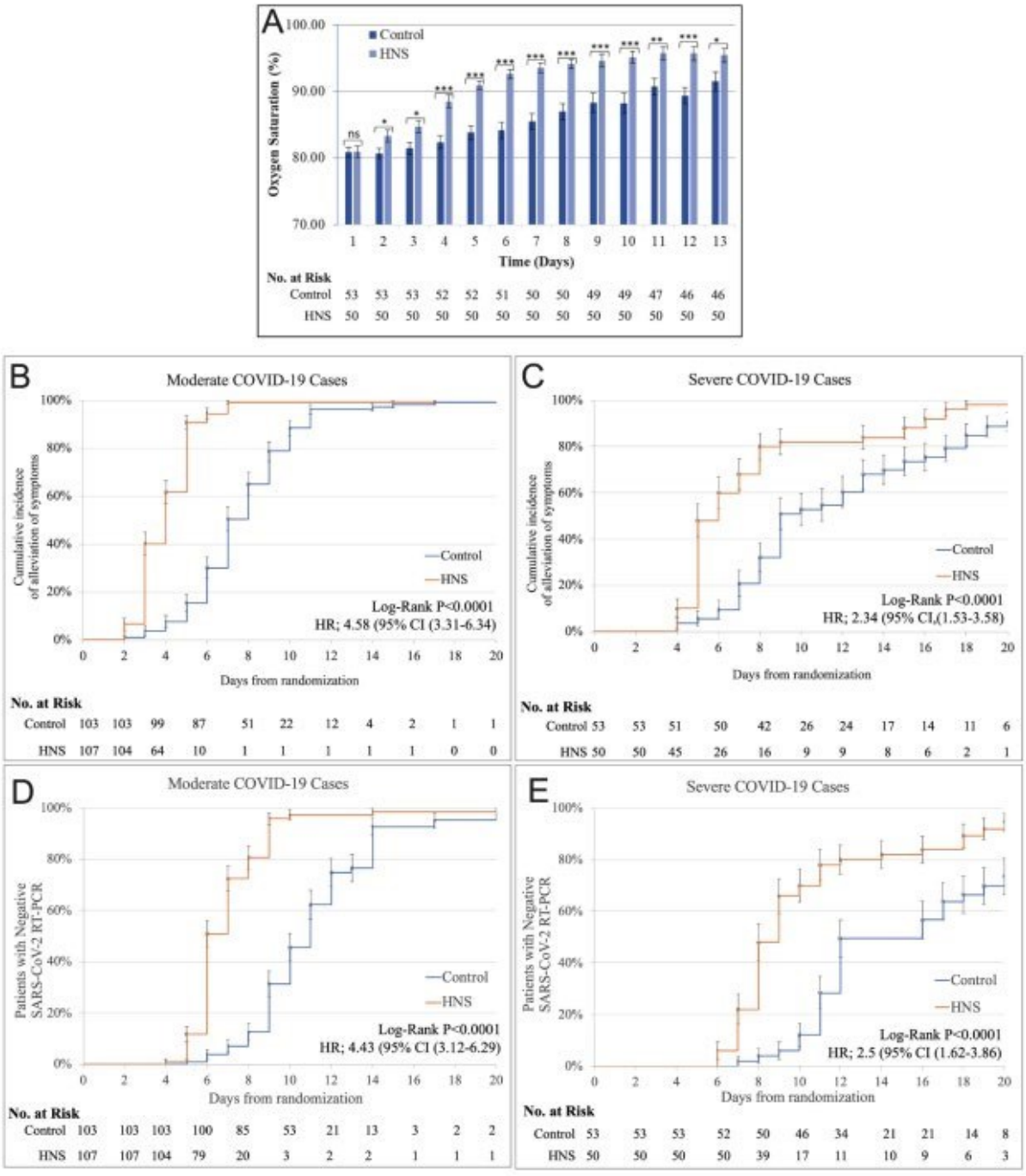
Akhtar et al. demonstrated the efficacy of single oral administration of NS powdered seeds and ethanolic extract (40 mg/kg body weight) in reducing the percentage of fecal eggs per gram in children who were infected with cestodes [83].

Fard et al. conducted a randomized trial in 100 women with Candida albicans vaginitis. The therapeutic effects of black seed capsules (500 mg twice daily) used together with clotrimazole vaginal cream were compared with those of placebo capsules (500 mg twice daily) used in combination with the same vaginal cream. After a 7-day course, symptoms of the infection such as vaginal itching, discharge, irritation were reduced more in the cohort receiving black seed capsules used with clotrimazole vaginal cream [84].

Blackseed study in Covid-19 patients:

In an investigator-initiated, open-label-placebo and randomized controlled trial conducted from April 30 to July 29, 2020 in four medical care facilities in Pakistan, 313 COVID-19 positive patients were stratified into two groups: mild to moderate (cough, fever, sore throat, nasal congestion, malaise and/or shortness of breath) and severe (fever and/or cough along with pneumonia, severe dyspnea, respiratory distress, tachypnea (>30 breaths/min) or hypoxia (SpO2 <90% on room air) (210 and 103 patients respectively) using the Clinical Management Guidelines for COVID-19 by the Ministry of National Health Services, Pakistan [114]. The patients within each of the two groups were randomly assigned to the treatment and control groups (Honey plus Nigella Sativa [HNS] and Placebo). The HNS group received honey (1 gm) plus Nigella sativa seeds (80mg) per kg body weight orally in 2-3 divided doses daily for upto 13 days while the control group received placebo (empty capsules). The primary outcomes were viral clearance (negative RT-PCR for the SARS-CoV-2 RNA), alleviation of clinical symptoms and the lowering of CGS on day 6. Secondary outcomes included reduction in fever degree (day 4), CRP levels (day 6), severity of symptoms (day 8), CGS score (day 10) and mortality on day 30. The study results showed that HNS helped with symptoms alleviation and viral clearance and reduced mortality in patients with moderate and severe disease. It was discovered that alleviation of COVID-19 symptoms for patients in the HNS groups occurred earlier than control groups: 4 versus 7 days for the moderate patients and 6 versus 13 days for the severe disease patients. Viral clearance (being negative for the SARS-CoV-2 RTPCR test) occurred 4 days sooner in the HNS group for both moderate and severe cases. A significant reduction in degree of fever was observed in the severe cases on day 4 (OR: 0.21; 95% CI: 0.09-0.46; P=0.0001). CRP levels decreased significantly (P <0.0001) on day 6 in both the HNS groups compared with their respective control groups. As per median degree of symptom severity on day 8, 98.13% patients were asymptomatic in HNS treated moderate cases in comparison to 56.31% in the control group (OR: 0.009; 95% CI: 0.001-0.08; P<0.0001). In severe cases, more patients were asymptomatic in the HNS group

while more had moderate symptoms (median) in the control arm (OR: 0.1; 95% CI: 0.040.24). By day 10, 96.26% of the moderate cases patients fully resumed normal activities with HNS compared to 68.93% in control group (OR: 0.07; 95% CI: 0.02-0.21). For the severe group, the median CGS at day 10 revealed that HNS cases resumed normal activities while control patients were still hospitalized requiring oxygen therapy (OR:0.05; 95% CI: 0.02-0.15). Thirty-day morality was 18.87% in control group and 4% with HNS therapy (OR: 0.18 95% CI: 0.02-0.92)[115].



A. Mean oxygen saturation spO2 over time in severe cases; Kaplan-Meier probability curves for time taken (in days) for alleviation of symptoms in moderate (B) and severe cases (C); Kaplan-Meier probability curves for time taken (in days) for viral clearance in moderate (D) and severe cases (E).ns = non-significant, *= P<0.05, **=P<0.001,***=P<0.0001.

To mask the after taste/unpleasant taste of the black seed oil in the TQ Formula, enteric coating will be applied on the drug (and the placebo). Previous clinical studies using fish oil have shown enteric coating

to be safe with the added benefit of improving compliance and enhancing gastric absorption by transient protection against gastric acidity [112].

Non-Clinical Studies

A molecular docking and molecular stimulation study by Elfiky et al., where natural product compounds were tested against the HSPA5 substrate binding domain, showed that the active components of cinnamon and the seeds of Nigella sativa may tightly bind to cell-surface HSPA5 (one of the host cell receptors recognized by the viral spike protein) and could be successful in contradicting SARS-CoV-2 spike recognition and attachment [63].

In a study by Ulasli et al., extracts prepared from the flowers and buds of Anthemis hyalina DC. (Asteraceae), the seeds of Nigella sativa L. (Ranunculaceae), and the peels of Citrus sinensis L. (Rutaceae) were tested against MHV-A59 type of betacoronavirus. The maximum non-toxic dose was found to be with 1/50 dilution of the extracts. Exposure of the extracts in virus-infected HeLa-CEACAM1a (HeLa-epithelial carcinoembryonic antigen-related cell adhesion molecule 1a) cells instigated a decrease in virus load. Following Ns treatment, the number of viruses was very low at 6 h post infections and it was 1/10th of the control amount by 8 h post infections. However, the reason for the decreased viral load is not known.[89] Salem et al. investigated the antiviral effect of black seed oil (BSO) from Nigella sativa using murine cytomegalovirus (MCMV) as a model. The viral load and innate immunity during early stage of the infection were analyzed. Intraperitoneal administration of BSO to BALB/c mice inhibited the virus titers in spleen and liver on day 3 of infection. This effect coincided with an increase in serum level of IFN-gamma. On day 10 of infection, the virus titer was undetectable in spleen and liver of BSO-treated mice, while it was detectable in control mice. The oil treatment increased IFN- gamma production and augmented numbers of CD4+ helper T cells, suppressor function and numbers of macrophages [90].

Umar et al. looked at the protective and antiviral activities of Nigella sativa against avian influenza (H9N2) in 130 non-vaccinated turkeys using 5 experimental groups of 30 turkeys each. Group A was kept as non-infected and a non-treated negative control while group B was kept as infected and non-treated positive control. Turkeys in groups A and B received normal commercial feed while turkeys of groups C, D and E were fed on diets containing 1.5%, 4% and 6% NS seeds, respectively, from day one through the entire experiment period. All groups were challenged with H9N2 AIV at 4th week of age except group A. Turkeys in group B showed more pronounced virus secretion than the turkeys in other groups receiving different levels of NS. Moreover, significantly higher antibody titer against H9N2 AIV in turkeys fed 6% NS seeds showed the immunomodulatory nature of NS. Similarly, increased cytokine gene expression suggested antiviral behavior of NS especially in a dose-dependent manner, leading to suppressed pathogenesis of H9N2 viruses. However, reduced virus shedding and enhanced immune responses were more pronounced in those turkeys received NS at the rate of 4% and 6% showing that supplement of NS would significantly enhance immune responsiveness and suppress pathogenicity of influenza viruses in turkeys [107].

In another study by Zaher et al., effect of Nigella sativa was tested using antiviral assays. Results showed that N Sativa has antiviral activity against Infectious Laryngotracheitis Virus (ILTV) at a concentration of 35µM [108].

In a study by Shahzad et al. [80], rats sensitized to ovalbumin and challenged intranasally with ovalbumin to induce an allergic inflammatory response were compared to ovalbumin-sensitized, intranasally ovalbumin-exposed rats pretreated with intraperitoneally administered black seed oil and to control rats. The levels of IgE, IgG1 and ova-specific T-cell proliferation in spleen were measured by ELISA. The pro-inflammatory cytokine IL-4, IL-5, IL-6 and TGF-beta1 mRNA expression levels were measured by reverse transcription polymerase chain reaction. The intraperitoneal administration of black seed oil inhibited the Th2 type immune response in rats by preventing inflammatory cell infiltration and pathological lesions in the lungs. It significantly decreased the nitric oxide production in BALF, total serum IgE, IgG1 and OVA-specific IgG1 along with IL-4, IL-5, IL-6 and TGF-beta1 mRNA expression [80].

In a similar study, Parlar et al. [61] looked at allergic airway inflammation induced by ovalbumin (OVA) challenge in sensitized-rats and effect of thymoquinone using 3 groups: **1.** Unsensitized control group (Control); **2.** Sensitized and challenged group (OVA): Rats were sensitized and challenged with OVA; **3.** TQ administrated group (TQ): Sensitized rats were treated with TQ, three times as 30 min, one and two days before the OVA provocation (Three doses of TQ- 1, 10, and 30 mg/kg). Inflammatory cells, interleukin (IL)-6 and TNF-α in bronchoalveolar lavage (BAL) fluid, and lipid peroxidation (LPO) in lung tissue were measured. Total inflammatory cells were significantly elevated in the OVA group compared with control group, mainly eosinophils and neutrophils. TQ treatment resulted in significantly reduced numbers of total inflammatory cells in the BALF from rat with OVA-induced allergic asthma, especially eosinophils and neutrophils. IL-6 level in BAL fluid was not significantly changed both in OVA and TQ groups as compared to control group. However, TNF-α level in BAL fluid was significantly elevated in the OVA group as compared to the control group ($p=0.011$), while TNF-α level in BAL fluid was lower in the TQ group than those in the OVA group.

In an in vitro study by Vaillancourt et al., thymoquinone significantly abolished LPS-induced proinflammatory cytokines such as interleukin1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), metalloproteinase-13 (MMP-13), COX-2, and prostaglandin E2 in an in vitro model of rheumatoid arthritis [62].

In a review done by Gholamnezhad et al. in 2018, anti-inflammatory effect of Thymoquinone was demonstrated in allergic lung inflammation- It was reported that TQ decreased IL-4, IL-5, and IL-13 but increased IFN-γ in BALF and lung homogenates [40] .

An in-vitro study in 2018, Cobourne-Duval et al. conducted a comparative quantitative proteomic analysis of LPS/IFNγ-activated BV-2 microglial cells (immortalized murine microglial cell line) with and without TQ treatment. The LPS/IFNγ-activated BV-2 cells showed significantly higher protein expression of several inflammatory cytokines compared to the controls: IL-2 = 127%, IL-4 = 151%, IL-6 = 670%, IL-10 = 133%, and IL-17a = 127%. The protein expression of the same inflammatory cytokines in the TQ treated, LPS/IFNγ-activated cells were reduced significantly ($P < .0001$) compared to the protein expression levels activated cells without TQ treatment: IL-2 = 38%, IL-4 = 19%, IL-6 = 83%, IL-10 = 23%, and IL-17a = 29% [43] Findings from the study showed TQ reduced the expression of several inflammatory cytokines in the LPS/ IFNγ activated BV-2 microglial cells, exhibiting an inhibitory effect on the expression of IL-2, IL-4, IL-6, IL-10, and IL-17a [43].

2.2.2 KNOWN POTENTIAL RISKS

The acute and sub-acute toxicity of N. sativa has been examined in various in-vitro and in-vivo studies. The seed extract and its constituents appear to have a low level of toxicity.

Ong et al. in 2016 examined the acute and subacute toxicity profiles of orally administered thymoquinone and thymoquinone-loaded nanostructured lipid carrier in BALB/c mice [37]. In this study, animal survival, body weight, organ weight-to-body weight ratio, hematological profile, biochemistry profile, and histopathological changes were analyzed. For acute toxicity study, twenty-seven female BALB/c mice were randomly assigned into nine groups (n=3), which were the control, two vehicle groups, and six treatment groups of TQNLC and TQ at three fixed doses (5, 50, and 300 mg/kg of body weight). The control received tap water. The two vehicle groups received olive oil and blank NLC, respectively, of the same volume as the treated groups. For treatment groups, TQ was diluted in olive oil, while TQNLC was diluted in deionized water. All the treatments were injected via oral administration on the first day only. For the subacute toxicity study, ninety BALB/c mice were randomly divided into nine groups (five males and five females): the control group, two vehicle groups, and six treatment groups of TQNLC and TQ with three escalating doses each (1, 10, and 100 mg/kg of body weight). The control received tap water, while the two vehicle groups received olive oil and blank NLC, respectively, at the same volume as the treated groups. For treatment groups, TQ was diluted in olive oil while TQNLC was diluted in deionized water. TQ and TQNLC were orally administered by gavage for 28 days and the mice were observed for any changes in the general physical conditions, such as appearance, fur condition, behavior, and mortality. For the acute toxicity study, 300mg/kg caused mortality to all the mice within 24 hours for TQ and only 1 mouse for TQNLC. No significant gross organ changes or weight changes were observed. For the subacute study, oral administration of TQNLC and TQ for 28 days did not cause any behavioral abnormalities of the mice at any time point. No mortality was observed during the experimental period. No significant gross organ changes or weight changes were observed. There were no significant changes in the hematological profiles of either male or female mice. No alterations were observed in the kidneys for all the treated groups in both sexes. The liver of both male and female mice treated with 100 mg/kg TQ and 100 mg/kg TQNLC showed areas of pyknotic nucleus and cell degeneration. Although the liver biochemical data were within the normal range, this suggests that the high dose of TQ and TQNLC may cause some toxic effects to the liver but not to the extent of altering the functions of the organ [37].

A study by Abukhader in 2012 showed that the MTD for the oral ingestion of TQ in both male and female rats was 250 mg/kg. With respect to rats which received oral ingestion of TQ, data collected from experimental observations and analysis suggested that single oral ingestion of TQ in doses lower than 500 mg/kg body weight could be nonlethal. Signs of transient toxicity were observed with 300 and 500 mg/kg such as weight loss, slight abdominal distention [44].

Zaoui et al. in 2002 investigated the toxicity of Nigella sativa L seeds in mice and rats through determination of LD50. The results on the acute toxicity study suggested that Nigella sativa oil has a large window of safety with a LD50 > 2000 mg/kg [38,39].

Datau et al. in a study performed on 39 centrally obese men demonstrated that intake of NS seeds (3 g/day for 3 months) had no detectable side effects [65]. Administration of 2g/day of NS seed for 6 weeks had no adverse effect on serum ALT or serum Cr in adult subjects [66]. Dehkordi et al. reported that the intake of NS extract (200 or 400 mg/day) for 2 months caused no observable complications in patients with mild hypertension [67]. NS at doses of 1, 2 and 3 g/day for 3 months had no adverse effect on either the renal or the hepatic functions of diabetic patients [68]. Patients with allergic rhinitis treated with NS seeds (250mg/day) for 2 weeks had no adverse effects [70], though some patients with allergic rhinitis

when treated using nasal drops of NS oil showed nasal dryness [73]. NS oil intake (equivalent to oil obtained from 0.7 g of seeds) for 40 days showed reasonable kidney and liver safety in patients with type 2 diabetes mellitus (DM) [74]. Whereas no severe side effects were reported in administration of NS oil (5 mL/day) to functional dyspeptic patients, some mild adverse impacts were observed, including nausea, bloating, and burning sensation [73]. Three controlled studies investigated the adjuvant use of NS in type-2 DM [68,77,78]. Nigella sativa administered at the daily dosages of 2 g/day and 3 g/day appeared to be well tolerated and was not associated with any adverse renal or hepatic functions throughout the study period.

In a patient suffering from DM, along with coronary artery disease and hypertension receiving NS at 2,000-2,500 mg/day, acute renal failure was reported to have occurred 6 days after the start of treatment [74]. However, it was surmised that this adverse effect could not have been related to NS since other clinical trials with many more human subjects had demonstrated the safety of NS in higher doses over longer periods of intake and that the adverse effect was probably attributable to contamination of the tablets with other products [75].
In yet another study by Tubesha et al., the acute toxicity of a nanoemulsion preparation of Thymoquinone was studied in rats. A test dose limit of 20ml of thymoquinone-rich fraction nanoemulsion (TQRFNE) (containing 44.5 mg TQ)/kg. TQRFNE and distilled water (DW) as a control were administered orally to both sexes of rats on Day 0 and observed for 14 days. All the animals appeared normal and healthy throughout the study. There was no observed mortality or any signs of toxicity during the experimental period [64].

Ali M. Al-Amri et al. conducted a phase I safety and clinical activity study of thymoquinone in patients with advanced refractory malignant disease. Patients who were at least 18 years of age with an Eastern Cooperative Oncology Group performance status (ECOG) of </= 2 received thymoquinone orally at a starting dose level of 3, 7, or 10mg/kg/day. Dose escalation proceeded according to a modified Fibonacci design. All 21 patients received at least one week of treatment, with a median of 3.71 weeks (range 1 week to 20 weeks). No side effects were reported though the maximum tolerated dose (MDT) was not identified [45].

2.2.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

To our knowledge, this will be the first IND study of TQ Formula (Black Seed Oil) in patients with positive Covid-19 diagnosis. However, Black Seed Oil/Nigella Sativa has been studied extensively over many years, and has been found to be relatively safe, with very few side effects.

Furthermore, Black seed (Black Cumin or Nigella Sativa) has been categorized by FDA under *"Spices and other natural seasonings and flavorings that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act" Title 21, Chapter I, Subchapter B, Sec. 182.10 Spices and other natural seasonings and flavorings.*
Website: *https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=182.10*

The aim of this study is to provide a potential treatment for outpatients with a positive Covid-19 diagnosis, where the current standard of care (SOC) is limited to pain/symptom management such as acetaminophen. Any potential decrease in symptom longevity will benefit this population; and the potential benefits outweigh the minor risks in this case. In addition, this small, randomized phase II

study will have a minimal number of patients exposed to the investigational product, and for a minimum duration.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES/Purpose	ENDPOINTS/Outcome Measures	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate if treatment with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) given orally on outpatient basis can significantly reduce median time to sustained clinical response compared to placebo in participants with COVID-19 infection treated in the outpatient setting.	Measurement of the difference in median time to sustained clinical response in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo. Sustained clinical response is defined as a reduction of scores to </= 2 on all symptoms of the Modified FLU-PRO Plus.	A reduction in time to sustained clinical response is a direct measure of treatment effectiveness.
Secondary		
1. To compare the viral load profile over time (from baseline through to Day 14) between treatment with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) given orally on outpatient basis and placebo in participants with COVID-19 infection.	Measurement of change in quantitative viral load from baseline, Day-7, and Day-14 using RT-PCR in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo with COVID-19 infection.	Faster decline in viral load is hypothesized to lead to faster recovery from the illness and less infectivity [109, 110]

OBJECTIVES/Purpose	ENDPOINTS/Outcome Measures	JUSTIFICATION FOR ENDPOINTS

2. To compare the percentage of RTPCR negative/undetectable (i.e., viral clearance) on Day 7 and Day 14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo	Percentage of negative/undetectable RT-PCR (i.e., viral clearance) on Day-7 and Day-14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo	Treatment with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) given orally on outpatient basis is hypothesized to result in higher percentages of viral clearance by RT-PCR Faster decline in viral load is hypothesized to lead to faster recovery from the illness and less infectivity [109, 110]
3. To compare the duration and severity of symptoms (measured by Modified FLU-PRO Plus) overtime from Day 1 through Day 14 in total Modified FLU-PRO Plus symptom severity score overall and in sub-domain scores (namely, Nose, Throat, Eyes, Chest/Respiratory, Gastrointestinal, Body/Systemic, Taste/Smell) , between treatment with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) given orally on outpatient basis and placebo in participants with COVID-19 infection	Measurement of severity of and change in Covid-19 symptoms per total score as well as sub-scores (Nose, Throat, Eyes, Chest/Respiratory, Gastrointestinal, Body/Systemic, Taste/Smell) measured through Modified FLU-PRO Plus from Day-1 through Day-14 in participants with COVID-19 infection treated either with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) or placebo	FLU-PRO is a validated measure that has been used on multiple virus studies [111]. The Modified FLUPRO Plus version has additional questions (Taste/Smell) that are Covid-19 specific. The Modified FLU-PRO Plus has been shortened to reduce number of symptoms.
4. To investigate if there exists an association between viral load and symptom severity by study arm and if such associations change overtime	Correlation Coefficient of quantitative viral load and symptom severity at baseline, at Day-7, and Day-14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo	Decreases in viral load are hypothesized to be correlated with better clinical outcomes [109, 110].
5. To evaluate the safety and tolerability of TQ Formula (500 mg oral capsule, 3 capsules BID) when given to participants with COVID-19 infection.	Number of overall adverse events, related adverse reactions, and hospitalizations reported in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo. All AEs/SAEs will be captured throughout the study as per schedule of assessments.	Comparing the number of AEs and SAEs and any relationship to IP is important for determining the safety profile of TQ Formula.

OBJECTIVES/Purpose	ENDPOINTS/Outcome Measures	JUSTIFICATION FOR ENDPOINTS
Tertiary/Exploratory		
1. To evaluate the basic pharmacokinetics of TQ Formula main active ingredient (thymoquinone) at same time points (Days 1, 7, and 14) in participants with COVID-19 infection.	Measurement of thymoquinone and metabolites' concentration in the plasma on day 1, Day 7 and 14 using HPLC in patients treated with TQ Formula .	Thymoquinone is the main active ingredient of TQ Formula and the pharmacokinetics of thymoquinone and its' metabolites in the plasma of treated patients are used to correlate with effectiveness of treatment
2. To explore the effect of TQ Formula on inflammatory cytokines, coagulation factors and effector immune cells at same time points (Days 1, 7, and 14) in participants with COVID-19 infection.	Measurement of the inflammatory cytokine production, coagulation factors and the various effector immune cell subsets in the Peripheral Blood Mononuclear Cells (PBMC) of these patients on Day 1, Day 7 and Day 14 using FACS.	The inflammatory cytokines and immunological markers are of importance because of their correlation with disease severity in COVID19.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This clinical research trial aims to demonstrate the safety of TQ Formula , as well as preliminary efficacy in treatment of Covid-19. The hypothesis is that treatment with TQ Formula will significantly reduce median time to sustained clinical response, defined as a reduction of scores to </= 2 on all symptoms of the Modified FLU-PRO Plus, compared with placebo. In addition, it is hypothesized that treatment with TQ formula will significantly reduce viral load and symptom burden from baseline to Day-7 compared to treatment with placebo.

This is a randomized, double-blind, placebo-controlled phase II study to assess the safety and efficacy of TQ Formula versus placebo in the treatment of Covid-19 in an outpatient setting. Participants will be randomized 1:1 to receive either 3 g per day of TQ Formula or placebo (identical in appearance). The participants will receive up to 14 days of dosing.

Screening Visit

The following evaluations should be completed at the Screening visit:

Informed Consent

The purpose and procedures of the study will be fully explained to potential participants. Those wishing to enroll in the study will sign a written informed consent prior to initiating any study related evaluations or procedures.

Demographics
Participant demographics, including age, race and gender will be captured and documented in the source and the electronic case report form (eCRF).

Medical History
Participant medical history, including any surgical procedures, will be captured and documented in the source and the electronic case report form (eCRF).

Inclusion/Exclusion Criteria Evaluation
Participants will be assessed to ensure that they meet all inclusion criteria and do not meet any of the exclusion criteria as listed in sections 5.1 and 5.2

Covid-19 Rapid Test (Antigen)
Participants will be given a CLIA waived rapid antigen Covid-19 test (per site standard procedures) in order to obtain same days results to use for randomization of participants. The test may be performed prior to informed consent as part of standard of care at the clinic.

Concomitant Medication Review
All concomitant medications, including vitamins and supplements, currently being taken by participants will be documented in source and in the eCRF.

Physical Exam
A complete physical exam of the following systems will be performed by the investigator or appropriately qualified designee and documented in the source and in the eCRF at Screen, Day 7 and Day 14. A complete physical examination, performed at screening and other specified visits, should include an evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, respiratory, gastrointestinal, and neurologic systems. Any abnormality identified at baseline should be recorded in the patient's medical records. Limited, symptom-directed physical examinations should be performed at specified postbaseline visits and as clinically indicated. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events in the patient's medical records.

Vital Signs
Vital signs, including blood pressure (BP), temperature and heart rate will be collected and documented in source and in the eCRF.

Pulse and Walking Oximetry
SpO2 and 6-minute walk test (walking oximetry) will be performed at screening.

Height and Weight
Height and weight of patient will be taken at screening.

Laboratory Evaluations (Hematology and Serum Chemistry)
Blood samples (approximately 10 ml in total) will be taken for the following laboratory evaluations: Albumin, alkaline phosphatase, total bilirubin, direct bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, AST, ALT, sodium, CBC/diff, LDH, , CRP, prothrombin time, activated partial prothrombin time, D-dimer

Pregnancy Test
Females of child-bearing potential will have a serum pregnancy test completed.

Baseline Visit

The following evaluations should be completed at the Baseline visit:

Inclusion/Exclusion Criteria Evaluation
Participants will be re-assessed to ensure that they meet all inclusion criteria and do not meet any of the exclusion criteria as listed in sections 5.1 and 5.2

Vital Signs
Vital signs, including blood pressure (BP), temperature and heart rate will be collected and documented in source and in the eCRF.

Sample for quantitative viral load testing (RT-PCR) and confirmation of Covid-19 positive result Participants will have a sample taken for RT-PCR quantitative viral testing.

Serum Collection (Immune monitoring)
Blood samples (approximately 15 ml) will be collected for immune monitoring

Randomization
Participants will be randomized to receive either TQ Formula + Standard of Care (SOC) or placebo +SOC

Administer Study Intervention
Participants will be provided with study treatment kit, with enough capsules for 14 days of treatment, 3 capsules BID.

Concomitant Medications and Adverse Events Review
Participants will be assessed for any new adverse events and any changes in concomitant medications

Patient Reported Outcomes
Participants will receive the Modified FLU-PRO Plus questionnaire to complete on a daily basis for the duration of the study, from randomization to final study visit (Day 21).

Subsequent Study Visits (Including Day 21 Final Visit)

Concomitant Medications and Adverse Events Review

Participants will be assessed for any new adverse events and any changes in concomitant medications at each study visit

Physical Exam
A complete physical exam will be performed by the investigator or appropriately qualified designee and documented in the source and in the eCRF at Day 7 and Day 14. A complete physical examination, performed at screening and other specified visits, should include an evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, respiratory, gastrointestinal, and neurologic systems. Any abnormality identified at baseline should be recorded in the patient's medical records. Limited, symptom-directed physical examinations should be performed at specified postbaseline visits and as clinically indicated. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events in the patient's medical records.

Vital Signs
Vital signs, including blood pressure (BP), temperature and heart rate will be collected and documented in source and in the eCRF at each study visit.

Serum Collection (Blood sample for immune monitoring)
Blood samples (approximately 15 ml) will be collected for immune monitoring at Day 7 and Day 14, in addition to the baseline visit.

Sample for quantitative viral load testing (RT-PCR)
Participants will have a sample taken for RT-PCR quantitative viral testing at Day 7 and again at Day 14, in addition to the baseline visit.

Laboratory Evaluations (Hematology and Serum Chemistry)
Blood samples (approximately 10ml in total) will be taken for safety laboratory evaluations (as listed in screening above) at Day 7 and 14 and as clinically indicated at any other study visits

Patient Reported Outcomes
Participants will receive the Modified FLU-PRO Plus questionnaire to complete on a daily basis for the duration of the study, from randomization to final study visit (Day 21).

Safety Follow Up Phone Call (Day 45)

Adverse Events Review
Participants will be called to assess status for any ongoing adverse events and/or serious adverse events

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Blackseed oil has been shown to possess anti-inflammatory properties, and anti-corona virus properties, based on our extensive literature and our pre-clinical studies that showed its activity in blocking viral entry. See sections 2.2 and 2.3 for details.
This study design (study drug versus placebo) was chosen as there are currently no known outpatient treatments for Covid-19. Participants can maintain their regular standard of care, irrespective of treatment allocation, and are allowed to take any additional vitamins or supplements.

The randomization of participants will allow researchers to determine any significant differences between treatment groups. Participants will be blinded to their treatment, in order to remove any reporting bias on the patient reported outcome questionnaire.

4.3 JUSTIFICATION FOR DOSE AND FORMULATION

TQ Formula will be administered as an oral dose daily. The dose planned in this study is 3 g per day (3 500mg capsules to be taken twice daily). The dosing decision is based on the mechanism of action, the pharmacology in non-human studies (the drug has extensive pre-clinical pharmacokinetic and pharmacodynamic testing) and human data for completed studies across a number of therapeutic indications (see references in toxicity section above). The proposed dose of 3 g per day has been used in a number of human trials reporting side effects ranging from absence of any side effects to mild side effects; otherwise, the drug has been well tolerated. Mahdavi et al. conducted a double-blind placebocontrolled randomized clinical trial to determine the effects of NS oil combined with a calorie-restricted diet on systemic inflammatory biomarkers in obese women. 90 volunteer obese women were enrolled. The intervention group received 3 g/day NS oil soft gel capsules (one capsule, three times a day, 30 minutes before each main meal), and the placebo group received similar amounts of sunflower oil as a placebo for eight weeks. Subjects reported no side effects during the intervention except mild gastrointestinal problems [81]. In a study by Bamosa et al., patients with type 2 diabetes mellitus were randomly divided into 3 groups to receive Nigella Sativa seed extracts at 1 gm, 2 gms and 3 gms per day respectively for 12 weeks. In the study, generally, the three doses of Nigella sativa were well tolerated with only three patients who experienced a mild epigastric discomfort that settled down after taking the capsules post meals [68]. In a study by Datau et al. performed on 39 centrally obese men assigned to receive NS seeds (3 g/day for 3 months) or placebo, subjects reported subjective complaints related to central obesity (body ache, weakness, sleeplessness, belching, decreased libido). All subjective complains reported by subjects in the treatment group disappeared in the first week of the study, but they all still persisted in the control group through the end of the study. [65].

Enteric coated formulation
To mask the after taste/unpleasant taste of black seed oil in TQ Formula, enteric coating will be applied on the drug (and the placebo). Previous clinical studies using fish oil have shown enteric coating to be safe with the added benefit of improving compliance and enhancing gastric absorption by transient protection against gastric acidity [112].

Dosing Modifications
Based upon data from previous studies using blackseed oil capsules, the doses proposed in this protocol are within the maximum tolerated doses. However, dose reductions will be allowed per protocol as below (Table 4.3.1).

If a patient experiences a Grade 3 or greater drug-related AE after a dose of blackseed capsules upon evaluation per protocol, then the next dose will not be administered. The patient may receive additional doses of blackseed capsules, at the discretion of the PI, if the Grade 3 or greater event has resolved or has been reduced sufficiently as assessed by the treating PI prior to administration of the additional doses of blackseed capsules and dose reduction will be allowed as per Table 4.3.1 below.

Table 4.3.1: Dose modifications

Agent	Starting Dose (0 Dose Level)	-1 Dose Level	-2 Dose Level
Blackseed Oil Capsules/Placebo	500 mg, 3 capsules, orally twice a day (total 3g daily)	500 mg, 2 capsules, orally twice a day (total 2g daily)	500 mg, 1 capsule, orally twice a day (total 1g daily)

For Grade 3 or greater treatment related toxicity that cannot be controlled with symptomatic treatment within 48 hours, such as nausea and/or vomiting that can be controlled by antiemetics or diarrhea that can be controlled by anti-diarrheal medicines, the dose level will be decreased to dose level -1. Similarly, patients who develop Grade 3 treatment related toxicity that cannot be controlled with symptomatic treatment within 48 hours at dose level -1 will have further decrease in dosage to dose level -2. Finally, patients who develop Grade 3 treatment related toxicity that cannot be controlled with symptomatic treatment within 48 hours at dose level -2 will discontinue therapy. If the patient experiences significant toxicity requiring a dose reduction, the patient will remain at the lower dose level for subsequent cycles, i.e., dose re escalation is not allowed.
Patients should terminate study treatment if further reduction is indicated beyond the –2 level.
Treatment may be delayed up to 10 days to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 10 day delay, all study treatment will be discontinued. Participant will remain on study for safety follow up per schedule of assessments.

Resuming therapy may not be allowed until treatment-related toxicities have resolved to baseline, unless the toxicities are considered by the Investigator unlikely to develop into serious or lifethreatening events. In this case, treatment may be continued at the same dose without reduction or interruption.

4.4 END OF STUDY DEFINITION

A participant is considered to have successfully completed the study if he or she has completed all phases of the study including the last visit shown in the Schedule of Activities (SoA), Section 1.3. An end of study form will be completed in EDC. Participants who terminate early will also have an end of study form completed.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

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

1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged 18 and over, presenting with recent mild to moderate clinical symptoms of Covid-19 infection (per FDA guidance – see appendix 3)
4. Positive COVID-19 infection confirmed with a rapid antigen test at screening and confirmed with a RT-PCR test at baseline
5. A score of ≥3 on a minimum of 2 symptoms on the Modified FLU-PRO Plus
6. Ability to take oral medication and be willing to adhere to the dosing regimen (Twice a day – BID for 14 days)
7. For females of reproductive potential: negative pregnancy test at screening and use of highly effective contraception method during study participation and for an additional 4 weeks after the end of study drug administration
8. For males of reproductive potential: use of condoms or other methods to ensure effective contraception with partner during study participation
9. Agreement to adhere to Lifestyle Considerations (see section 5.3) throughout study duration

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current or recent (within 4 weeks) treatment with any corticosteroids; however, high-dose inhaled steroids, which are used to treat acute or chronic bronchial inflammation, will be permitted
2. Current or recent (within 4 weeks) treatment with any antivirals
3. Room air oxygen saturation (SaO2) < 94% at screen
4. Walking oximetry < 90% or participant unable to complete 6-minute walking oximetry test at screen
5. Severe Covid-19 symptoms (severe per FDA classification – see appendix 3)
6. Requires immediate admission to hospital for any reason
7. Pregnancy or lactation
8. Known allergic reactions to components of black seed oil, or thymoquinone
9. Treatment with another investigational drug or other investigational intervention within 2 weeks of study start and throughout study duration.
10. Significant hepatic disease (ALT/AST> 4 times the ULN); any laboratory parameter >/= 4 times the ULN
11. History of severe chronic kidney disease or history of liver disease

12. Patients with inflammatory bowel disease (such as Crohn's) that could affect the intestinal absorption of TQ Formula enteric coated capsules.
13. Known HIV or Hepatitis C infection
14. Influenza diagnosis (confirmed by testing) during screening or within prior 14 days
15. Any uncontrolled condition(s) or diagnosis, both physical or psychological, or physical exam finding that precludes participation, as per investigator

5.3 LIFESTYLE CONSIDERATIONS

It is recommended that doses be taken with food, approximately 12 hours apart. Participants may continue with their normal standard of care, including any supplements or vitamins.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an initial negative Covid-19 test may be rescreened. Rescreened participants should be assigned a new participant number, with a source note indicating that the participant is being re-screened.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Participants will be identified through Covid-19 screening tests at participating study sites and potential referral centers. Individuals who test positive for Covid-19 will be contacted and asked if they wish to participate in a research study. Upon signing of the informed consent, each patient will undergo screening procedures to determine eligibility for the study. Each study site may develop a site-specific recruitment plan if required.

Up to 60 participants will be equally randomized to receive either TQ Formula 3g (3x 500 mg capsules, BID) or matching placebo capsules (See section 9.2 for sample size determination details). There will be between 2-4 US sites participating in this study. The sites will be outpatient clinics that are currently testing/treating Covid-19 patients.

It is anticipated that each site will recruit approximately 4-5 participants per week. Based on this anticipated accrual rate, 2 sites should be able to complete recruitment for this study within approximately 4-5 weeks.

Participants will be compensated a reasonable amount for their study time and out of pocket costs such as parking, transportation or food on visit days will be reimbursed.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The study intervention is TQ Formula capsules, 500 mg per capsule. The control product is placebo capsules, identical in appearance to TQ Formula capsules but with no active ingredients.

Black seed oil is currently available as a food supplement, with 500 mg capsules being sold over the counter. For the purposes of this trial, both TQ Formula and placebo capsules will be manufactured as over-encapsulated capsules. A specific batch and lot will be produced, with appropriate stability testing.

6.1.2 DOSING AND ADMINISTRATION

All study participants will receive the same dose of either TQ Formula (total dosing of 3 g per day) or placebo for the duration of the study. Study participants should take 3 capsules orally (500 mg each), approximately 12 hours apart, two times per day (BID). Each dose should be taken preferably with food. Missed doses will not be made up; however, the window for dosing is +/- 6 hours. If longer than 6 hours since missed dose, participants should continue with next scheduled dose. There are no dose limiting effects anticipated with this investigational drug.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

A central IP depot will provide the pre-labelled, pre-packaged investigational product to the study sites. Sites will receive an initial shipment and possibly a second shipment (if required). All used, unused and/or expired product will be returned to the IP depot or destroyed onsite if study site meets regulatory requirements for destruction and can provide appropriate documentation for sponsor.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The investigational drug consists of 500 mg of black seed oil per capsule. Placebo consists of similar oil without active ingredient. Investigational drug will be undistinguishable from placebo; the appearance of the capsules and all packaging will be identical. Investigational product will be packaged as 500 mg/placebo capsules; each participant will receive a total of 84 capsules in order to take 6 capsules per day for the 14-day study treatment period. Investigational product will be labelled per CFR regulations.

6.2.3 PRODUCT STORAGE AND STABILITY

Both study and placebo product capsules should be stored at ambient temperature (15°C to 25°C) and protected from sunlight exposure. Investigational product should be stored in a locked, limited-access room. Only designated study personnel should dispense investigational product.

6.2.4 PREPARATION

There is no preparation of study or placebo product required by the study site. All pre-packaged and pre-labelled kits with appropriate number of capsules for 14 days of dosing for each patient are provided to the sites. Sites will be provided with a randomization number for each subject that will correspond to a kit number on site.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Study participants will be randomized in a one to one fashion (1:1) to receive either 3 g/day of TQ Formula (500 mg capsules x 3, BID) or placebo capsules (identical in appearance to active product) for 14 days. Participants will receive the full study supply (14-day medication) at randomization. Study capsules should be stored at ambient temperature (15°C to 25°C), away from direct sunlight.

The study is double blinded; all subjects, investigators, and study personnel involved in the conduct of the study, including data management and primary biostatistician, will be blinded to treatment assignment. There will be an independent unblinded statistician . The unblinded study statistician will not otherwise participate in study procedures or data analysis.

A randomization list will be prepared by the unblinded statistician and provided to the investigational product (IP) depot for labelling. Sponsor and CRO study staff will remain blinded to study treatment. Randomization numbers will be assigned through the Electronic Data Capture (EDC) system.

Participants will complete a daily questionnaire on Covid-19 symptoms, which will be used to determine the primary endpoint for the study. For this reason, it is important that participants remain blinded to treatment allocation. Participants will, however, have access to their viral test results after Day 14, if a test is completed as part of standard of care.

Unblinding
In the unlikely event that unblinding is required, the principal investigator can contact the unblinded statistician to unblind treatment. Treatment unblinding is discouraged if knowledge of the treatment assignment will not materially change the planned management of a medical emergency.
Unblinding should be discussed in advance with the medical monitor if possible. If the investigator is not able to discuss treatment unblinding in advance, then he/she must notify the medical monitor as soon as possible about the unblinding incident without revealing the participant's treatment assignment.

6.4 STUDY INTERVENTION COMPLIANCE

Investigators will assess compliance to study treatment dosing during each visit with participants. In addition, participants will be asked to return all investigational product with them to each on-site visit for compliance verification. Investigator or designee will perform IP accountability and document on appropriate study logs and source documentation on site. Patient compliance must remain between 80% and 120% throughout the course of the study. Patients who miss more than 6 consecutive doses will be labelled as non-compliant and will not be included in the per protocol population. These patients will be included in the safety analyses.

6.5 CONCOMITANT AND PROHIBITED THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Electronic Case Report Form (eCRF) are concomitant prescription medications, over-the-counter medications and supplements.

Concomitant medication usage will be collected at screening and throughout the course of the study at each study visit. Any medication currently being taken at the time of screening will be recorded at the screening visit. Any changes in concomitant medication usage will be documented throughout the course of the study during study visits.

All standard of care medication to treat Covid-19 is allowed per investigator discretion.

Prohibited Medications

All standard of care medication is allowed during the course of the clinical trial. The use of any drugs approved to treat severe Covid-19 requiring hospitalization, such as: corticosteroids, antivirals, Hydroxychloroquine is prohibited for entry into the study. The use of these medications is considered inpatient regimen for severe Covid-19, and these patients are excluded from this trial. If any participant requires these medications due to worsening of Covid-19 symptoms, study treatment will be stopped and participant will continue to be followed for safety (See Appendix 4).

Investigators should carefully assess patients who are currently being treated with any drugs that are metabolized by CYP2C9 due to potential for interaction with Thymoquinone (See Appendix 5).

6.5.1 RESCUE MEDICINE

This section is not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

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

The data to be collected at the time of study intervention discontinuation will include the following:

- Reason for discontinuation
- Adverse events
- Changes to concomitant medications

If a participant discontinues investigational product, all study visits will continue as per the Schedule of Assessments (SOA).

Participants who are randomized but who subsequently have a negative RT-PCR Covid-19 test, will discontinue investigational product, but will continue to be followed up for safety and efficacy.

7.2 PARTICIPANT TREATMENT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

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

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

The reason for participant treatment discontinuation or withdrawal from the study will be recorded in the source documents and on the Electronic Case Report Form (eCRF).

For any participants who withdraw from the study, all attempts should be made to ensure that a safety follow up visit is completed (Day 21 procedures).

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to attend 3 consecutive scheduled visits (either telehealth or in person) and is unable to be contacted by the study site staff.

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

- The site will attempt to contact the participant and reschedule the missed visit within 1 day and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

Administrative Procedures
Informed consent
Inclusion/exclusion criteria
Demographics and medical history
Prior and concomitant medication review
Randomization and Study intervention
Modified FLU-PRO Plus Questionnaire
Complete case report forms
Clinical Procedures/Assessments
Review adverse events and concomitant medications
Physical examination
Height, weight, and vital signs (T, P, RR, BP)
Pulse and Walking Oximetry
LOCAL Laboratory Assessments
Covid-19 Rapid Antigen Test
Pregnancy test (only for females of child-bearing potential)
Hematology (CBC with differential)
Chemistry panel
CENTRAL Laboratory Assessments
RT-PCR sample for quantitative viral load analysis
Whole blood for biomarker/immune studies (serum and plasma)

8.1 EFFICACY ASSESSMENTS

Covid-19 Quantitative Viral Load (RT-PCR)
Participants will have an RT-PCR sample taken at baseline, day 7 and day 14 to measure quantitative viral load and viral load clearance. These samples will be sent to a central laboratory for analysis.

Patient Reported Outcomes
Participants will receive the Modified FLU-PRO Plus questionnaire (Appendix 2) to complete on a daily basis for the duration of the study, from randomization to final study visit. The responses will be used to measure severity and change in Covid-19 symptoms from baseline.

Serum Collection (Blood sample for immune monitoring)
Participants will have blood collected for immune monitoring. This testing will explore pharmacokinetics and inflammatory cytokines, coagulation factors and effector immune cells in samples. These samples will be sent to a central laboratory for analysis.

8.2 SAFETY AND OTHER ASSESSMENTS

Physical Exam
A complete physical exam of the following systems will be performed by the investigator or appropriately qualified designee and documented in the source and in the eCRF at Screening, Day 7 and Day 14. A complete physical examination, performed at screening and other specified visits, should include an evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, respiratory, gastrointestinal, and neurologic systems.

Vital Signs
Vital signs, including blood pressure (BP), temperature, respiratory rate and heart rate will be collected throughout the course of the study.

Laboratory Evaluations (Hematology and Serum Chemistry)
The following laboratory evaluations will be tested locally at screening, Day 7 and Day 14: Albumin, total protein, alkaline phosphatase, total bilirubin, direct bilirubin, ALT, AST, sodium, potassium, chloride, bicarbonate, BUN, creatinine, phosphorus, calcium, glucose, CBC/diff, LDH, CRP, prothrombin time, activated partial prothrombin time, D-dimer. In the event of elevated liver enzymes, please see section 8.5 for the drug induced liver injury plan.

Adverse Event Review and Evaluation
Participants will be assessed for any new adverse events throughout the course of the study. Participants will be encouraged to contact the study site immediately if they observe any worsening symptoms or health concerns.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

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

For this study, adverse events will be captured from the time of informed consent until 7 days after last dose of investigational product (30 days after last dose for SAEs)

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

SAEs will be captured from the time of informed consent until 30 days after the last dose of investigational product. For information on SAE reporting and associated procedures and forms, refer to the study Safety Plan.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

All AEs will be assessed for severity by the investigator or designated sub-investigator. The severity of all AEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v. 5.0)
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

For adverse events (AEs) not included in the CTCAE, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.

- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

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

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship possible, but improbable, and in which other drugs or chemicals or underlying disease may provide plausible explanations. There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication) or a temporal relationship cannot be ruled out. However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events).
  Note: Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Not Related** – The AE is completely independent of study intervention administration (e.g., the event did not occur within a reasonable time after administration of the study intervention), and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 EXPECTEDNESS

The principal investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected; however, sponsor will make the final determination for reporting purposes to regulatory

authorities. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate electronic case report form (eCRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline medical history and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as a new AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset, severity, and duration of each episode.

The principal investigator or designee will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last dose of study drug. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization. A final follow-up phone call will be performed at Day 45 in order to assess status of any ongoing AEs or SAEs. Any AEs that are considered unresolved at Day 45 will be followed clinically by the investigator until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

<u>Adverse Event</u>

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution. This includes the following:

1. AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms that were not present prior to the AE reporting period
2. Pre-existing medical conditions judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period

3. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms and are considered clinically significant

8.3.6 SERIOUS ADVERSE EVENT REPORTING

Generally, any AE considered serious by the PI or Sub-investigator or which meets the definition of an SAE included in **Section 8.3.2, Definition of Serious Adverse Events** must be submitted on an SAE form to the Tranquil Clinical Research pharmacovigilance group (PVG).

The principal investigator or designee must immediately report to the sponsor any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event [21 CFR 312.64(b)].

Upon identification, all SAEs will be reported by the site within 24 hours using the Initial SAE Report Form. The Initial SAE Report Form should be submitted Tranquil Clinical Research (CRO) within 24 hours either by e-mail (preferable) or by phone using the following contact information:

Email : *Safety@tranquilconsulting.com* Phone
: +1 713-898-2137

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

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor becomes aware and determines that the information qualifies for reporting.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Participants will be made aware of any significant changes in safety information that results in a change to the informed consent form. All study participants will be re-consented with the new form as soon as possible.

8.3.8 EVENTS OF SPECIAL INTEREST

This section is not applicable.

8.3.9 REPORTING OF PREGNANCY

In the event that a participant becomes pregnant while on study, study treatment will be discontinued, and a pregnancy form will be completed.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- 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.

An incident, experience, or outcome that meets the definition of an UP noted above may warrant consideration of changes to the protocol or consent in order to protect the safety, welfare, or rights of participants or others.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Sponsor/CRO. The UP report will include the following information:

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

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

- UPs that are serious adverse events (SAEs) will be reported according to the timelines noted in section 8.3.6.
- Any other UP will be reported to the IRB (per IRB policy) and to the CRO/study sponsor within a reasonable time period of the investigator becoming aware of the problem.

- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) as applicable, within the appropriate timeline (per institution policy) of the IRB's receipt of the report of the problem from the investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

If an unanticipated problem results in a change to the informed consent form, all study participants will be provided with the updated information and re-consented with the new form.

8.5 DRUG INDUCED LIVER INJURY PLAN

If patient reports symptoms compatible with DILI (early and nonspecific symptoms include anorexia, nausea, fatigue, right upper abdominal discomfort, vomiting), liver enzyme measurements should be made immediately, regardless of when the next visit or monitoring interval is scheduled.

As part of the study screening and monitoring criteria, liver enzymes are assessed at screening, Day 7 (Study Visit 3) and Day 14 (Study Visit 5). Finding of the following will require further follow up:

Finding of 3-fold or greater elevations above the ULN of ALT or AST in subjects with normal values before drug exposure OR

2-fold increases of ALT or AST above baseline values for subjects with AT values between 3 and 4 times ULN before drug exposure

→ Repeat testing within 48 to 72 hours of all four of the usual serum measures (ALT, AST, ALP, and TBL) to confirm the abnormalities and to determine if they are increasing or decreasing values between 3 and 4 times ULN before drug

If symptoms persist or repeat testing shows AT >3xULN for subjects with normal baseline measures or 2fold increases above baseline values for subjects with elevated values before drug exposure, it is appropriate to initiate close observation to determine whether the abnormalities are improving or worsening as follows:

Repeating liver enzyme and serum bilirubin tests two or three times weekly.

→ Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic.

- Obtaining a more detailed history of symptoms and prior or concurrent diseases.
- Obtaining a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; NASH (Non-alcoholic steatohepatitis); hypoxic/ischemic hepatopathy; and biliary tract disease.
- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
- Considering gastroenterology or hepatology consultations

Discontinuation of treatment will be considered if:

- ALT or AST >8xULN
- ALT or AST >5xULN for more than 2 weeks
- ALT or AST >3xULN and (TBL >2xULN or INR >1.5)
- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

9 STATISTICAL CONSIDERATIONS

A separate Statistical Analysis Plan will be written to describe the statistical analysis procedures addressing each primary, secondary and exploratory aims in detail.

9.1 STATISTICAL HYPOTHESES

The primary statistical hypotheses are:

1. Treatment with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) given orally on outpatient basis (study drug) will significantly reduce median time to sustained clinical response compared to placebo in participants with COVID-19 infection treated in the outpatient setting. Sustained clinical response is defined as a reduction of scores to </= 2 on all symptoms of the Modified FLU-PRO Plus.

The secondary statistical hypotheses are as follows:

1. Study drug will significantly reduce quantitative viral load by Day-14 compared to placebo in participants with COVID-19 infection treated in the outpatient setting.

2. Study drug will significantly increase the percentage of negative/undetectable RT-PCR viral load at Day 7 and Day 14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo in participants with COVID-19 infection

3. Study drug will significantly reduce duration and severity of symptoms overall and within each symptom domain overtime from Day 1 to Day 14 compared to placebo in participants with COVID-19 infection

4. There is a significantly positive correlation between quantitative viral load and symptom severity at baseline, at Day 7, and Day 14 in patients with COVID-19 infection

5. Study drug will lead to acceptable AE and SAE profiles compared to placebo

- Primary Efficacy Endpoint(s):

1. Measurement of the difference in median time to sustained clinical response in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo.

- Secondary Efficacy Endpoint(s):

1. Measurement of change in quantitative viral load from baseline, Day 7, and Day 14 using RTPCR in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo with COVID-19 infection.

2. Percentage of negative/undetectable RT-PCR (i.e., viral clearance) on Day-7 and Day-14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo

3. Measurement of severity of and change in Covid-19 symptoms per total score as well as sub-scores (Nose, Throat, Eyes, Chest/Respiratory, Gastrointestinal, Body/Systemic, Taste/Smell) measured through Modified FLU-PRO Plus from Day 1 through Day 14 in participants with COVID-19 infection treated either with 3 g TQ Formula (500 mg per capsule, 3 capsules BID) or placebo

4. Correlation Coefficient of quantitative viral load and symptom severity at baseline, at Day 7, and Day 14 in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo

5. Number of overall adverse events, related adverse reactions, and hospitalizations reported in participants taking 3 g TQ Formula (500 mg per capsule, 3 capsules BID) versus participants taking placebo. All AEs/SAEs will be captured throughout the study as per schedule of assessments.

9.2 SAMPLE SIZE DETERMINATION

For statistical analysis purposes, sustained clinical response is defined as a reduction to a score of </= 2 on all symptom scores on the Modified FLU-PRO Plus questionnaire.

Based on the median time to sustained clinical response reported by CDC [122], the expected median time to response in the placebo arm will be approximately 16 days from the onset of symptoms in outpatients. With a sample size of 20 in each arm, a difference in median time to sustained clinical response of 10 days between the intervention arm and the placebo arm will be able to be detected with 81.5% statistical power with 5% Type-1 error rate. Median time to sustained clinical response will be estimated based on 21-day follow-up. With an attrition expectation of around 20%, the planned accrual is 26 patients in each arm and potentially up to 30 patients.

Patients will be randomly assigned to one of the two study arms, using a block-randomization with a block size of 4, stratified by the enrolling sites. No other stratifications will be used. Randomization block size will be kept fixed at 4 as this is a small study and making the block size random as well is not needed. An unblinded statistician will generate a randomization list.

Definition of Evaluability for Primary Objectives: The double blind, placebo controlled two-arm clinical trial is designed to enroll up to 60 patients equally randomized between the two arms of the study. An evaluable patient is defined as a patient who has received at least one dose of the intervention drug or placebo.

9.3 POPULATIONS FOR ANALYSES

- Full Analysis Dataset: The Full Analysis Set (FAS) is defined as all randomized participants who receive at least one dose of study intervention.
- Final Dataset for Primary Objectives (FDPO): FDPO is defined as all evaluable participants according to the evaluability definition above.
- Safety Analysis Dataset: The Safety Analysis Set is defined as participants who take at least one dose of study intervention.
- Per-Protocol Analysis Dataset: The Per-Protocol Analysis Set (PP) is defined as all participants in the FAS who complete all visits up to and including Day 14, are compliant with study medication and do not have any major protocol violation. Major protocol violations will be identified prior to breaking the blind.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Categorical variables will be presented as frequencies and percentages with column and row percentages in 2x2 or RxC tables as appropriate. Association between two nominal variables will be investigated using Chi-Square tests and/or Fisher's Exact test as needed. Cochran Armitage trend-test may be included among the possible tests to investigate the possible trend between one ordinal factor and one binary factor. Association between two ordinal variables may be investigated through Non-zero Correlation test. Continuous variables will be presented as mean and standard deviation as well as five-number summary (minimum, first quartile, median, third quartile, and maximum). Range and inter-quartile range may also be explicitly provided as needed. Distribution of a continuous variable among the levels of a binary or polynomial factor will be investigated graphically through boxplots and through Wilcoxon-MannWhitney test and/or Kruskal Wallis tests. Association between two continuous variables will be described through scatter plots and Pearson's and Spearman's correlation coefficients as appropriate. When needed, normality assumption of a given continuous variable will be tested using Shapiro-Wilk Normality test. The details of the statistical models addressing each and every primary, secondary, and exploratory objectives along with primary predictors and control variables will be provided in a corresponding Statistical Analysis Plan (SAP). In this protocol document, a general analysis and statistical testing approach will be provided, and the details will be left for the study SAP.

In all comparisons and tests, a two-sided p-value will be used and a Type-1 error rate of 0.05 will be used to indicate significance. No multiplicity correction will be employed for statistical tests addressing the two primary objectives. Similarly, no multiplicity correction will be employed for statistical tests addressing the secondary and exploratory objectives as the results from these objectives must be considered within the hypotheses generating context.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary endpoint of this clinical trial is the estimate of the median time to sustained clinical response for the treatment and placebo arms. Kaplan-Meier method will be used to estimate the time to sustained clinical response distribution and log-rank test will be utilized to compare the time to sustained clinical response distributions between the two study arms. Events will be defined as cases that attain clinical response while on the follow-up window and time to event will be calculated as the days from symptom onset to the resolution of symptoms to zero based on the Modified FLU-PRO Plus questionnaire. Patients who do not attain to clinical response while on the follow-up window will be censored on Day-21 or on the day they are off study before Day-21 due to other reasons.
Although all analyses will be conducted based on the intent-to-treat context, sensitivity analyses based on the adherence to the protocol regimen will also be carried out for the primary as well as secondary objectives.
When appropriate, Last-Value-Carried-Forward (LVCF) imputation approach will be utilized for the missing values. As this is a small study that has a short span of enrollment and treatment windows, we do not anticipate excessive missing values; however, missingness structure will still be analyzed as to whether or not the missingness is Missing Completely at Random (MCAR), or Missing at Random (MAR), or Not-Missing at Random (NMAR). In the even of MCAR and MAR, sensitivity analyses will be carried out by using Multiple Imputation (MI) techniques.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

None of the following endpoints depend on the findings of the primary endpoint.
The first secondary endpoint of this clinical trial is the differential change of viral load distribution overtime between the two-study arm. As the viral load will be measured at Baseline, on Day-7, and on Day-14 (and potentially for some patients in Day-4 and Day-10 visits), the outcome variable of interest will be a longitudinal interval-scale measure. To assess the differential behaviour of viral load overtime between the two study arms, Random Coefficients Models will be utilized where viral load will be the dependent variable and study arm and time will be main predictors. Viral load measures may be subjected to some data transformation (e.g., log transformation) to improve the model fit and assumptions as necessitated by model diagnostics. In addressing this objective, all available data will be utilized; that is, in addition to the evaluable patients, data from non-evaluable patients such as data only at baseline visit or data at baseline and Day-10 for example will be used to improve the local fit of the underlying model. To assess differential behavior in total symptom severity score, time*study-arm interaction will be investigated as well.

The percentages of RT-PCR negative/undetectable viral loads between the two study arms will be compared using Fisher's Exact test at Day-7 and Day-14 visits.

The third secondary endpoint of this clinical trial is the differential change of total and subdomain symptom severity score measured by Modified FLU-PRO Plus overtime between the two-study arm. As the total and subdomain symptom severity score will be measured at Baseline (Day-1) and on each day throughDay-14, the outcome variables of interest will be a longitudinal interval-scale measures. To assess the differential behaviour of total and subdomain symptom scores overtime between the two study arms, Random Coefficients Models will be utilized where total and each of the subdomain symptom severity score will be the dependent variables and study arm and time will be main predictors. Total and subdomain symptom severity scores may be subjected to some data transformation (e.g., log transformation) to improve the model fit and assumptions as necessitated by model diagnostics. In addressing this objective, all available data will be utilized; that is, in addition to the evaluable patients, data from non-evaluable patients such as data only at baseline visit or data just at baseline and Day-10 for example will be used to improve the local fit of the underlying model. To assess differential behavior in total symptom severity score, time*study-arm interaction will be investigated as well. When scientifically indicated, aggregated symptom severity scores may be defined through combining a set of subdomains such as Nose, Eyes. And Throat.

Association between quantitative viral load and symptom severity will be investigated through regression models, where total symptom severity score will be the dependent variable predicted by the quantitative viral load measures in the model at each visit. Study-Arm*viral load interaction will be added to the model to investigate if any association between viral load and symptom severity is mitigated by the study treatment.

Last but not the least secondary endpoint is the comparison of Adverse Events (AE) and Serious Adverse Events (AE) between the two study arms. AEs and SAEs are nominal variables described by grade and attribution to the protocol therapy. For the presentation purposes, we will define 'Toxicity' as an AE with at least possible attribution of the therapy. Summary tables of frequencies and percentages of AEs will be generated as number of episodes within number of patients (N-episodes/N-subjects) by grade and attribution. When frequencies allow, percentages of patients experiencing an AE, or a set of AEs will be compared between the two arms using Fisher's Exact test. In doing so, some aggregation can be done at the grade level such as 'Grade 3+' AEs or categories along these lines. Such comparisons will be done for toxicities of interest as well.

Analysis details for the exploratory aims will be provided in the study SAP.

9.4.4 SAFETY ANALYSES

Although this is not a dose-escalation trial, the following safety monitoring scheme will be employed in the initial enrollment phase of the trial:
The safety monitoring phase of the study will consist of up to 12 patients, whose adverse event profiles will be monitored continuously. If two or less number of patients in this initial cohort of 12 patients experience Grade 3+ adverse events attributable to the study regimen and not resolved through symptomatic treatments within 48 hours, the safety monitoring phase will be considered complete and the enrollment will continue. If three or more patients in this initial cohort of 12 patients experience Grade 3+ adverse events attributable to the study regimen and not resolved through symptomatic treatments within 48 hours, then the enrollment will be halted to discuss the safety measure of the protocol and possible amendments will be developed to address the safety concerns. As the adverse event profiles of the patients in this safety monitoring phase are followed closely, the safety monitoring

phase will be halted once the number of patients with Grade 3+ adverse events attributable to the study regimen and not resolved through symptomatic treatments within 48 hours go beyond two cases; therefore, the patient enrollment may be halted for safety evaluation before enrolling 12 patients. The above safety monitoring rules will be employed regardless of patient evaluability for the primary endpoints; however, to be included in the safety monitoring, it will be required that the patient received at least one dose of the study regimen and attribution of a given adverse event to the study regiment is clearly established as 'at least possibly attributable to the study regimen'.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

9.4.6 PLANNED INTERIM ANALYSES

This section is not applicable for the analysis of the primary and secondary objectives; however, there will be safety interim analyses after the initial cohort of 12 patients have been enrolled and have received at least one dose of the assigned drug.

9.4.7 SUB-GROUP ANALYSES

In analyses for primary and secondary endpoints, age, gender, race and other demographics and clinical factors and markers may be used as control variables as appropriate; however, the statistical design of this study does not include any planned subgroup analyses. All subgroup analyses if found appropriate will be considered exploratory to generate hypotheses for future prospective studies.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Patient specific data will be presented by study arm, including patient demographics and baseline clinical factors and measures.

9.4.9 EXPLORATORY ANALYSES

The study includes exploratory objectives for the PK profile of the study drug and measurement of the immune response through cytokine production, coagulation factors and various effector immune cell subsets in the PBMC of these patients on Day 1, Day 7 and 14 using FACS. The details of the analytic approaches for these exploratory objectives will be shared in the study SAP. Analyses to address these two exploratory aims will be detailed in the study SAP.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

In obtaining and documenting informed consent, the investigator must comply with applicable regulatory requirements (e.g., 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56) and should adhere to ICH GCP.

Prior to the beginning of the trial, the investigator should have the IRB's written approval for the protocol and the written informed consent form(s) and any other written information to be provided to the participants.

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

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, the Investigational New Drug (IND) sponsor and regulatory authorities.

If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for

the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

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

- Determination of unexpected, significant, or unacceptable risk to participants i.e.- If three or more patients in the initial cohort of 12 patients experience Grade 3+ adverse events attributable to the study regimen and not resolved through symptomatic treatments within 48 hours, then the enrollment will be halted to discuss the safety measure of the protocol and possible amendments will be developed to address the safety concerns.
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

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

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

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

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

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

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Samples collected for this study will be analyzed and stored at an immune monitoring laboratory. After the study is completed, the de-identified, archived data will be transmitted to and stored by the study Sponsor for use by other researchers including those outside of the study. Permission to transmit samples to the immune monitoring laboratory will be included in the informed consent.

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

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator (s)	**Medical Monitor**
Nahla Salem, MD *Hassan Bencheqroun, MD*	*Emmanuel Iyoha, MD* *Tranquil Clinical Research* E-mail: emmanueli@tranquilconsulting.com

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of the medical monitor and the principal investigators at the sites. A Data and Safety Monitoring Board (DSMB) will not be used for this study, due to the short recruitment/treatment periods and the pre-established safety profile for Black Seed Oil.

10.1.7 CLINICAL MONITORING

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

- Monitoring for this study will be performed by Tranquil Clinical Research.
- *On-site monitoring visits will occur at pre-defined intervals. In the event that on-site monitoring is prohibited, remote monitoring will be performed at the same intervals. Comprehensive 100% source data verification will be performed by the clinical research associates (CRAs) or designees.*

- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. When appropriate, Last-Value-Carried-Forward (LVCF) imputation approach will be utilized for the missing values. As this is a small study that has a short span of enrollment and treatment windows, we do not anticipate excessive missing values; however, missingness structure will still be analyzed as to whether or not the missingness is Missing Completely at Random (MCAR), or Missing at Random (MAR), or Not-Missing at Random (NMAR). In the even of MCAR and MAR, sensitivity analyses will be carried out by using Multiple Imputation (MI) techniques.

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

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

It is recommended that each site have SOPs for quality management. Some of the SOPs may describe the following:

- How data and biological specimens (when applicable) will be evaluated for compliance with the protocol, ethical standards, regulatory compliance, and accuracy in relation to source documents. • The documents to be reviewed (e.g., CRFs, clinic notes, product accountability records, specimen tracking logs, questionnaires, audio or video recordings), who is responsible, and the frequency for reviews.
- Who will be responsible for addressing QA issues (e.g., correcting procedures that are not in compliance with protocol) and QC issues (e.g., correcting errors in data entry).
- Staff training methods and how such training will be tracked.
- If applicable, calibration exercises conducted prior to and during the study to train examiners and maintain acceptable intra- and inter-examiner agreement.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH GCP and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of the sponsor, CRO, and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity. Describe in this section who will have access to records.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Electronic source data are data initially recorded in electronic form. Examples of source data include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, participants' memory aids or evaluation checklists, pharmacy dispensing records, audio recordings of counseling sessions, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

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

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Source data should meet ALCOAC standards: attributable, legible, contemporaneous, original, accurate and complete.

Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including but not limited to patient demographics, vital signs, medical history, adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data as per the schedule of assessments will be entered into a 21 CFR Part 11-compliant electronic data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

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

These practices are consistent with ICH GCP:

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

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations in a timely manner and when possible in advance of the scheduled protocol-required activity.
All deviations must be addressed in study source documents and reported to site CRA and/or sponsor. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the monitoring and/or project plans.

There will be no waivers for protocol deviations granted for this study.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

This trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 5 years after the completion of the primary endpoint by contacting the sponsor.

The sponsor will be responsible for developing publication procedures and resolving authorship issues. Please refer to your specific contract, grant, and/or Clinical Trials Agreements.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed.

Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

This section is not applicable.

10.3 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FAS	Full Analysis Set
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDPO	Final Dataset for Primary Objectives
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
LCVF	Last-Value-Carried-Forward
MAR	Missing at Random

MCAR	Missing Completely at Random
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple Imputation
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NMAR	Not-Missing at Random
OHRP	Office for Human Research Protections
NMAR	Not-Missing at Random
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RT-PCR	Reverse transcription polymerase chain reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	Standard of Care
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.0	13-Nov-2020	Initial Final Version	
2.0		Primary endpoint changed per FDA recommendations Influenza test and RT-PCR test at screen removed to reduce patient burden Replacement of patients removed per FDA recommendation Modified FLU-PRO Plus - modified to reduce the number of symptoms and ensure that the scale is consistent across all symptoms and to reduce patient burden Drug Induced Liver Injury plan added	Changes per FDA Pre-IND response recommendations

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12 APPENDIX 1 – COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (CTCAE) V5.0

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

CTCAE_v5_Quick_Reference_5x7.pdf

13 APPENDIX 2 – MODIFIED FLU-PRO PLUS©QUESTIONNAIRE

Participant ID: _____ **Participant Initials:**_____ **Date:**____/____/____

Modified FLU-PRO Plus©

People experience Covid-19 in different ways. We would like to know about the symptoms you have been experiencing during the past 24 hours. For each symptom, please mark one box ☐ under the response that best matches your experience. Mark the "Not at all" box if you did not have that symptom in the past 24 hours.

Please complete this questionnaire each evening.

What time is it?_____AM/PM (please circle)

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Sore or painful throat	☐	☐	☐	☐	☐
Difficulty swallowing	☐	☐	☐	☐	☐
Trouble breathing	☐	☐	☐	☐	☐
Chest congestion	☐	☐	☐	☐	☐
Chest tightness	☐	☐	☐	☐	☐
Dry or hacking cough	☐	☐	☐	☐	☐
Wet or loose cough	☐	☐	☐	☐	☐
Felt nauseous (feeling like you wanted to throw-up)	☐	☐	☐	☐	☐
Stomach ache	☐	☐	☐	☐	☐
Felt dizzy	☐	☐	☐	☐	☐
Head congestion	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐

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**Modified for clinical trial use

Participant ID: _____ **Participant Initials:**_____ **Date:**____/____/____

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Lack of appetite	☐	☐	☐	☐	☐
Sleeping more than usual	☐	☐	☐	☐	☐
Body aches or pains	☐	☐	☐	☐	☐
Weak or tired	☐	☐	☐	☐	☐
Chills or shivering	☐	☐	☐	☐	☐
Sneezing	☐	☐	☐	☐	☐
Coughing	☐	☐	☐	☐	☐
Coughed up mucus or phlegm	☐	☐	☐	☐	☐
Loss of smell	☐	☐	☐	☐	☐
Loss of taste	☐	☐	☐	☐	☐

In the past 24 hours, how often have you had any of the following symptoms?

	0 times	1 time	2 times	3 times	4 or more times
How many times did you vomit?	☐	☐	☐	☐	☐
How many times did you have diarrhea?	☐	☐	☐	☐	☐

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14 APPENDIX 3 – BASELINE COVID-19 SEVERITY CATEGORIZATION *

SARS-CoV-2 infection without symptoms

- Positive testing by standard reverse transcription polymerase chain reaction (RT-PCR) assay or equivalent test
- No symptoms

Mild COVID-19

- Positive testing by standard RT-PCR assay or equivalent test
- Symptoms of mild illness with COVID-19 that could include fever, cough, sore throat, malaise, headache, muscle pain, gastrointestinal symptoms, without shortness of breath or dyspnea
- No clinical signs indicative of Moderate, Severe, or Critical Severity

Moderate COVID-19

- Positive testing by standard RT-PCR assay or equivalent testing
- Symptoms of moderate illness with COVID-19, which could include any symptom of mild illness or shortness of breath with exertion
- Clinical signs suggestive of moderate illness with COVID-19, such as respiratory rate ≥ 20 breaths per minute, saturation of oxygen (SpO2) > 93% on room air at sea level, heart rate ≥ 90 beats per minute
- No clinical signs indicative of Severe or Critical Illness Severity

Severe COVID-19

- Positive testing by standard RT-PCR assay or an equivalent test
- Symptoms suggestive of severe systemic illness with COVID-19, which could include any symptom of moderate illness or shortness of breath at rest, or respiratory distress
- Clinical signs indicative of severe systemic illness with COVID-19, such as respiratory rate ≥ 30 per minute, heart rate ≥ 125 per minute, SpO2 ≤ 93% on room air at sea level or PaO2/FiO2 < 300
- No criteria for Critical Severity

Critical COVID-19

- Positive testing by standard RT-PCR assay or equivalent test
- Evidence of critical illness, defined by at least one of the following:
  - Respiratory failure defined based on resource utilization requiring at least one of the following:

Endotracheal intubation and mechanical ventilation, oxygen delivered by high- flow nasal cannula (heated, humidified, oxygen delivered via reinforced nasal cannula at flow rates > 20 L/min with fraction of delivered oxygen ≥ 0.5), noninvasive positive pressure ventilation, ECMO, or clinical diagnosis of respiratory failure (i.e., clinical need for one of the preceding therapies, but preceding therapies not able to be administered in setting of resource limitation)

– Shock (defined by systolic blood pressure < 90 mm Hg, or diastolic blood pressure < 60 mm Hg or requiring vasopressors) – Multi-organ dysfunction/failure

NOTE: A clinical diagnosis of respiratory failure (in the setting of resource limitation) in which the management deviates from standard of care should be recorded as part of formal data collection.

Corticosteroids

- hydrocortisone (Cortef)
- cortisone
- ethamethasoneb (Celestone)
- prednisone (Prednisone Intensol)
- prednisolone (Orapred, Prelone)
- triamcinolone (Aristospan Intra-Articular, Aristospan Intralesional, Kenalog)
- Methylprednisolone (Medrol, Depo-Medrol, Solu-Medrol)
- dexamethasone (Dexamethasone Intensol, DexPak 10 Day, DexPak 13 Day, DexPak 6 Day
- betamethasone

Antivirals

- Abacavir
- Acyclovir (Aciclovir)
- Adefovir
- Amantadine
- Ampligen
- Amprenavir (Agenerase)
- Umifenovir (Arbidol)
- Atazanavir
- Atripla
- Baloxavir marboxil (Xofluza)
- Biktarvy
- Bulevirtide
- Cidofovir
- Cobicistat (Tybost)
- Combivir
- Daclatasvir (Daklinza)

- Darunavir
- Delavirdine
- Descovy
- Didanosine
- Docosanol
- Dolutegravir
- Doravirine (Pifeltro)
- Edoxudine
- Efavirenz
- Elvitegravir
- Emtricitabine
- Enfuvirtide
- Entecavir
- Etravirine (Intelence)
- Famciclovir
- Fomivirsen
- Fosamprenavir
- Foscarnet
- Ganciclovir (Cytovene)
- Ibacitabine
- Ibalizumab (Trogarzo)
- Idoxuridine
- Imiquimod
- Imunovir
- Indinavir
- Lamivudine
- Letermovir (Prevymis)
- Lopinavir
- Loviride
- Maraviroc
- Methisazone
- Moroxydine
- Nelfinavir
- Nevirapine
- Nexavir (Kutapressin)

- Nitazoxanide
- Norvir
- Oseltamivir (Tamiflu)
- Penciclovir
- Peramivir
- Penciclovir
- Peramivir (Rapivab)
- Pleconaril
- Podophyllotoxin
- Raltegravir
- Remdesivir (Veklury)
- Ribavirin
- Rilpivirine (Edurant)
- Rilpivirine
- Rimantadine
- Ritonavir
- Saquinavir
- Simeprevir (Olysio)
- Sofosbuvir
- Stavudine
- Taribavirin (Viramidine)
- Telaprevir
- Telbivudine (Tyzeka)
- Tenofovir alafenamide
- Tenofovir disoproxil
- Tipranavir
- Trifluridine
- Trizivir HIV
- Tromantadine
- Truvada
- Umifenovir
- Valaciclovir (Valtrex)
- Valganciclovir (Valcyte)
- Vicriviroc
- Vidarabine

- Zalcitabine
- Zanamivir (Relenza)
- Zidovudine

Hydroxychloroquine

Baricitinib (Olumiant)

Convalescent Plasma

*_____**This listing is not intended to be exhaustive.**

16 APPENDIX 5 - COMMON SUBSTRATES FOR CYP2C9 BY THERAPEUTIC CLASS*

Class	**Antiinflammatories**	**Oral Hypoglycemics**	**Oral Anticoagulants**	**Diuretics and uricosurics**	**Angiotensin II blockers**
Substrates	Flurbiprofen Diclofenac Naproxen Piroxicam Suprofen Ibuprofen Mefenamic acid Celecoxib	Tolbutamide Glyburide Glipizide Glimepiride	(S)-Warfarin (S)-Acenocoumarol (Phenprocoumon) **	Torsemide Ticrynafen ** Sulfinpyrazone sulfide	Losartan Irbesartan Candesartan
Class (con't.)	**Antiasthmatics**	**Anticonvulsants**	**Anticancer agents**	**Endogenous compounds**	**Miscellaneous**

Substrates (con't)	Zafirlukast (Zileuton)	Phenytoin (Phenobarbital) (Trimethadione)	Cyclophosphamide (Tamoxifen)	Arachidonic acid 5-Hydroxytryptamine Linoleic acid	Mestranol Fluvastatin _9-Tetrahydrocannabinol (Benzopyrene) (Pyrene) (Fluoxetine) (Sildenafil) (Rosiglitazone)

*This listing is not intended to be exhaustive.

**Parentheses indicates that (where known) other P450s or metabolic pathways play a major role in clearance.

Black Seed Oil_Protocol_V4.0_25Mar2021_Fina
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