A Randomized, Double-Blind, Placebo-Controlled, Multicenter, Phase IIa/IIb Study to Evaluate the Safety, Tolerability, and Efficacy of NP-101 in Treating High-Risk Participants who have Tested Positive for Novel Coronavirus 2019 (BOSS-Covid-19-002)

Protocol Number: BOSS-Covid-19-002

Principal Investigators:
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IND Sponsor: Novatek Pharmaceuticals, Inc.
IND Number: 152687

Version Number: 5.0
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Approved by:

Dr. Ahmed Kaseb	Professor		
Name	*Title*	*Signature*	*Date*

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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; if a new consent form is developed and approved by the 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, Multicenter, Phase IIa/IIb Study to Evaluate the Safety, Tolerability, and Efficacy of NP-101 in Treating High-Risk Participants who have Tested Positive for Novel Coronavirus 2019 (BOSS-COVID-19-002).
Study Description:	This is a Phase IIa/IIb multicenter, interventional, randomized, double-blind, placebo-controlled study designed to evaluate the safety, tolerability, and efficacy of NP-101 in outpatient high-risk COVID-19 positive participants. Blinding roles: Participants, Investigators and Sponsor The study is comprised of two parts (Phase IIa and Phase IIb) and four cohorts. The first part of the trial (IIa) involves three cohorts and is a dose escalation study designed to select the minimum effective dose (optimal dose) for use in the second part (IIb) of the study. In the dose escalation (Phase IIa) portion of the trial (n= 60), qualified participants will be enrolled in a parallel dose escalation design and randomized in a 3:1 [active+best supportive care (BSC):placebo+BSC] ratio to one of three cohorts of 20 participants each (15 active +BSC, 5 placebo + BSC). Cohort 1 (15 participants taking 600 mg capsules for a total daily dose of 3 grams of NP-101 plus BSC and 5 participants taking placebo plus BSC), and Cohort 2 (15 participants taking 600 mg capsules for a total daily dose of 4.8 g of NP-101+ BSC and 5 participants taking placebo plus BSC will run concurrently since acceptable safety data for the 3 g dose was obtained in an earlier phase II study. Cohort 1 will receive either 3 g Total Daily Dose (TDD) of NP-101 + BSC or Placebo+ BSC, Cohort 2 will receive either 4.8 g TDD of NP-101 +BSC or placebo + BSC) and Cohort 3 (15 participants taking 600 mg capsules for a total daily dose of 6 g of NP-101 + BSC and 5 participants taking placebo plus BSC.) Safety will be evaluated according to the terms of the Statistical Analysis Plan (SAP). In the second portion of the trial (Phase IIb), Participants enrolled in Cohort 4 (n= 248) will be randomized in a ratio of 1:1 (active+BSC : placebo+BSC) and will receive the Minimum Effective Dose (MED) as determined by the parameters set by the SAP and approved by the DMC. Study assessments, length of subject participation and receipt of BSC (Best Supportive Care) will remain the same in all four cohorts. A detailed schematic describing all visits and a schedule of activities is included in the Schema and Schedule of Activities, Sections 1.2 and 1.3, respectively.

Study Population:	Up to 308 (up to 60 in dose-escalation Phase-IIa and up to 248 during Phase-IIb) participants will be randomized to receive either NP-101 capsules + best supportive care (BSC) or placebo capsules + BSC
	Efforts will be made to ensure that the diversity in the study population mirrors that of the population as a whole. Males and females aged 18 years and up, who satisfy the high-risk inclusion criteria for Covid-19 infection will be eligible for enrollment.
Phase:	Phase IIa/Phase IIb
Study Sites/Facilities Enrolling Participants:	Up to 8 centers located in and outside of the United States
Description of Study Intervention:	NP-101 600 mg capsules versus placebo capsules, Part 1 (Phase IIa) – (3 AM, 2 PM), (4 BID) or (5 BID) capsules will be taken as directed depending on the Cohort. Part 2 (Phase IIb) – MED (3 AM, 2 PM), (4 BID) or (5 BID) capsules will be taken as directed depending on the MED as determined per parameters of the SAP and as confirmed by the DMC. All participants will receive BSC treatment as required.
Study Duration:	Estimated up to 4 months for Phase-IIa and up to 6 months for Phase-IIb. Actual study duration will depend on recruitment rate.
Participant Duration:	Up to 45 days

Hypothesis, Objectives, and Endpoints:

Hypothesis: NP-101 is safe, tolerable, and superior to placebo as assessed by the percentage of participants who have reached sustained clinical recovery on Day 5 after randomization

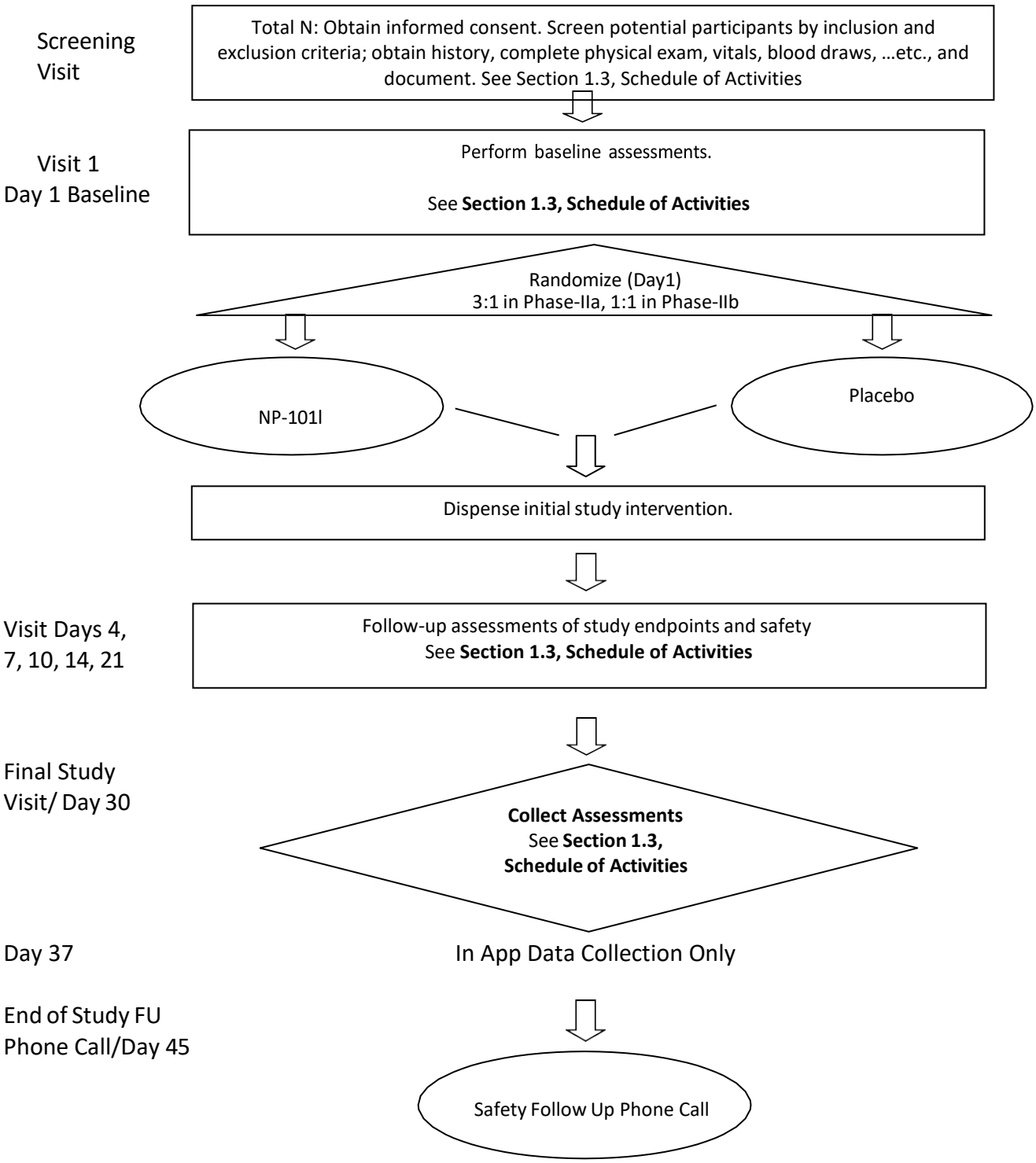
Primary Objectives	Primary Endpoints
Primary Safety Objective (for phase IIa): **1.** To identify the Minimum Effective Dose (MED) of NP-101 in high-risk Covid-19 patients treated on an outpatient basis.	Primary Safety Endpoint (for phase IIa): **1.** Number of Dose Limiting Toxicities (DLT) in the NP-101 arm at each dose level compared to placebo and the safety threshold.
Primary Safety Objectives (for phases IIa and IIb): 1. To evaluate the safety and tolerability of NP-101 compared to placebo.	Primary Safety Endpoints (for phases IIa and IIb): 1. Number of overall adverse events, related adverse reactions, adverse events leading to discontinuation of study drug and hospitalizations, or deaths reported in participants taking the MED of NP-101 versus participants taking placebo. All AEs/SAEs will be captured throughout the study as per schedule of Activities. As part of the primary safety assessment, treatment discontinuations will be compared between participants taking the MED of NP-101 versus participants taking placebo

Primary Efficacy Objectives (Only Phase IIb MED): 2. To compare the rate of Sustained Clinical Recovery on Day 5 after randomization in participants taking the MED of NP-101 as compared to placebo. * **Sustained Clinical Recovery (SCR)** is defined as the continuous alleviation or resolution of all COVID-19 PRO Symptom Survey scores to ≤1 ("none" or "mild"), starting on or before Day 5 after randomization and remaining so for at least 3 consecutive days and without subsequent relapse, defined as not progressing to "moderate" or "severe" for at least two consecutive days.	Primary Efficacy Endpoints (Only Phase IIb MED): 2. Measurement of the difference of Sustained Clinical Recovery rates on Day 5 in participants taking the MED of NP-101 as compared to placebo.
Secondary Objectives	**Secondary Endpoints**
1. To compare the change in viral load and cycle threshold profiles from Randomization to Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo.	1. Measurement of the difference of the average viral load and cycle threshold measures from Randomization to Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo.
2. To compare the percentage of RT-PCR negative/undetectable (i.e., viral clearance) on Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo.	2. Measurement of the difference in the percentage of negative/undetectable RT-PCR (i.e., viral clearance) on Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo.
3. To compare the distribution of days to Sustained Clinical Recovery, days to Complete Clinical Recovery, and days to Complete Clinical Resolution, between participants with taking the MED of NP-101 as compared to placebo. **The time to Sustained Clinical Recovery is defined *as*** the number of days from randomization to the first of 3 consecutive days of alleviation or resolution of all symptoms to Mild or None. **First Day of Complete Clinical Recovery is defined as** the first day from randomization when all symptoms scored as moderate or severe at study entry are scored as mild or absent AND all symptoms scored mild or absent at study entry are scored as absent and remain so afterwards until Day 30. **First Day of Complete Clinical Resolution is defined as** the first day from randomization	3. Estimate of the difference in days to Sustained Clinical Recovery, days to Complete Clinical Recovery, and days to Complete Clinical Resolution survivorship functions in participants taking the MED of NP-101 as compared to placebo. The time to sustained clinical recovery is defined as the number of days from randomization to the first of 3 consecutive days of alleviation or resolution of all symptoms to Mild or None.

when all symptoms are scored as absent and remain so afterwards until Day 30.	
4. To compare the change in effector immune cells, specifically CD4+ and CD8+ T lymphocytes, from Randomization to Day 7 and Day 14 in in participants taking the MED of NP-101 as compared to placebo.	4. Measurement of the difference of the average level of effector immune cells, specifically CD4+ and CD8 T-cells, from Randomization to Day 7 and 14 in participants taking the MED of NP-101 versus those taking placebo.

5. To compare the rate of disease progression measured by the worsening of the Covid-19 symptoms on Day 5 from "none" or "mild" to "moderate" or "severe" and remaining at that level for at least two days before alleviation or resolution, or the experience of hospitalization or death due to Covid-19 symptom worsening in participants taking the MED of NP-101 versus placebo.	5. Measurement of the difference in the percentage of disease progression on Day 5 measured by the worsening of the Covid-19 symptoms from "none" or "mild" to "moderate" or "severe" and remaining at that level for at least two days before alleviation or resolution, or the experience of hospitalization or death due to Covid-19 symptom worsening in participants taking the MED of NP-101 versus placebo.
6. To compare the rate of Long-Covid symptoms in participants taking the MED of NP-101 versus placebo. **Long-Covid Definition:** A patient will be classified as having experienced Long-Covid if they reported any mild, moderate, or severe symptoms on the PRO Symptom Survey Part II as new or ongoing symptoms on any of Day 30 ± 1 days, Day 37 ± 1 days, or Day 45 ± 1 days.	6. Measurement of the difference in the percentage of patients with Long-Covid symptoms in participants taking the MED of NP-101 versus placebo.
Exploratory Objectives (Phase IIb only)	**Exploratory Endpoints (Phase IIb only)**
1. To compare the change in inflammatory cytokines and coagulation factors from Randomization (Day 1) to Day 7 and Day 14 in in participants taking the MED of NP-101 as compared to placebo.	1. Measurement of the difference between the average level of inflammatory cytokines and coagulation factors, from Randomization to Day 7 and 14 in participants taking the MED of NP-101 versus those taking placebo.
2. To construct a risk prediction model for Sustained Clinical Recovery based on known risk factors as well as other patient and disease characteristics.	2. Significance assessment of each risk factor individually in predicting the Sustained Clinical Recovery and its adjusted significance controlling for the effects of other risk factors in the model
3. To compare the proportions of patients developing any feature of conjunctivitis between NP-101 and placebo and to compare the time to conjunctivitis and time to resolution among those who developed conjunctivitis.	3. Measurement of the difference in the percentage of conjunctivitis symptoms in participants taking the MED of NP-101 versus placebo and assess their time of onset and resolution.

1.2 SCHEMA (For all Cohorts)



1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	**Screen Visit**	**Baseline Visit** [d]	**Study Visit 2** [f]	**Study Visit 3**[g]	**Study Visit 4** [f]	**Study Visit 5**[g]	**Study Visit 6**[f]	**Final Study Visit** [f]	**Data Collection via APP only**	**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**	**Days 30 +/- 1 day**	**Days 37 +/- 1 day**	**Day 45 +/- 1 day**
Informed consent	X									
Demographics	X									
Medical history, including Covid vaccination (type and dates)	X									
Inclusion/Exclusion Criteria Evaluation	X	X								
Nasal swab: RT-PCR sample for quantitative viral load analysis		X[c]		X		X				
Blood Collection (including sample for immune monitoring)		X		X		X				
Physical exam (complete at screening and baseline, focused on day 7 and day 14)	X	X		X		X				
Vital signs	X	X		X		X				
Pulse Oximetry	X	X		X		X				
Height	X									
Weight	X									
Hematology	X		X[e]	X	X[e]	X	X[e]	X[e]		
Serum chemistry [a]	X		X[e]	X	X[e]	X	X[e]	X[e]		
Best Supportive Care	X	X	X	X	X	X	X	X		
Pregnancy test [b]	X					X				
Randomization and dispense IP, first dose taken at visit		X								
Drug Accountability		X		X		X	X if not returned at Day 14			
Concomitant medication Review, including medications for Covid symptoms	X	X		X X						X
Adverse event review/evaluation	X	X	X	X	X	X	X	X		X
Patient Reported Outcomes (PRO Symptom Survey, Parts I and II and Conjunctivitis Questionnaire*	X+	X	X	X	X	X	x	X	X	X
Complete Case Report Forms (CRFs)	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, (Optional: LDH, CRP, prothrombin time, activated partial prothrombin time, and D-dimer)
b: Serum or urine pregnancy test (women of childbearing potential). c: Covid-19 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 and Day 30 (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, or if patients can't make it to clinic, e.g. due to sickness, visits on Day 7(Visit 3) and Day 14 (Visit 5) could also be replaced by home health visit for sample collection and telehealth visit for clinical evaluation.
g. Long-Covid symptoms will be collected at Baseline, Day 14, Day 30 and Day 45 through the app.
*Administered everyday
+ PRO Symptom Survey at Screen will be completed on site to determine if patient meets inclusion criterion

2.1 BACKGROUND

2 INTRODUCTION

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 SARS-CoV-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 (G-CSF), 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 COVID-19 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 [27,30,29]. 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. Reference: https://doi.org/10.3390/pathogens10030379 [43]

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.

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 2.3.2Novatek Pharmaceuticals, Inc. recently completed a study, " A Randomized, Double-Blind, Placebo- Controlled, Multicenter Study to Evaluate the Safety and Efficacy of ThymoQuinone Formula (TQF) for Treating outpatient SARS-CoV-2"; clinicaltrials.gov reference:

https://clinicaltrials.gov/ct2/show/NCT04914377.

The results of this trial have been published and can be found at: https://www.mdpi.com/2076-0817/11/5/551/htm. Please note that TQ Formula is also known as NP-101.

Study summary:

Preclinical and clinical studies were conducted to determine the safety and preliminary efficacy of oral TQ Formula (TQF), a drug derived from *Nigella Sativa*, in the treatment of outpatient SARS-CoV-2. In a double-

blind, placebo-controlled phase 2 trial, we randomly assigned (1:1 ratio) non-hospitalized, adult (>18years), symptomatic SARS-CoV-2 patients to receive oral TQF (500 mg, 3 capsules, BID for 14 days) or placebo capsules. Primary endpoints were safety [rate of adverse events (AEs)/serious AEs (SAEs)], and median time-to-sustained clinical response (SCR). Primary analysis was based on intent-to-treat. Secondary/exploratory endpoints included comparing the effect of TQF on individual and total symptom burden (TSB), and on effector immune cells. TQF was tested preclinically in murine leukemia pseudovirus transfected particles. 55 patients were eligible for enrollment and randomized to receive TQF (n=29) or placebo (n=26). The median time-to-Sustained Clinical Response (SCR) was 6 days in TQF arm vs 8 days in the placebo arm (p=0·77), and 5 days in TQF arm vs 7.5 days in the placebo arm in the high-risk cohort, HR 1.55 (95% CI: 0.70, 3.43, p=0.25). No significant difference was found in the rate of AEs (p=0·16); TQF arm had no SAEs either. TQF led to a significantly faster decline in the TSB (p<0.001), and a significant increase in cytotoxic CD8+(p=0·042) and helper CD4+(p=0·042) central memory T lymphocytes. TQF exhibited an *in-vitro* inhibitory effect on entry of five SARS-CoV-2 variants, including Omicron. TQF was very well-tolerated. While median time-to-SCR did not reach statistical significance: it was shorter in the TQF arm and preclinical/clinical signals of TQF activity across multiple secondary and exploratory endpoints were significant. Therefore, the current study, BOSS-Covid-19-002, will serve to confirm findings from the first trial, as well as provide additional data related to the safety and efficacy of NP-101.

The data from the current study, BOSS-Covid-19-002, will be important because it may confirm emerging data showing that CD4+ and CD8+ T lymphocytes play a critical role in COVID-19 immunity. (1) CD4+ T cells activate B cells to promote the production of virus-specific antibody, while CD8+ T cells are primary cytotoxic T cells, which can kill viral infected cells. (2) Results from recent studies confirmed specific phenotypic features of the T cell response against COVID-19 in which upregulation of IFN- gamma rather than IL4 or IL-17, suggesting that Th1 CD4+ cell response was associated with recovery from COVID-19. (3) Therefore, increasing CD4+ and CD8+ T lymphocytes will enhance the protective cellular mediated immunity against COVID-19.

2.2 RATIONALE AND RISK/BENEFIT ASSESSMENT

Rationale:

The aim of this study is to continue development of an oral treatment for high-risk, Covid positive outpatients for whom the available treatment options and drug formulations are currently limited. Physicians can offer patients in this population pain/symptom management, such as acetaminophen, as well as the currently authorized and approved treatments for COVID-19, as listed in the EUA website (https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs) . NP-101 is unique in its ability to induce significant immunomodulatory effects in the form of increased CD4 and CD8 lymphocytes, as demonstrated in Novatek's initial phase II trial. Additionally, unlike many treatments which are now deemed obsolete as reported in the EUA website, NP-101's effect appears to be variant independent given its mechanism of action as described later in this section. None of the authorized or approved medications for the high risk, covid positive population appear to be naturally derived and none of them appear to meet vegetarian standards. NP-101 is a naturally derived, oral medication encased in a vegetarian shell which has been shown in clinical studies to enhance immune response in COVID patients and the API has been shown to be effective against multiple variants of SARS-COV-2, including omicron in preclinical studies. Thus, NP-101 offers value as a treatment option due to its preliminary safety and efficacy profiles, as well as its unique mechanism of action. Therefore, the current study is warranted to confirm the preliminary findings as described above. After gaining informed consent, all participants will be evaluated and eligible to start on the appropriate best supportive care (BSC), including emergency use authorized medications and symptomatic therapy, even before randomization, if clinically indicated. Once randomized, all participants, regardless of treatment arm, will be continuously assessed to determine whether BSC needs to be adjusted.

Synthetic Compounds:

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 COVID-19, as indicated by overall mortality, initiation of ventilation and duration of hospital stay [113]. Remdesivir [57,58] which also showed promise was the first FDA approved COVID antiviral therapy [114] under emergency use authorization (EUA), followed by other agents, including malnupiravir and nirmatrelvir/ritonavir oral formulations. Currently, several vaccines are authorized and recommended to prevent COVID-19 [116] including:

- Pfizer-BioNTech COVID-19 vaccine
- Moderna's COVID-19 vaccine
- Johnson and Johnson's/ Janssen

Role of Traditional Herbal Medicines:

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].

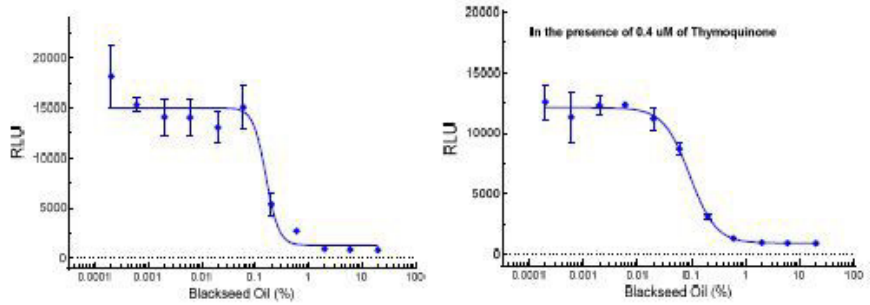
Among various medicinal plants, Nigella sativa (N. sativa) (Family Ranunculaceae) is emerging as an herb which many research studies have revealed its wide spectrum of pharmacological potential. N. sativa is commonly known as black seed. N. sativa is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, and 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. Nigella sativa 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, anti-inflammatory, gastroprotective, hepatoprotective, renal protective [42]. Most of the therapeutic properties of this plant are believed to be 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. This includes our most recent review article about its potential anti- COVID-19 effects (Link: https://pubmed.ncbi.nlm.nih.gov/35462919/).

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].

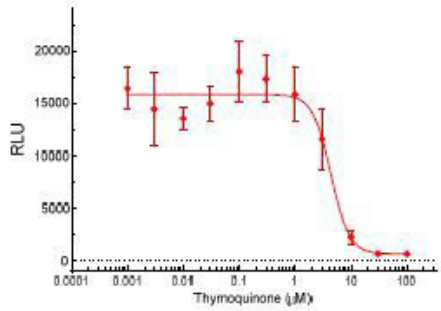
NP-101 offers promising potential as a therapeutic option in management of SARS-CoV-2 as it is derived from Nigella sativa and its active pharmaceutical ingredients have been shown to possess beneficial properties like anti-viral effects (see sections 2.2.1 and 2.2.2).Recently, and in collaboration with CODEX Bio Labs, NP-101 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 (CB- 80552-010, Codex BioSolutions Inc). It was observed that both Blackseed Oil and Thymoquinone seemed to block viral infection (see graphs below)

Results:

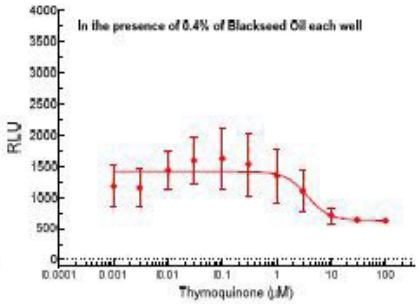


Bottom	1285
Top	15023
LogIC50	-0.7970
HillSlope	-3.617
IC50	0.1596
Span	13738

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



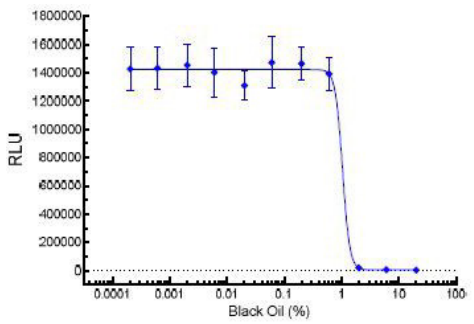
Bottom	677.8
Top	15861
LogIC50	0.6379
HillSlope	-2.643
IC50	4.344
Span	15184



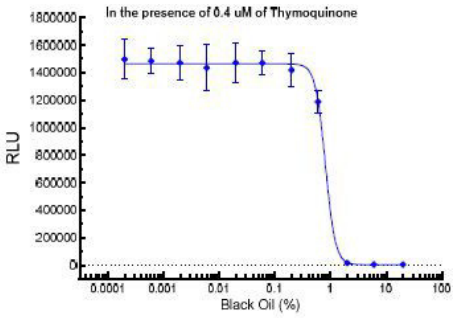
Bottom	628.6
Top	1417
LogIC50	0.5617
HillSlope	-2.149
IC50	3.645
Span	788.6

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

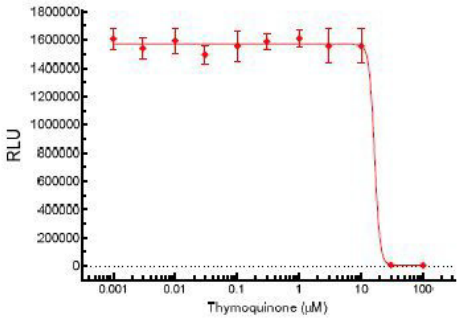
However, it was found that at high concentrations, Blackseed Oil and Thymoquinone caused cell death which indicates that both may have cell toxicity. To confirm it, cell growth assay was performed in the presence of Blackseed Oil or Thymoquinone with Codex's EnerCount cell growth assay kit which measures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



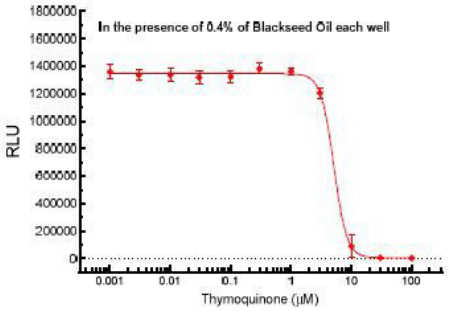
Bottom	5316
Top	1423711
LogIC50	0.01500
HillSlope	-6.938
IC50	1.035
Span	1418395



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

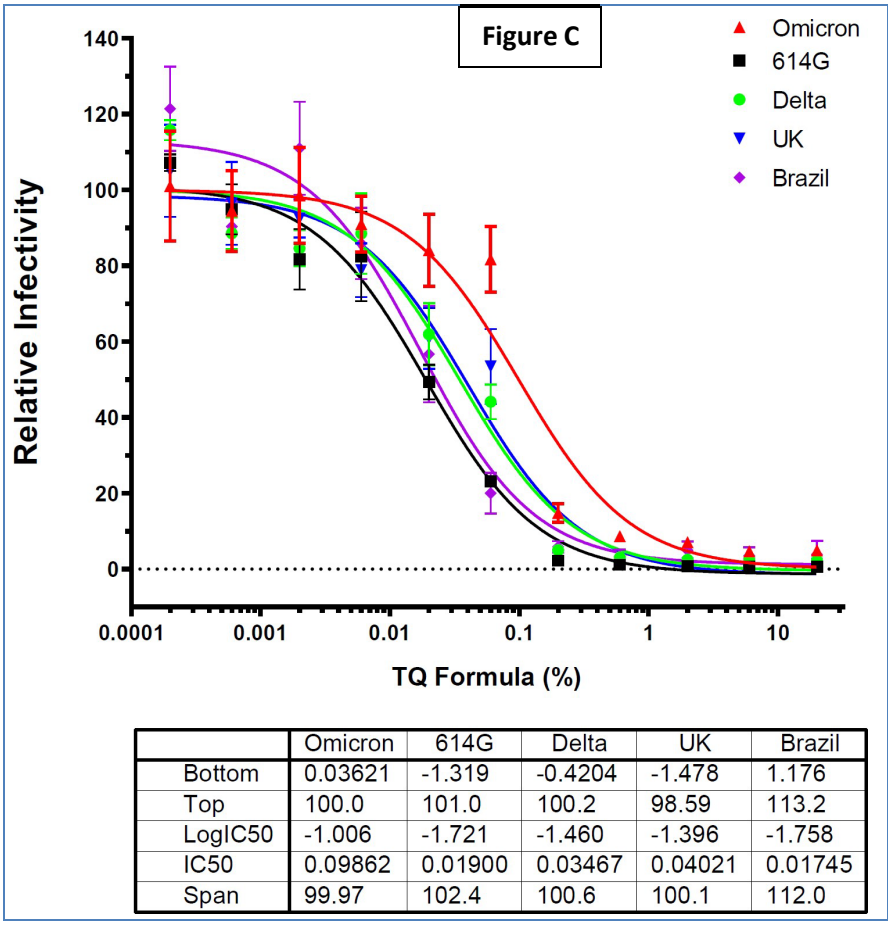
Conclusion: By comparing Fig. A and Fig. B, it was concluded that at certain concentrations, Blackseed oil (0.06%-0.6%) and Thymoquinone (1-10 μ M) can block the SARS-CoV-2 MLV-PP infection without any cell toxicity.

More recently, As shown in Fig. C, NP-101 showed inhibitory effect on all five SARS-CoV-2 variants, including Omicron (614D, Delta, UK, Brazil, and Omicron) with an IC50 value range between 0·01%-0·04%. These studies strongly suggested a significant effect of NP-1o1 in inhibiting SARS-CoV-2 infection. The effect was significant and universal against all tested SARS-CoV-2 variants.

Figure C. Inhibition of SARS-CoV-2 pseudoviruses infection by NP-101 (TQ Formula):

Serial dilutions of NP-101 were tested for inhibition against an MLV-based pseudotyped virus using four different SARS-CoV-2 variant spike protein constructs (614D, Delta, UK, Brazil, and Omicron), in the infection of HEK293-ACE2 cells. X-axis: compound concentration. Y-axis, relative luminescence unit (RLU), reflecting the luciferase activity and the viral infectivity. The experiments were repeated three times with independent samples giving similar results. For all panels, data points shown are mean and standard deviation (s.d.) for n = 3 technical replicates. IC50 values derived from curve fitting are listed in the Table below each Graph.

To further investigate whether NP-101 and TQ can inhibit SARS-CoV-2 entry via ACE2 receptor, we modified the construct by adding beta lactamase to the N-terminus of MLV-gag-pol protein (bLac-gag-pol) (4). The fluorescent signals were indication of viral entry into cells. As shown in Fig. 2, both TQ Formula and TQ can inhibit SARS-CoV-2 614D variant entry into ACE2-expressing HEK293 cells.



	Omicron	614G	Delta	UK	Brazil
Bottom	0.03621	-1.319	-0.4204	-1.478	1.176
Top	100.0	101.0	100.2	98.59	113.2
LogIC50	-1.006	-1.721	-1.460	-1.396	-1.758
IC50	0.09862	0.01900	0.03467	0.04021	0.01745
Span	99.97	102.4	100.6	100.1	112.0

Potential mechanism of actions of NP-101 (TQ Formula)

Molecular modeling outcomes

The analysis showed that thymoquinone caused significant disruption of viral spike-ACE2 receptor interaction (Figure 3): TQ caused reduced the number of hydrogen bonds from 37 to 24 (about 35.0% reduction); and reduced the number of salt bridges from 16 to 5 (about 69.0% reduction). Overall, interfacing bonds decreased by about of 44.0% which is significant and can provide a meaningful prophylactic and therapeutic effect.

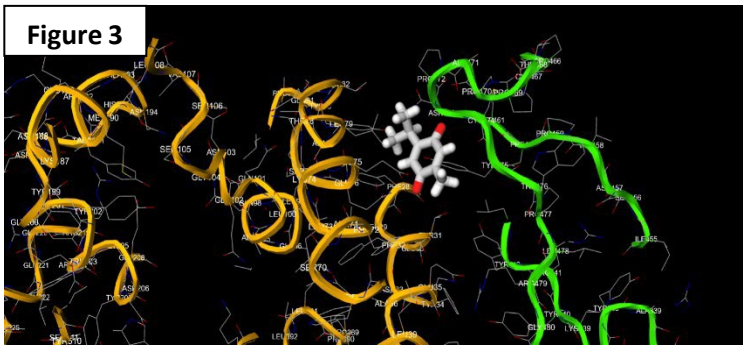


Figure 3: 3-dimensional modeling of viral spike protein (green), angiotensin-converting enzyme 2 (ACE2) receptor (yellow) with thymoquinone (white)

2.2.1 KNOWN POTENTIAL BENEFITS

Clinical Studies

Abdel-Moneim et al. in a study conducted on HCV participants 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 participants [88]. Decreased viral load and improved liver function were similarly reported in another study by Barakat et al., where HCV participants 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 participants 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].

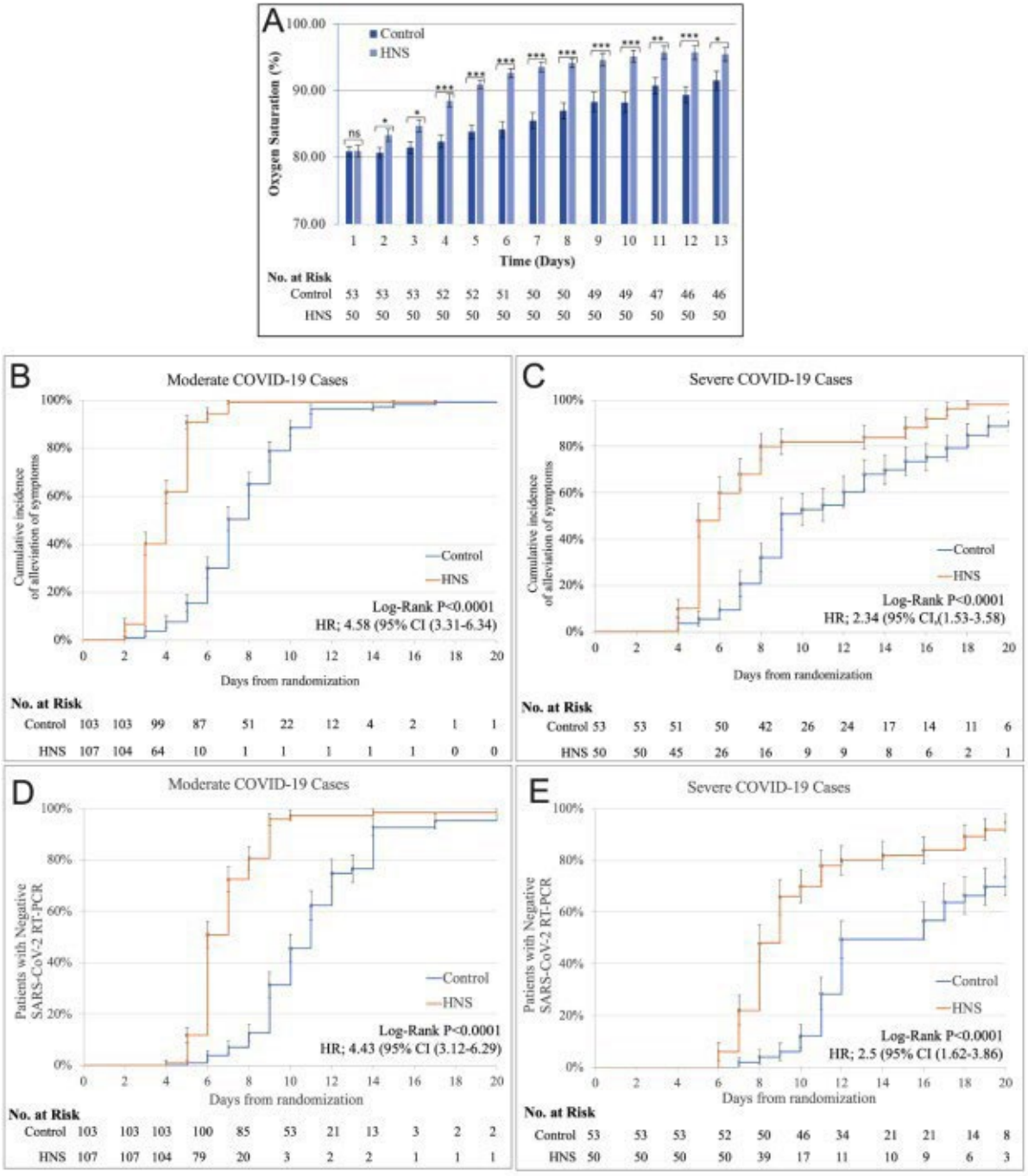
Fard et al. conducted a randomized trial in 100 women with Candida albicans vaginitis. The therapeutic effects of black seed capsules (500 mg twice daily) used together with clotrimazole vaginal cream were compared with those of placebo capsules (500 mg twice daily) used in combination with the same 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 participants:

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 participants 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 participants respectively) using the Clinical Management Guidelines for COVID-19 by the Ministry of National Health Services, Pakistan [114]. The participants 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 up-to 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 participants with moderate and severe disease. It was discovered that alleviation of COVID-19 symptoms for participants in the HNS groups occurred earlier than control groups: 4 versus 7 days for the moderate participants and 6 versus 13 days for the severe disease participants. Viral clearance (being negative for the SARS-CoV-2 RT- PCR 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% participants 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 participants were asymptomatic in the HNS group while more had moderate symptoms (median) in the control arm (OR: 0.1; 95% CI: 0.04-0.24). By day 10, 96.26% of the moderate cases participants 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 participants were still hospitalized requiring oxygen therapy (OR:0.05; 95% CI: 0.02-0.15). Thirty-day mortality 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 parent compound, black seed oil, in the NP-101, both the investigational product and the placebo will be enteric. 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 the turkeys receiving 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].

In Conclusion, these results suggest that black seed oil (BSO) and its main active ingredient, thymoquinone (TQ's), well-documented anti-inflammatory and antimicrobial properties, as well as their immunomodulatory effects, both in vitro and in vivo, are evident. Thus, they could potentially have a beneficial effect in T-cell modulation and can potentially be used as an immunostimulant to enhance the cell-mediated immune response by activating cytotoxic T lymphocytes that can be used against infectious diseases. Notably, to our knowledge, our completed BOSS-COVID-19-001 study of TQ Formula (NP-101) in COVID-19 patients (link: https://www.mdpi.com/2076-0817/11/5/551/htm) is the only study that aimed at studying immune modulatory effects in outpatient COVID-19 patients. We explored the effect of TQ Formula on effector immune cells in participants with COVID-19 infection. Flow cytometry assessments of COVID-19-specific CD4+ and CD8+ T cells circulating in the bloodstream from all randomized patients were analyzed at the same time points (Days 1, 7, and 14), based on Intention-to-treat analysis. This exploratory analysis of effector T lymphocytes showed a significant increase in cytotoxic CD8+ T lymphocytes (p = 0.042) and helper CD4+ T lymphocytes (p = 0.042) with native/central memory phenotype (CD45RA+CCR7+), from baseline to day 14, in treatment arm (TQ Formula) as compared to placebo. This suggests that TQF supports the recovery of the immune system against SARS-CoV-2, which may help prevent the progression to severe SARS-CoV-2.Thus, at day 14, there was a significant increase in the phenotypes of spike-specific T effector cells including Central memory cells marker; CD45RA+CCR7+-expressing T-lymphocytes associated with faster symptom recovery, suggesting that TQF enhanced specific SARS-CoV-2-CD4+/CD8+ T cells. These results are compelling since these effects could protect patients from future infections, however, they warrant confirmation through further evaluation in larger studies. This is going to be one of our secondary aims of the current BOSS-COVID-19-002 trial.

2.2.2 ASSESSMENT OF 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 30 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 30 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 participants [66]. Dehkordi et al. reported that the intake of NS extract (200 or 400 mg/day) for 2 months caused no observable complications in participants 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 participants [68]. Participants with allergic rhinitis treated with NS seeds (250mg/day) for 2 weeks had no adverse effects [70], though some participants 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 participants 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 participants, 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 participants 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 participants with advanced refractory malignant disease. Participants 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 participants 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

This will be the second IND study of NP-101 (Black Seed Oil) in participants 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. 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*

As previously stated in the Rationale section, NP-101 has been shown in clinical studies to enhance immune response in COVID patients and the API has been shown to be effective against multiple variants of SARS-COV-2, including omicron, in preclinical studies. Thus, NP-101 provides an alternative treatment option due to its preliminary safety and efficacy profiles, as well as its unique mechanism of action. The potential benefits of treatment with NP-101 outweigh the minor risks for this population, providing participants with an oral, naturally derived, vegetarian treatment option. To further guarantee the safety of this high-risk patient population, after gaining informed consent, all participants will be evaluated and eligible to start on the appropriate best supportive care (BSC), including emergency use authorized medications and symptomatic therapy, even before randomization, if clinically indicated. Once randomized, all participants, regardless of treatment arm, will be continuously assessed to determine whether BSC needs to be adjusted. BSC allows treatment with any approved or authorized treatment for Covid, as well as treatment for symptom alleviation. As available treatments and regimens continually shift in the COVID space, we will use the NIH's *Coronavirus Disease 2019 (COVID 19) Treatment Guidelines* as an aid to investigators in determining BSC.

Best Supportive Care (BSC): The *Coronavirus Disease 2019 (COVID-19) Treatment Guidelines*, https://www.covid19treatmentguidelines.nih.gov/about-the-guidelines/whats-new/, is published in an electronic format that can be updated in step with the rapid pace and growing volume of information regarding the treatment of COVID-19. The COVID-19 Treatment Guidelines Panel (the Panel) is committed to updating this document to ensure that health care providers, patients, and policy experts have the most recent information regarding the optimal management of COVID-19 (see the Panel Roster for a list of Panel members). New Guidelines sections and recommendations and updates to existing Guidelines sections are developed by working groups of Panel members. All

recommendations included in the Guidelines are endorsed by a majority of Panel members (see Guidelines Development for additional details on the development process). The study team will be asked to stay up to date on treatments and treatment options as relayed through the NIH website as well as the FDA's EUA website, (https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs.

3 OBJECTIVES AND ENDPOINTS AND HYPOTHESES

Primary Efficacy Objectives (Only Phase IIb MED)	Primary Efficacy Endpoints (Only Phase IIb MED)	
2. To compare the rate of Sustained Clinical Recovery on Day 5 after randomization in participants taking the MED of NP-101 as compared to placebo. * **Sustained Clinical Recovery (SCR)** is defined as the continuous alleviation or resolution of all COVID-19 PRO Symptom Survey scores to ≤1 ("none" or "mild"), starting on or before Day 5 after randomization and remaining so for at least 3 consecutive days and without subsequent relapse, defined as not progressing to "moderate" or "severe" for at least two consecutive days.	2. Measurement of the difference of Sustained Clinical Recovery rates on Day 5 in participants taking the MED of NP-101 as compared to placebo.	To assess the efficacy of NP-101 in treating COVID- 19 high risk participants treated in an outpatient setting in terms of percentage of participants recovering from moderate to severe symptoms by Day 5 compared to placebo. The choice of Day 5 Sustained Clinical Recovery was mainly based on our previous Phase-II study of NP-101 (BOSS-001), that showed that the greatest difference was seen around Day 5 from randomization and also based on the CDC's 5-day isolation requirement.
Secondary Objectives	**Secondary Endpoints**	**Justification for Endpoints**
1. To compare the change in viral load and cycle threshold profiles from Randomization to Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo.	1. Measurement of the difference of the average viral load and cycle threshold measures from Randomization to Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo	Faster decline in viral load and faster incline in cycle threshold are hypothesized to be indications for faster recovery from the illness and less infectivity [109, 110]

2. To compare the percentage of RT-PCR negative/undetectable (i.e., viral clearance) on Day 7 and Day 14 in participants taking the MED of NP-101 as	2. Measurement of the difference in the percentage of negative/undetectable RT-PCR (i.e., viral clearance) on Day 7 and Day 14 in participants taking	Treatment with the MED of NP-101 given orally on outpatient basis is hypothesized to result in higher percentages of viral

Overall Hypothesis: NP-101 is safe, tolerable, and superior to placebo as assessed by the percentage of participants who have reached sustained clinical recovery on Day 5 after randomization

Objectives/Purpose	Endpoints/Outcome Measures	Justification for Endpoints
Primary Safety Objective (for phase IIa)	**Primary Safety Endpoint (for phase IIa)**	
1. To identify the Minimum Effective Daily Dose (MED) of NP-101 in high-risk Covid-19 patients treated on an outpatient basis.	**1.** Number of Dose Limiting Toxicities (DLT) in NP-101 arm at each dose level compared to placebo and the safety threshold.	Identifying a MED is important for describe the safety profile of NP-101 and it will be used in the Phase-IIb portion of the study.
Primary Safety Objectives (for phases IIa and IIb)	**Primary Safety Endpoints (for phases IIa and IIb)**	
1. To evaluate the safety and tolerability of NP-101 compared to placebo.	1. Number of overall adverse events, related adverse reactions, adverse events leading to discontinuation of study drug and hospitalizations, or deaths reported in participants taking the MED of NP-101 versus participants taking placebo. All AEs/SAEs will be captured throughout the study as per schedule of activities. As part of the primary safety assessment, treatment discontinuations will be compared between participants taking the MED of NP-101 versus participants taking placebo	Comparing the number of AEs and SAEs and any relationship to IP is important for determining the safety profile of NP-101. We hypothesize that NP-101 will not have significantly higher frequency of AEs, SAEs and treatment discontinuation incidences. All AEs/SAEs and treatment discontinuation will be captured throughout the study as per schedule of activities.

compared to placebo.	the MED of NP-101 as compared to placebo.	clearance by RT-PCR. Faster decline of viral load is hypothesized to lead to faster recovery from the illness and less infectivity on Day 5 and Day 14 [109, 110]
3. To compare the distribution of days to Sustained Clinical Recovery, days to Complete Clinical Recovery, and days to Complete Clinical Resolution, between participants with taking the MED of NP-101 as compared to placebo. ***The time to sustained clinical recovery is defined as*** the number of days from randomization to the first of 3 consecutive days of alleviation or resolution of all symptoms to Mild or None. **First Day of Complete Clinical Recovery is defined as** the first day from randomization when all symptoms scored as moderate or severe at study entry are scored as mild or absent AND all symptoms scored mild or absent at study entry are scored as absent and remain so afterwards until Day 30. **First Day of Complete Clinical Resolution is defined as** the first day from randomization when all symptoms are scored as absent and remain so afterwards until Day 30.	3. Estimate of the difference in days to Sustained Clinical Recovery, days to Complete Clinical Recovery, and days to Complete Clinical Resolution survivorship functions in participants taking the MED of NP-101 as compared to placebo. The time to sustained clinical recovery is defined as the number of days from randomization to the first of 3 consecutive days of alleviation or resolution of all symptoms to Mild or None.	We hypothesize that the distribution of days to Sustained Clinical Recovery, days to Complete Clinical Recovery, and days to Complete Clinical Resolution will be significantly shorter than that of placebo.
4. To compare the change in effector immune cells, specifically CD4+ and CD8+ T lymphocytes, from Randomization to Day 7 and Day 14 in in participants taking the MED of NP-101 as compared to placebo.	4. Measurement of the difference of the average level of effector immune cells, specifically CD4+ and CD8 T-cells, from Randomization to Day 7 and 14 in participants taking the MED of NP-101 versus those taking placebo.	We hypothesize that participants treated with NP-101 will have significantly higher CD4+ and CD8+ T-cell counts on average compared to placebo, which will be a sign of stronger immunity build-up against the infection.

5. To compare the rate of disease progression measured by the worsening by the Covid-19 symptoms from "none" or "mild" to "moderate" or "severe" and remaining at that level for at least two days before alleviation or resolution, or the experience of hospitalization or death due to Covid-19 symptom worsening in participants taking the MED of NP-101 versus placebo.	5. Measurement of the difference in the percentage of disease progression measured by the worsening by the Covid-19 symptoms from "none" or "mild" to "moderate" or "severe" and remaining at that level for at least two days before alleviation or resolution, or the experience of hospitalization or death due to Covid-19 symptom worsening in participants taking the MED of NP-101 versus placebo.	We hypothesize that treatment with NP-101 will lower the likelihood of disease progression and hospitalization.
6. To compare the rate of Long-Covid symptoms in participants taking the MED of NP-101 versus placebo. **Long-Covid Definition:** A patient will be classified as having experienced Long-Covid if they reported any mild, moderate, or severe symptoms listed by CDC as new or ongoing symptoms on any of Day 30 ± 1 days, Day 37 ± 1days, or Day 45 ± 1 days	6. Measurement of the difference in the percentage of patients with Long-Covid symptoms in participants taking the MED of NP-101 versus placebo.	We hypothesize that participants treated with NP-101 will experience lower rate of Long-Covid symptoms compared to placebo.
Exploratory Objectives (Phase IIb only)	**Exploratory Endpoints (Phase Iib only)**	
1. To compare the change in inflammatory cytokines and coagulation factors from Randomization to Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo.	1. Measurement of the difference between the average level of inflammatory cytokines and coagulation factors, from Randomization to Day 7 and 14 in participants taking the MED of NP-101 versus those taking placebo.	The inflammatory cytokines and immunological markers are of importance because of their correlation with disease severity in COVID-19.
2. To construct a risk prediction model for Sustained Clinical Recovery based on known risk factors as well as other patient and disease characteristics.	2. Significance assessment of each risk factor individually in predicting the Sustained Clinical Recovery and its adjusted significance controlling for the effects of other risk factors in the model	Identifying which risk factor is more clinically and statistically important may help guide in generating closer follow-up of such participants.

3. To compare the proportions of patients developing any feature of conjunctivitis between NP-101 and placebo and to compare the time to conjunctivitis and time to resolution among those who developed conjunctivitis.	3. Measurement of the difference in the percentage of conjunctivitis symptoms in participants taking the MED of NP-101 versus placebo and the difference in time to onset of conjunctivitis and time to resolution of it.	We hypothesize that participants treated with NP-101 will have significantly lower rate of conjunctivitis symptoms (which are evolving with new COVID-19 variants) compared to placebo, and longer time to onset and shorter time to resolution of such symptoms with treatment with NP-101 compared to placebo

4 STUDY DESIGN

4.1 OVERALL DESIGN

This clinical research trial aims to demonstrate the safety of NP-101, find the optimum dose (i.e., the recommended minimum effective daily dose for phase Iib dose or "MED"), as well as efficacy against Covid-19 in the outpatient setting. **The hypothesis** is that NP-101 is safe, tolerable, and superior to placebo as assessed by the percentage of participants who have reached sustained clinical recovery on Day 5 after randomization. **Sustained Clinical Recovery (SCR)** is defined as the continuous alleviation or resolution of all COVID-19 PRO Symptom Survey scores to ≤1 ("none" or "mild"), starting on or before Day 5 after randomization and remaining so for at least 3 consecutive days and without subsequent relapse, defined as not progressing to "moderate" or "severe" for at least two consecutive days. In addition, it is hypothesized that treatment with NP-101 will significantly reduce viral load, proportion of participants with disease progression, proportion participants with RT-PCR Positive by Day 7 and Day 14, median days to Sustained Clinical Recovery, and will significantly increase the effector immune cells, specifically CD4+ and CD8 T-cells, on Day 7 and Day 14.

This is a randomized, double-blind, placebo-controlled phase Iia/Iib study to assess the safety and efficacy of NP-101 versus placebo in the treatment of Covid-19 in high -risk participants in the outpatient setting. In the first part of the study (phase Iia), high risk Covid-19 participants will be randomly assigned in a 3:1 ratio to dose specific cohorts Cohort 1 (3 g), Cohort 2 (4.8 g) and Cohort 3 (6 g). Cohort I and 2 will be evaluated concurrently, followed by Cohort 3 in the second part of the study (Iib), high risk participants will be randomly assigned to the MED or Placebo in a 1:1 ratio. The participants will receive up to 14 days of dosing.

Screening Visit: ≤72 hours before Baseline

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 an electronic or 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, COVID-19 vaccination type and dates, 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 (Nasal Swab: RT-PCR for quantitative viral load analysis)
Participants will be given a Nasal Swab RT-PCR COVID-19 test (per site standard procedures) to obtain same day results to document COVID-19 diagnosis or Participants can present documentation of a positive RT-PCR test obtained within the prior 5 days of the day of randomization.

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, with a focused examination on 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, 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/or preferably directly into the electronic database.

Pulse Oximetry (Oxygenation saturation)
SpO2 will be performed at screening. Participants are required to have SpO2 of >93% on room air prior to randomization which has not increased since onset of COVID-19 signs/symptoms) obtained at rest by study staff at Day 1 of randomization.

PRO Symptom Surveys Part I and II and Conjunctivitis Survey

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, phosphorus, potassium, total protein, AST, ALT, sodium, CBC/diff, LDH, and optional: LDH, CRP, prothrombin time, activated partial prothrombin time, D-dimer

Pregnancy Test
Females of child-bearing potential will have either a serum or urine pregnancy test completed at baseline.

Baseline Visit - Randomization (Day 1 of therapy)

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.

Nasal Swab Sample for quantitative viral load testing (RT-PCR)
Participants will have a sample taken for RT-PCR quantitative viral testing.

Whole Blood Collection
Blood samples (approximately 15 ml) will be collected for biomarkers such as immune monitoring and inflammatory cytokines testing

Randomization
Participants will be randomized on Day 1 of study to receive either NP-101 + Best Supportive Care (BSC) or placebo + BSC

Distribute IP
Participants will be provided with study treatment kit, with enough capsules for 14 days of treatment plus spares.

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

Patient Reported Outcomes and Conjunctivitis Questionnaires via the APP
Participants will complete the PRO Symptom Survey questionnaire Part I and Part II on a daily basis for the duration of the study, from Day 1 of randomization to final study visit (Day 30), as well as Day 37 and Day 45.

Subsequent Study Visits (Including Day 21 and Final Visit Day 30)

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 including medications to treat COVID-19 symptoms such as analgesics and antipyretics anti-diarrhea, currently being taken by participants will be documented in source and in the eCRF. Efforts will be made by investigators to document the name, dose, dosage form, date and time of administration of

medications to treat COVID-19 symptoms. The incidence of hospitalization for any cause will be defined as ≥24 hours of acute care in a hospital or any similar facility or death through day 45. Every effort, including electronic alerts and reminders, will be made per protocol procedures and as needed to remind participants to ascertain data collection and ascertain survival and hospitalization status through day 45 from randomization for participants who discontinued participation early.

Physical Exam

A focused 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. Limited, symptom-directed physical examinations should be performed at day 7 and day 14 specified post-baseline 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 clinic study visit.

Whole Blood Collection

Blood samples (approximately 15 ml) will be collected for biomarkers such as immune monitoring and inflammatory cytokines testing at Day 7 and Day 14, in addition to the baseline visit.

Nasal Swab 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 10 ml in total) will be taken for safety laboratory evaluations (as listed in screening visit above) at Day 7 and 14 and as clinically indicated at any other study visits.
If subjects with abnormal baseline liver indices develop elevations of AST or ALT >2x baseline or total bilirubin > 1.5x baseline values during the study, repeat testing should be performed within 48-72 hours. If there are persistent elevations (AST or ALT>2x baseline or TBL >1.5 baseline values) upon repeat testing, then close observation (testing and physical examination 2-3 times per week) should be implemented, and discontinuation of drugs should be considered.

Patient Reported Outcomes and Conjunctivitis Questionnaire

Participants will complete the PRO Symptom Survey Pt I and II electronically on a daily basis, and will measure body temperature orally around same time daily (efforts will be made for reminders such as text messages, phone calls or emails, to be sent daily around same time of 4-5 pm for participants to complete PRO Symptom Survey, and record verbal responses for those who are unable to self-record because of illness or other circumstances) for the duration of the study, from randomization to final study visit (Day 30), as well as day 37 and day 45. Reasons for missing data will be documented. Efforts will be made to ascertain vital status for all COVID-19 trial subjects even after a subject decides to discontinue treatment or discontinue participation in the trial, including follow-up for key outcomes.

The PRO Symptom Survey part I includes the following three global impression items, as well as a question related to the ease of use of the survey:

- In the past 24 hours, have you returned to your usual health (before your COVID-19 illness)? Yes or No
- In the past 24 hours, have you returned to your usual activities (before your COVID-19 illness)? Yes or No
- In the past 24 hours, what was the severity of your overall COVID-19-related symptoms at their worst? None, Mild, Moderate, or Severe

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

PRO Symptom Survey Pt 1, Pt 2 and the conjunctivitis questionnaire will be completed at Screening, Baseline, Day 1-Day 30, Day 37 and Day 45 via the app.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Blackseed oil, the active ingredient of NP-101 has been shown to possess anti-inflammatory properties, and anti-coronavirus 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) and endpoints were chosen based on our preliminary data from our completed small randomized phase 2 study (Link: https://www.mdpi.com/2076-0817/11/5/551/htm) to confirm the safety and efficacy of treatment with NP-101 against coronavirus versus placebo. Participants can maintain their regular standard of care, irrespective of treatment allocation, and are allowed to take any additional vitamins or supplements. Concomitant medications at each study visit including medications to treat COVID-19 symptoms such as analgesics and antipyretics or anti-diarrhea, currently being taken by participant swill be documented in source and in the eCRF. Use of therapies intended as Covid-19 treatments (including any monoclonal antibodies and remdesivir) will be allowed.

The randomization of participants will allow researchers to determine any significant differences between treatment groups. Participants, Investigators and Sponsor will be blinded to their treatment, to remove any reporting bias on the patient reported outcome questionnaire, the PRO Symptom Survey.

4.3 JUSTIFICATION FOR DOSE AND FORMULATION

NP-101 will be administered as an oral dose daily. The doses planned in the Phase-IIa portion of this study are 3 g per day, 4.8 g and 6 g per day with 600 mg capsules. Based on the protocol dose-escalation scheme, one of these three dose levels will become the recommended dose, known as the MED, for the IIb portion of the trial. Safety and efficacy will be monitored by the DMC throughout the entire study, including the planned interim analysis.

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 several therapeutic indications (see references in toxicity section above).

The proposed initial dose of 3 g per day has been used in several 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 placebo- controlled 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. Participants reported no side effects during the intervention except mild gastrointestinal problems [81]. In a study by Bamosa et al., participants with type 2 diabetes mellitus were randomly divided into 3 groups to receive Nigella Sativa seed extracts at 1 g, 2 g and 3 g per day respectively for 12 weeks. In the study, generally, the three doses of Nigella Sativa were well tolerated with only three participants experiencing 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, participants reported subjective complaints related to central obesity (body ache, weakness, sleeplessness, belching, decreased libido). All subjective complaints reported by participants in the treatment group disappeared in the first week of the study, but they all persisted in the control group through the end of the study. [65].

Enteric Formulation
To mask the after taste/unpleasant taste of black seed oil in NP-101 and its matching placebo are enteric.. Previous clinical studies using fish oil have shown enteric capsules to be safe with the added benefit of improving compliance and enhancing gastrointestinal 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 investigational product, then the PI will decide whether or not to decrease the dose in accordance with the protocol derived safety algorithm for escalation and de-escalation. This information will be captured in the EDC, as well as the safety database. The EDC team will then update the app to ensure electronic messaging that is consistent with the site's direction to the participant.

Table 4.3.1: Dose modification

Agent	Starting Dose (0 Dose Level)	-1 Dose Level	-2 Dose Level

Blackseed Oil Capsules NP-101 Capsules/Placebo if MED= 3g daily	600 mg, 5 capsules, orally 3 in the morning, 2 in the evening (total 3 g daily)	600 mg, 4 capsules, orally 2 in the morning, 2 in the evening (total 2.4 g daily)	600 mg, 3 capsules, orally 2 in the morning, 1 in the evening (total 1.8 g daily)
Blackseed Oil Capsules NP-101/Placebo if MED= 4.8g daily	600 mg, 8 capsules, orally 4 in the morning, 4 in the evening (total 4.8 g daily)	600 mg, 6 capsules, orally 3 in the morning, 3 in the evening (total 3.6 g daily)	600 mg, 4 capsules, orally 2 in the morning, 4 in the evening (total 2.4 g daily)
Blackseed Oil NP-101 Capsules/Placebo if MED= 6g daily	600 mg, 10 capsules, orally 5 in the morning, 5 in the evening (total 6 g daily)	600 mg, 8 capsules, orally 4 in the morning, 4 in the evening (total 4.8 g daily)	600 mg, 6 capsules, orally 3 in the morning, 3 in the evening (total 3.6 g daily)

For Grade 3 or greater treatment related toxicity that cannot be controlled with symptomatic treatment within 48 hours of beginning the symptomatic treatment, such as nausea and/or vomiting that cannot be controlled by antiemetics or diarrhea that cannot be controlled by anti-diarrheal medicines, the dose level will be decreased to dose level -1. Similarly, participants who develop Grade 3 or greater treatment related toxicity that cannot be controlled with symptomatic treatment within 48 hours of beginning symptomatic treatment at dose level -1 will have further decrease in dosage to dose level -2. Finally, participants who develop Grade 3 or greater 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.

Participants should terminate study treatment if further reduction is indicated beyond the –2 level. Treatment may be delayed up to 7 days to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 7-day delay, all study treatment will be discontinued. Participant will remain on study for safety follow up per schedule of activities.

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 life-threatening 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 along with assessments through Day 30.

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. A resting SpO2 of >93% on room air.
3. Stated willingness to comply with all study procedures and availability for the duration of the study
4. Male or female, aged 18 and over, presenting with mild to moderate clinical symptoms of Covid-19 infection (per FDA guidance – see appendix 3) with symptom onset within 5 days prior to the day of randomization
5. Positive COVID-19 infection confirmed by RT-PCR within 5 days prior to the day of randomization. Note: RT-PCR is the preferred method; however, with evolving approaches to laboratory confirmation of SARS-CoV-2 infection, other molecular or antigen tests that detect viral RNA or protein are allowed. The test result must be available to confirm eligibility. Participants may be enrolled based on positive results of a rapid SARS-CoV-2 antigen test performed at screening.
6. A score of $\geq$2 (moderate) on a minimum of 1 symptom on the PRO Symptom Survey on the day of randomization
7. Ability to take oral medication and be willing to adhere to the dosing regimen (Twice a day – BID for 14 days)
8. 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
9. For males of reproductive potential: use of condoms or other methods to ensure effective contraception with partner during study participation and for an additional 4 weeks after the end of study drug administration
10. Agreement to adhere to Lifestyle Considerations (see section 5.3) throughout study duration
11. To be considered high risk, participants should have at least one of the following conditions:
    - age ≥50 years;
    - active cancer
    - chronic kidney disease;
    - chronic lung disease including COPD
    - Obesity (BMI ≥30)
    - serious heart conditions [heart failure, coronary artery disease, or cardiomyopathies];
    - Diabetes (type 1 or type 2)
    - Immunocompromised state (weakened immune system or autoimmune diseases)
    - Chronic liver disease
    - Cystic fibrosis
    - HIV infection
    - Smoking, current or former
    - Sickle cell disease or thalassemia
    - Solid organ or blood stem cell transplant
    - Stroke or cerebrovascular disease
    - Asthma

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, inhaled steroids, which are used to treat acute or chronic bronchial inflammation, will be permitted
2. Severe Covid-19 symptoms (severe per FDA classification – see appendix 3)
3. Requires immediate admission to hospital for any reason
4. Pregnancy or lactation
5. Known allergic reactions to components of black seed oil or thymoquinone
6. Treatment with another investigational drug or other investigational intervention within 2 weeks of study start and throughout study duration.
7. Significant hepatic disease (ALT/AST> 2.5 times the ULN); any laboratory parameter >/= 4 times the ULN or platelet count <100,000/µ L or neutrophilic granulocyte absolute count <500/mm3
8. History of moderate to severe CKD, (i.e. on hemodialysis or has an estimated glomerular filtration rate less than 45mL/min) at the time of enrollment
9. Participants with inflammatory bowel disease (such as Crohn's) that could affect the intestinal absorption of NP-101 enteric coated capsules.
10. Known uncontrolled HIV (with a recent viral load > 50 copies/mL or CD4<200 cells/mm3 or known active uncontrolled Hepatitis B (defined as HBsAg-positive or detectable HBV DNA viral load) or known active Hepatitis C (defined as detectable HCV RNA viral load) infection
11. Influenza diagnosis (confirmed by testing) during screening or within prior 14 days
12. Any uncontrolled condition(s) or diagnosis, both physical or psychological, or physical exam finding that precludes participation, as per investigator
13. Current treatment with CYP2C9 substrates (see Appendix 5)

5.3 LIFESTYLE CONSIDERATIONS

It is recommended that doses be taken with food, twice a day. Participants may continue with their normal standard of care, including any supplements, vitamins, and symptomatic therapy such as anti-pyretics, but those must be captured in the concomitant medications.

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

Recruitment Method

This hybrid digital study will recruit through the efforts of retailer(s) and pharmacies, in addition to the efforts of each site and investigator.

Participants will be identified through Covid-19 screening tests at participating study sites and through selected retailers and pharmacies. 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 determined necessary

Up to 60 participants will be equally randomized in a 3:1 ratio to receive either NP-101 or matching placebo capsules during the Phase-IIa portion of the study, and up to 248 participants will be equally randomized in a 1:1 ratio to receive either NP-101 at the MED or matching placebo capsules during the Phase-IIb portion of the study (See section 9.2 for sample size determination details). There will be at most 8 sites participating in this study and three sites will receive recruitment assistance through retailers and pharmacies. The sites will be outpatient clinics that are currently testing/treating COVID-19 participants.

It is anticipated that each site will recruit approximately 4-5 participants per week. Based on this anticipated accrual rate, a least 2 active sites should be able to complete recruitment for the Phase-IIa study within approximately 6-7 weeks, and considering the 45-day follow-up, the Phase-IIa portion of the study may be completed within 3-4 months. At the same recruitment rate, recruitment to the Phase-IIb portion of the study may be completed within 25 weeks (~6 months). The actual recruitment speed will depend on how many active sites can contribute to the study, the Covid-19 infection rates and overall severity burden of the infection with changing Covid-19 variants and the effectiveness of our recruitment strategy.

Since COVID-19 disproportionately affects racial and ethnic minorities, these populations should be represented in clinical trials, study sponsor is planning to ensure that clinical trial sites will include geographic locations with a higher concentration of racial and ethnic minorities (such as Houston, TX , Miami, FL and Chicago, Illinois) to recruit a diverse study population which accurately mirrors the population as a whole. Decentralized sites might also be utilized.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTIONS

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.

The study intervention is NP-101 capsules, 600 mg per capsule and placebo capsules which are identical in appearance to NP-101 capsules but with no active ingredients.

For the purposes of this trial, both NP-101 and placebo capsules will be manufactured with enteric 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 NP-101 at the MED or placebo for the duration of the study. Study participants should take the NP-101 according to the starting dose specifications as outlined in Table 4.3.1. Dose Modification tables. 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 600 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 600 mg active/placebo capsules.

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 labelled bottles with the appropriate number of capsules for 14 days of dosing for each participant are provided to the sites. Sites will be provided with a code for each subject that will correspond to a bottle on site.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Study participants in Cohorts 1-3 will be randomized in a three to one fashion (3:1) to receive either 3 g, 4.8 g or 6 g day of NP-101 (600 mg capsules x (3 AM,2 PM), (4 BID) or (5 BID) dependent upon cohort) or placebo capsules (600 mg capsules x (3 AM,2 PM), (4 BID) or (5 BID) dependent upon cohort, 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 participants, 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 system generated analyses provided to the DMC for review and decision making in accordance with the SAP between cohorts of phase IIa, after Phase-IIa is completed, and at the time of the interim analysis of Phase-IIb. The final analyses will be conducted after the data lock for both Phase- IIa and IIb.

A randomization list will be prepared electronically by the EDC vendor and provided to the IP depot. Sponsor and PIs will remain blinded to study treatment. Randomization codeswill be assigned through the electronic system.

Participants will complete the PRO Symptom Survey, 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.

Unblinding

If unblinding is required, the principal investigator can unblind through the electronic system, 24 hours, 7 days a week. Treatment unblinding is discouraged if knowledge of the treatment assignment will not materially change the planned management of a medical emergency. It is preferred that a non-clinical team member have access to the system, perform the unblinding and let the investigator know the treatment assignment of the patient.

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 enter the number of capsules taken twice daily into the App and they will also bring all investigational product with them to each on-site visit for compliance verification. Investigator or designee will perform IP accountability, reconcile and document appropriately. Patient compliance must remain 85% throughout the course of the study. Participants who miss more than 6 consecutive doses (i.e., 3-days of therapy) will be labelled as non-compliant and will not be included in the per protocol analysis set while they will be included other full set analyses for both safety and efficacy endpoints.

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 reviewed at screening and throughout the course of the study at each study visit and captured electronically daily. Any medication 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 authorized and approved medications available to treat Covid-19 and its symptoms are allowed as recommended the investigator

Prohibited Medications
The use of drugs approved or authorized to treat severe Covid-19 requiring hospitalization, such as: corticosteroids and some antivirals is prohibited for entry into the study. However, if any participant requires these medications due to worsening of Covid-19 symptoms once in the study, the study treatment will be stopped, but participant will continue to be followed through Day 30. Investigators should not include any participants who are currently being treated with any drugs that are metabolized by CYP2C9 due to potential for interaction with Thymoquinone (See Appendix 5).

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 Activities (SOA).

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: Importantly, patients who progress will undergo medical evaluation by treating physicians (principal investigators) to evaluate their candidacy for currently available COVID-19 management approaches including referral to emergency room for inpatient admission and management, if warranted, based on their vital signs and pulse oximetry for example. Additionally, those patients who do not meet admission criteria and need further

outpatient management, will be evaluated for outpatient therapies for COVID-19, including emergency use authorized medications as medically warranted per treating physicians' discretion.

- 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 on the Electronic Database.

For any participants who discontinue from the study, all attempts should be made to ensure that study procedures and follow-up visits are completed per protocol.

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. Sponsors and investigators should make efforts to minimize the amount of missing data.
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 at the listed contact information 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, telephone calls, text messages or emails, and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should bedocumented 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.

Administrative Procedures
Informed consent
Inclusion/exclusion criteria
Demographics and medical history
Prior and concomitant medication review
Randomization and Study intervention
PRO Symptom Survey
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 Oximetry
LOCAL Laboratory Assessments
Covid-19 RT-PCR 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 immune studies/biomarkers (serum and plasma)

8 STUDY ASSESSMENTS AND PROCEDURES

Covid-19 Quantitative Viral Load (RT-PCR)
Participants will have an RT-PCR sample taken at baseline (day 1 of randomization), day 7 and day 14 to measure quantitative viral load and viral load clearance. These samples will be tested at the study site if PCR machine is available or sent to a central laboratory for analysis.

Patient Reported Outcomes
Participants will complete the PRO Symptom Survey Parts I and II (Appendix 2) electronically daily for the entirety of the study, from randomization to final study visit Day 30, Day 37 and Day 45. The responses will be used to measure severity and change in Covid-19 symptoms throughout the study.

Conjunctivitis Questionnaire
Participants will complete the conjunctivitis questionnaire daily until Day 30, at Day 37 and Day 45.

Day 0: Most current symptom assessment before randomization. In most cases, it is expected to be on the day of randomization, right before randomization to assess the eligibility
Day 1: Symptom assessment within the first day after receiving the first dose of the medication.
Day X: Symptom assessment after X days after receiving the first dose of the medication. Thus, **Day 5** will be the assessment on Day 5 from the therapy start date, which is ideally the day of randomization.

Blood samples Collection (including 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 or tested at study site if Flow Cytometer is available. See laboratory manual for further details.

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 1 of randomization, Day 7 and Day 14. A complete physical examination, performed at screening/Day 1 of randomization and other specified visits, should include an evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, 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, and optional: 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 during the active treatment period (up to 14 days) and for 7 days after the end of the treatment period. Additionally, data on adverse events considered by the investigator to be related to the study drug will be collected until the end of study. Participants will be encouraged to contact the study site immediately if they observe any worsening symptoms or health concerns at any time point of the study.

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 considered intervention-related (21 CFR 312.32 (a)).

For this study, adverse events will be captured from the time of informed consent through the entirety of the study.

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 (Day 45, during end of study follow up call). 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 in the electronic database . 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 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

Adverse Event

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

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 to the contracted pharmacovigilance group, Prime Vigilance via directions provided in the Safety Plan.

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 electronic database , which will trigger notifications to the Medical Monitor and the pharmcovigilence team at Prime Vigilance. The Initial SAE Report should be submitted to the selected pharmacovigilance group within 24 hours either by e-mail using the contact information provided on the form and in the Safety Plan.

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 pharmacovigilance team on behalf of 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 the participant will be followed until delivery.

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** 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. 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 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

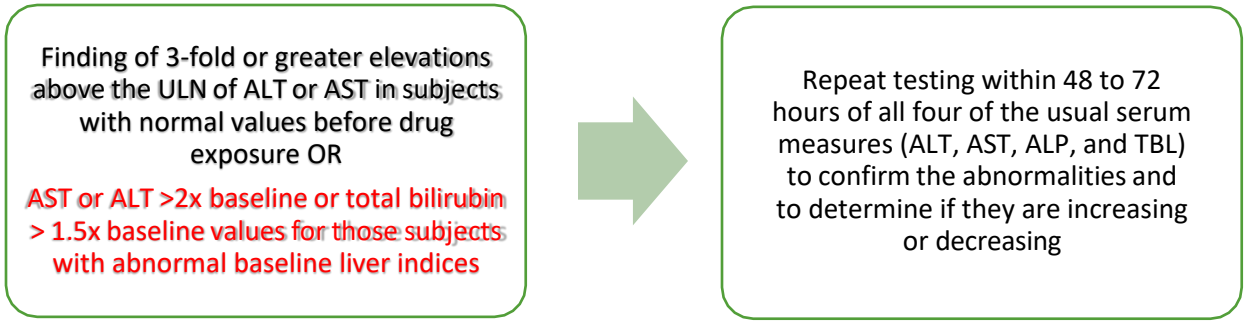
written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) as applicable, within the appropriate timeline (per institution policy) of the IRB's receipt of the report of the problem from the investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

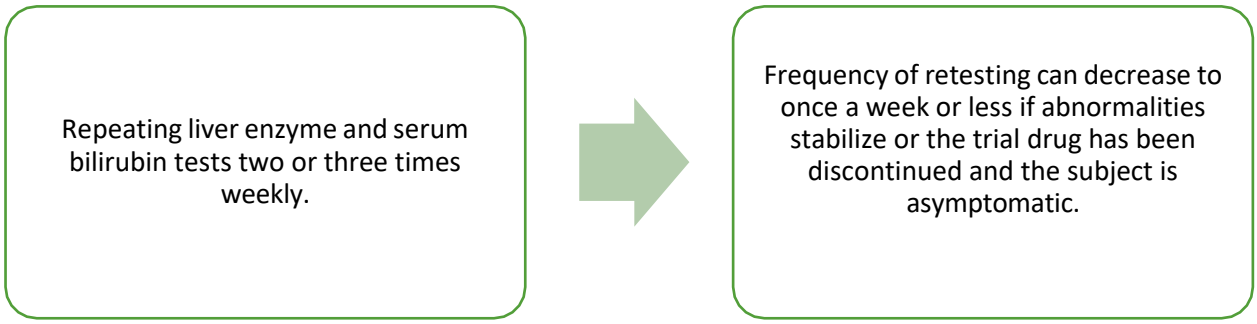
If an unanticipated problem results in a change to the informed consent form, all study participants will be provided with the updated information and re-consented with the new form.

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.
If subjects with abnormal baseline liver indices develop elevations of AST or ALT >2x baseline or total bilirubin > 1.5x baseline values during the study, repeat testing should be performed within 48-72 hours. If there are persistent elevations (AST or ALT>2x baseline or TBL >1.5 baseline values) upon repeat testing, then close observation (testing and physical examination 2-3 times per week) should be implemented, and discontinuation of drugs should be considered.
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:



If symptoms persist or repeat testing shows AT >3xULN for participants with normal baseline measures or AST or ALT>2x baseline or TBL >1.5 baseline values for participants with elevated values before drug exposure, it is appropriate to initiate close observation (testing and physical examination 2-3 times per week) to determine whether the abnormalities are improving, or worsening (or consider drug discontinuation) as follows:



- 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. **Dose Escalation**: Considering the nature of NP-101, Dose Level 6g is expected to be safe and to be declared as the minimum effective daily dose (MEDD) for the Phase IIb trial.
2. **Safety**: Safety and Tolerability of treatment with NP-101 at the MED given orally on an outpatient basis (study drug) will be comparable with taking placebo in participants with COVID-19 infection treated in the outpatient setting.
3. **Efficacy**: Treatment at the MED given orally on outpatient basis (study drug) will significantly increase the Day 5 Sustained Clinical Recovery rate compared to placebo in participants with COVID-19 infection treated in the outpatient setting.

Sustained Clinical Recovery (SCR) is defined as the continuous alleviation or resolution of all COVID-19 PRO Symptom Survey scores to ≤1 (“none” or “mild”), starting on or before

Day 5 after randomization and remaining so for at least 3 consecutive days and without subsequent relapse, defined as not progressing to "moderate" or "severe" for at least two consecutive days.

The secondary statistical hypotheses are as follows:

1. Study drug will significantly differentially reduce quantitative viral load from Randomization to Day 7 and Day 14 compared to placebo in participants with COVID-19 infection treated in the outpatient setting.
2. Study drug will significantly differently increase the percentage of negative/undetectable RT-PCR viral load at Day 7 and Day 14 in participants taking MED of NP-101 versus participants taking placebo in participants with COVID-19 infection
3. Study drug will significantly differently reduce the days to Sustained Clinical Recovery, days to Complete Clinical Recovery, and days to Complete Clinical Resolution compared to placebo in participants with COVID-19 infection.
4. Study drug will significantly differently increase the CD4+ and CD8+ T-cell concentrations on Day 7 and/or Day 14 compared to placebo in participants with COVID-19 infection.
5. Study drug will significantly reduce the disease progression defined as the worsening of the Covid-19 symptoms on Day 5 from "none" or "mild" to "moderate" or "severe" and remaining at that level for at least two days before alleviation or resolution, or the experience of hospitalization or death due to Covid-19 symptom worsening in participants taking the MED of NP-101 versus placebo.
6. Study drug will reduce the rate of Long-Covid symptoms compared to placebo.

- Primary Safety and Efficacy Endpoint(s) (Phase IIa and IIb):

1. **Dose Finding (Phase IIa)**: Number of Dose Limiting Toxicities (DLT) in NP-101 arm at each dose level compared to placebo and the safety threshold.
2. **Safety:** Number of overall adverse events, related adverse reactions, adverse events leading to discontinuation of study drug and hospitalizations, or deaths reported in participants taking the MED of NP-101 versus participants taking placebo. All AEs/SAEs will be captured throughout the study as per schedule of activities. As part of the primary safety assessment, treatment discontinuations will be compared between participants taking the MED of NP-101 versus participants taking placebo
3. **Efficacy:** Measurement of the difference of Sustained Clinical Recovery rates on Day 5 in participants taking the MED of NP-101 as compared to placebo.

- Secondary Efficacy Endpoint(s):

1. Measurement of the difference of the average viral load and cycle threshold measures from Randomization to Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo.

2. Measurement of the difference in the percentage of negative/undetectable RT-PCR (i.e., viral clearance) on Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo.
3. Estimate of the difference in days to Sustained Clinical Recovery, days to Complete Clinical Recovery, and days to Complete Clinical Resolution survivorship functions in participants taking the MED of NP-101 as compared to placebo. The time to sustained clinical recovery is defined as the number of days from randomization to the first of 3 consecutive days of alleviation or resolution of all symptoms to Mild or None.
4. Measurement of the difference of the average level of effector immune cells, specifically CD4+ and CD8 T-cells, from Randomization to Day 7 and Day 14 in participants with the MED of NP-101 versus those taking placebo
5. Measurement of the difference in the percentage of disease progression on Day 5 measured by the worsening of the Covid-19 symptoms from "none" or "mild" to "moderate" or "severe" and remaining at that level for at least two days before alleviation or resolution, or the experience of hospitalization or death due to Covid-19 symptom worsening in participants taking the MED of NP-101 versus placebo
6. Measurement of the difference in the percentage of Long-Covid symptoms in participants taking the MED of NP-101 versus placebo.

9.2 SAMPLE SIZE DETERMINATION

Phase-IIa:

Sample size for the dose-escalation phase of the trial will be 16 participants for each cohort and dose level of 3 g, 4.8 g and 6 g total daily doses, where 20 participants will be randomized in a 3:1 ratio (active:placebo) resulting in 15 participants assigned to theNP-101 arm and 5 to the Placebo arm such that at least 16 safety evaluable participants (12 NP-101 and 4 Placebo) can be obtained based on an attrition rate of 20-25%.

Phase-IIb:

1) For statistical analysis purposes, **Sustained Clinical Recovery (SCR)** is defined as the continuous alleviation or resolution of all COVID-19 PRO Symptom Survey scores to ≤1 ("none" or "mild"), starting on or before Day 5 after randomization and remaining so for at least 3 consecutive days and without subsequent relapse, defined as not progressing to "moderate" or "severe" for at least two consecutive days.

Phase IIb power calculation is based only on the second primary objective of the trial. Based on the Sustained Clinical Recovery rates estimated for high-risk participants treated in the prior BOSS-001 protocol Phase-II trial of TQ Formula (NP-101), a sample size of 200 participants randomized equally during phase IIb part of the study to NP-101 and Placebo Arms will yield at least 80% power to detect a 20% improvement in Sustained Clinical Recovery rate in participants treated at the MED of NP-101 compared to those treated with Placebo, in a group sequential design with one interim analysis when 100 evaluable participants are enrolled and completed their data for the primary objectives, with a total of 2.5% final Type-1 error rate. The reason for the interim analysis is to determine whether or not to stop the trial early if NP-101 does not provide sufficient evidence of activity or has an unacceptable safety profile, or stopping the trial early if NP-101 has higher efficacy than anticipated based on the literature and the earlier Phase-II study (BOSS-001) so that it can be made available to high-risk outpatient Covid-19 patients in a more timely manner. This prespecified interim analysis for early efficacy or futility will be carried out once 50% of the target enrollment of evaluable patients (n=100) in phase IIb had been followed through Day 30. This analysis will be based on the primary efficacy and safety endpoints according to prospectively set-stopping boundaries for efficacy and futility that will be

reviewed by regulatory agencies and reviewed by the DMC.

Considering a conservative attrition expectation of around 20% to account for patient dropouts and rare missing data for the primary endpoint, the planned total accrual for the primary efficacy objective is 248 participants, equally split as 124 participants in each arm. .

Definition of Evaluability for Primary Objectives: This double-blind, placebo-controlled, two-arm clinical trial is designed to enroll up to 248 patients during phase IIB part, 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 and have sufficient symptom data to assess the Day 5 Sustained Clinical Recovery status. Although all patients regardless of whether or not they receive the assigned medicine will be included in the primary analyses based on the intent-to-treat principle, evaluability- and adherence-based analyses will also be carried out.

Here are some endpoint combinations justifying our sample size for this study:

Table 9.2.1. Group Sequential Design scenarios with varying effect sizes

Response Rate in Placebo	Response Rate in NP-101	Minimum Sample Size Required	Sample Size with 20% Attrition	Sample size for the Interim Analysis	Stop Early for Futility (Z-Test)	Stop Early for Efficacy (Z-Test)	Final Decision Boundary (Z-Test)
0.20	0.40	168	208	104	0.688	2.737	1.935
0.25	0.45	182	224	112	0.71	2.722	1.935
0.30	0.50	192	240	120	0.693	2.736	1.935
0.35	0.55	198	248	124	0.712	2.723	1.935
0.40	0.60	200	248	124	0.695	2.736	1.935
0.45	0.65	198	248	124	0.712	2.723	1.935

The early stopping probabilities for a set of selected response combinations are provided below. At the targeted effect size, the early stopping probability for efficacy is 24%, for futility is 9%.

Table 9.2.2. Early Stopping probabilities with varying effect sizes

Response Rate Difference (Active-Placebo)	Early Stopping Probability for Efficacy	Early Stopping Probability for Futility	Total Early Stopping Probability
0	0.003	0.757	0.760
0.1	0.043	0.374	0.417
0.2	0.243	0.090	0.333
0.3	0.626	0.009	0.635

Participants will be randomly assigned to one of the two study arms. No stratifications for randomization will be used, however each enrollment center will receive the investigational products in sets of randomized blocks of 4. As a member of the DMC, an unblinded statistician will generate a randomization list and carry out the interim analysis as the study biostatistician will remain blinded to the study data until after the final database lock or until early efficacy or early futility decisions are recommended by the independent statistician.

9.3 POPULATIONS FOR ANALYSES

- Full Analysis Set (FAS): All randomized participants regardless of whether they receive any study intervention. This corresponds to Intent-to-treat (ITT) analysis without any

modification.

- Full Analysis Set for the subset of randomized participants whose COVID-19 symptoms started within 3 days from randomization (sub-FAS): All participants who were randomized within 3 days of Covid-19 symptom onset regardless of whether they receive any study intervention.
- Safety Analysis (SA) Dataset: All randomized participants who receive at least one dose of study intervention.
- Safety Analysis with Covid-19 related concomitant medications (SA-ConMed) Analysis Dataset: These safety analysis datasets may have multiple versions depending on the combination of Covid-19 related concomitant medications that the patients used along with NP-101 from enrollment date to off-study date. Due to randomization, concomitant medication types and dosages are expected to be distributed similarly between the two study arms.
- Modified Intent-to-Treat-1(mITT1): All randomized participants regardless of whether they receive any study intervention, who had sufficient self-reported symptom data so that the sustained clinical recovery on Day 5 can be assessed.
- Modified Intent-to-Treat-2(mITT2): All participants who were randomized within 3 days of Covid-19 symptom onset regardless of whether they receive any study intervention, who had sufficient self-reported symptom data so that the sustained clinical recovery on Day 5 can be assessed.
- Per-Protocol (PP) Analysis Dataset: The Per-Protocol Analysis Set (PP) is defined as all participants in the FAS who completed all visits up to and including Day 14, and were compliant with study medication at ≥ 85% adherence and do not have any major protocol violation. Major protocol violations will be identified prior to breaking the blind.
- Per-Protocol (PP) Analysis with Covid-19 related concomitant medicines (PP-ConMed) Analysis Datasets: These analysis datasets may have multiple versions depending on the combination of Covid-19 related concomitant medications that the patients used along with NP-101 from enrollment date to off-study date.

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 Fisher's Exact test. Cochran Armitage trend-test may be included among the possible tests to investigate the 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 (namely, minimum, first quartile (Q1), median, third quartile (Q3), 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-Mann-Whitney 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), which will be prepared, finalized, and submitted to FDA before database lock. In this protocol document, a general analysis and statistical testing approach will be provided, and the details will be left for the study SAP. Should the analyses specified in the SAP differ from those described in the protocol, the methodology in the SAP will prevail.
In all comparisons and tests for the secondary and exploratory aims, a two-sided p-value will be used and a Type-1 error rate of 0.05 will be used to indicate significance. As there is a single estimand for the primary efficacy objective, no multiplicity correction is needed, and conclusions will be based on the decision boundaries provided as part of the statistical design presented above in Section 9.2 Sample Size Determination.

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 SAFETY AND EFFICACY ENDPOINT(S)

Primary analysis for Phase-IIa:
Primary Estimand for Phase-IIa is the number of Dose Limiting Toxicities (DLT) in NP-101 arm compared to Placebo and the safety threshold. Based on the number of DLTs observed, a Minimum Effective Daily Dose (MED) of NP-101 will be determined.
Phase-IIa portion of this study is a randomized dose-escalation trial, where the enrollment for the initial two dose levels, namely, 3g and 4.8g daily dose, will be started simultaneously based on safety data in literature as well as our previous randomized Phase-II study utilizing 3g daily dose of NP-101.

Definition of Dose Limiting Toxicity (DLT): A Dose Limiting Toxicity (DLT) is defined as any Grade 3+ adverse events that occurred within the first 21 days from randomization and are found to be attributable to the study regimen and not resolved through symptomatic treatments within 48 hours.
DLT Observation Period: In this study, DLT observation period will be the initial 21 days from randomization.
Evaluability for Safety Assessment: Any patient who received at least one dose of the assigned study medication will be considered evaluable for the NP-101 safety assessment.

At each dose level, up to 20 participants will be randomly assigned in a 3:1 ratio to NP-101 and Placebo in a double-blind fashion, resulting in up to 15 participants being treated with NP-101 and up to 5 patients being treated with Placebo. Number of patients with DLTs will be counted dynamically without unblinding the random assignments on a continuous basis. At the same time, the study database will be also counting the number of evaluable patients with DLTs in NP-101 arm ($N_{DLT,3g,\ NP101}$, $N_{DLT,4.8g,\ NP101}$, $N_{DLT,6g,\ NP101}$, respectively for dose levels 3g, 4.8g. and 6g, which are back-end variables only visible to PV Committee) dynamically and independently at each dose level being investigated.
A dose level with at most two NP-101 patients having DLTs will be considered safe, and a dose-escalation or MED decision will be made automatically by the database system and a decision report will be provided to the PV Committee and the study team accordingly.

- If 3 g is found to be NOT safe, dose de-escalation to 2.4 g will occur.

- If Dose Level 3g is found to be safe and Dose Level 4.8g is found to be NOT safe, then Dose Level 3g will be considered to be MED and enrollment in Phase-IIb trial will be initiated automatically at Dose Level 3g.
- If both Dose Levels 3g and 4.8g are found to be safe, enrollment to Dose Level 6g will be initiated automatically.
- If both Dose Level 3g and 4.8g are found to be safe while Dose Level 6g was found to be NOT safe, then Dose Level 4.8g will be considered to be MED and enrollment in Phase-IIb trial will be initiated automatically at Dose Level 4.8g.
- If all dose levels are found to be safe, then Dose Level 6.0 will be considered to be MED and enrollment in Phase-IIb trial will be initiated automatically at Dose Level 6g.
- In the very unlikely event that Dose 4.8g is found to be safe while Dose 3g is found to be UNSAFE, the DMC will more closely investigate the attribution of these DLTs to NP-101 considering detailed patient characteristics; this may require protocol amendment to add further safety measures to address these safety concerns. The DMC will provide their final decision of dose escalation, de- scalation, or declaring a dose-level to be the MED to the study team based on their assessment and this decision will be manually coded in the study database to manage the future enrollment.

Decision flow-chart for dose-finding:

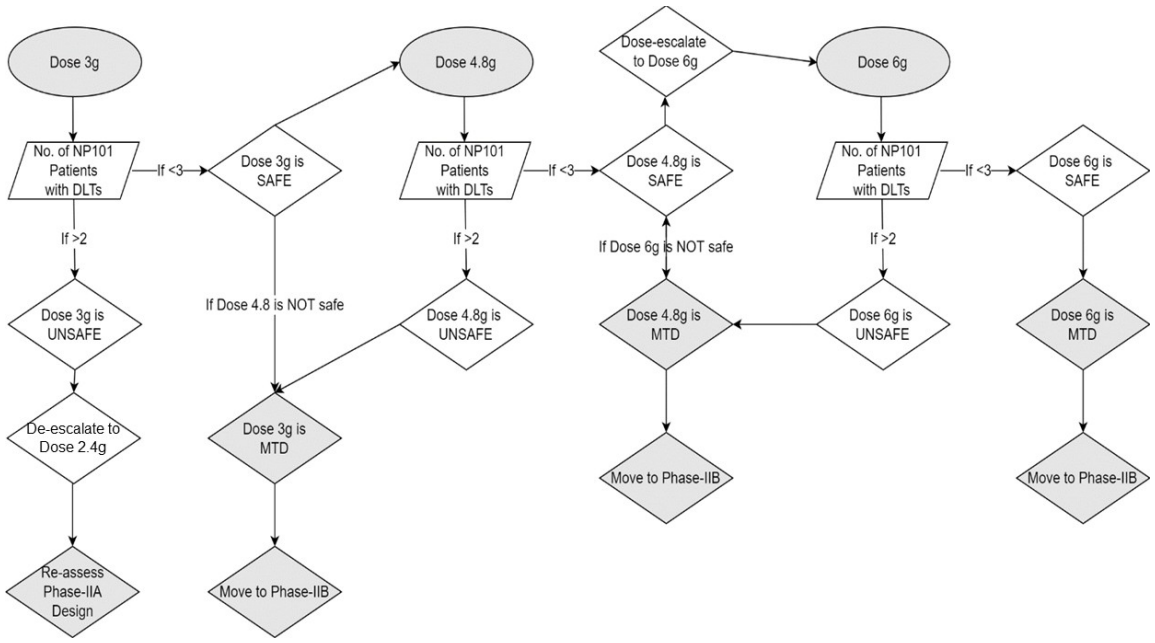


Figure 9.4.2.

The above DLT 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.The study protocol database will be built on a flexible data-capture and decision-making platform. The following detailed decision-making algorithm will be utilized to generate seamless DLT monitoring and safety summary reports for the review of the PV committee (unblinded to the random assignments) as well as the study team without unblinding. Details of the decision algorithm for the dose-finding phase of the protocol has been provided in the Statistical Analysis Plan (SAP)

As part of the Phase-IIa safety assessment, frequencies of overall adverse events, related adverse

reactions, adverse events leading to discontinuation of study drug and hospitalizations, or deaths reported in participants taking the Selected Dose of NP-101 versus participants taking placebo will be tabulated and compared for each dose level investigated.

Primary analysis for Phase-IIa/IIb:

Primary Estimand-1 for the safety objective is the difference of frequencies of overall adverse events, related adverse reactions, adverse events leading to discontinuation of study drug and hospitalizations, or deaths reported in participants taking the MED of NP-101 versus participants taking placebo regardless of whether they actually receive their assigned therapy (ITT) and assuming that their hospitalization and treatment discontinuation rates will be similar. Possible intercurrent events for this estimand can be, though expected to be quite rare, hospitalization (i.e., transitioning from outpatient status to inpatient status) and treatment discontinuation, both of which may lead to potential missing data or change in the primary measures. Every effort will be put forward to capture any adverse events from Day 1 to Day-21 as well as on the final Safety call on Day 45 from randomization, especially during. possible intercurrent events. Therefore, hospitalization-free, while-on-study and per-protocol treatment versions of the above estimand will also be considered within each population definitions above. Safety cohorts will be redefined based on Covid-19 related concomitant medications combinations and the safety endpoints will be compared between the two arms of the study in these ConMed-specific subcohorts as well if the size of these safety sub-cohorts allows such analyses.

Primary estimand-2 aims at the assessment of efficacy of the proposed therapy and is defined as difference of Sustained Clinical Recovery rates on Day 5 in participants taking the MED of NP-101 as compared to placebo regardless of whether they actually receive their assigned therapy (ITT) and assuming that their hospitalization and treatment discontinuation rates will be similar. Possible intercurrent events for this estimand can be, though expected to be quite rare, hospitalization (i.e., transitioning from outpatient status to inpatient status) and treatment discontinuation, both of which may lead to potential missing data or change in the primary measures. The study participants will be reminded to submit their symptom questionnaires even during and after these intercurrent events, if any, to avoid missing data as much as practically possible. As the patient-reported symptom outcomes are captured through eCRFs online, such missing data is expected to be limited. Hospitalization-free, while-on-study and per-protocol treatment versions of the above estimand will also be considered as defined above.

Sustained Clinical Recovery rate will be estimated at Day 5 as the ratio of those who achieve improvement of all symptoms to Mild and None and remained so for at least three days to all participants treated in each arm. 95% Clopper-Pearson Confidence Intervals will be constructed for Sustained Clinical Recovery Rate for each arm, Wald Asymptotic Conference Interval will be constructed for the Sustained Clinical Recovery Rate difference between the two arms, and Z-test will be used to assess the significance of the difference between the two arms. Although all analyses will be conducted based on the intent-to-treat context, sensitivity analyses based on the adherence to the protocol regimen and based on different missing data imputation approaches will also be carried out for the primary as well as secondary objectives. In addition, per-protocol efficacy cohorts will be redefined based on Covid-19 related concomitant medications combinations and the efficacy endpoints will be estimated and compared between the two arms of the study in these ConMed-specific subcohorts if the size of these efficacy sub-cohorts allows such analyses.

Missing Data Handling Strategy for the Primary Objective

We do not expect excessive missing data for the primary objective data considering the safeguards in

place in the protocol as described in Section 4.1 (subsection Patient Reported Outcomes). When appropriate, Last-Value-Carried-Forward (LVCF) imputation approach will be utilized for the missing symptom values especially in the symptom questionnaire as the patient-reported symptoms will be captured from randomization through Day 30 and intermittent missing values can be imputed via the LVCF approach. For the primary efficacy objective, symptoms reported up to Day 5 will be critical to assess clinical recovery and the symptoms reported from Day 5 to Day-8 are critical to assess the 'sustained' response. As this is a study with a short span of enrollment and treatment windows (14-day therapy, 21-day symptom assessment), we do not anticipate excessive missing values in symptoms especially within the first 8 days where the participants are expected to be more engaged with the study; however, missing data 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 event of MCAR and MAR, sensitivity analyses will be carried out by using Multiple Imputation (MI) techniques when appropriate. In the presence of missing data, we will generate at least 20 multiply-imputed samples using the MI procedure of SAS ® and will combine the estimates using the MIANALYZE procedure of SAS ® Version 9.4 (Cary, North Carolina, USA). Sensitivity analyses will also include hospitalization-free, while-on-study, and per-protocol treatment cohorts as described above.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

None of the following secondary endpoints depend on the findings of the primary endpoint.

The first secondary objective is "To compare the change in viral load and cycle threshold profiles from Randomization to Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo. Secondary estimand-1 is defined as the difference of the average viral load and cycle threshold measures from Randomization to Day 7 and Day 14 in participants taking the MEDNP-101 as compared to placebo regardless of whether they actually receive their assigned therapy (ITT) and assuming that their hospitalization and treatment discontinuation rates will be similar. The viral load and cycle threshold will be measured at Baseline, on Day 5, and on Day 14; therefore, the outcome variable of interest will be a longitudinal ratio-scale measure. The difference of viral load and cycle threshold between the two arms of the study will be separately compared using Wilcoxon-Mann-Whitney test for Day 5 and Day 14. Although the baseline distributions of viral load and cycle threshold are expected to be similar due to randomization, Analysis of Covariance (ANCOVA) models will also be established for the log (viral load) and cycle threshold according for the baseline measurements. The above mentioned intercurrent events, namely, hospitalization (i.e., transitioning from outpatient status to inpatient status) and treatment discontinuation, are applicable for Secondary estimand-1 as well. Therefore, hospitalization-free, while- on-study and per-protocol treatment versions of the above estimand will also be considered within each analysis set defined above.

Secondary estimand-2 is defined as the measurement of the difference in the percentage of negative/undetectable RT-PCR (i.e., viral clearance) on Day 7 and Day 14 in participants taking the MED of NP-101 as compared to placebo regardless of whether or not they actually receive their assigned therapy (ITT) and assuming that their hospitalization and treatment discontinuation rates will be similar. To address this estimand, the difference of RT-PCR negative/undetectable rates between the two study arms will be estimated along with 95% Wald Asymptotic Conference Interval, and Z-test will be used to assess the significance of the difference between the two arms on Day 5 and Day 14 visits. The above mentioned intercurrent events are applicable for Secondary estimand-2 as well. Therefore, hospitalization-free, while-on-study and per-protocol treatment versions of the above estimand will also be considered within each analysis set defined above.

Secondary estimand-3 will be the difference in days of Sustained Clinical Recovery survivorship function in participants taking the MED of NP-101 as compared to placebo. Distribution of the days to Sustained

Clinical Recovery for the treatment and placebo arms will be estimated using the Kaplan-Meier method and compared using the Log-rank test between the two study arms.

If the proportional hazards assumption is not satisfied, Wilcoxon-Gehan test will be used. Events will be defined as participants that attain Sustained Clinical Recovery while on the follow-up window and days to event will be calculated as the days from randomization to the first of the three consecutive days of Clinical Recovery, that is, the first day when all symptoms are resolved to at most “mild” or “none” and remained so for at least two additional days for a total of three consecutive days.

Participants who do not attain clinical response while on the follow-up window will be censored on Day 30 or on the day when they are taken off study before Day 30 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 with respect to the analysis datasets defined above.

Secondary estimand-4 is the difference between the average level of effector immune cells, specifically CD4+ and CD8 T-cells, from Randomization to Day 7 and Day 14 in participants taking the MED of NP-101 versus those taking placebo. These markers will be compared for between the arms using Wilcoxon-Mann-Whitney test at each visit. To compare the change over time in these markers, Random Coefficients Models will be utilized where each of these markers will be the dependent variable and study arm and time (i.e., days from enrollment) will be main predictors.

The secondary estimand-5 is the difference in the percentage of disease progression on Day 5 measured by the worsening of the Covid-19 symptoms from “none” or “mild” to “moderate” or “severe” and remaining at that level for at least two days before alleviation or resolution, or the experience of hospitalization or death due to Covid-19 symptom worsening in participants taking the MED of NP-101 versus placebo. To address this estimand, the disease progression rate difference between the two study arms will be estimated along with 95% Wald Asymptotic Conference Interval, and Z-test will be used to assess the significance of the difference between the two arms on Day 5. These analyses will be conducted under each analysis datasets defined above.

The secondary estimand-6 is the difference in the percentage of Long-Covid symptoms in participants taking the MED of NP-101 versus placebo. To address this estimand, the Long-Covid symptom rates difference between the two study arms will be estimated along with 95% Wald Asymptotic Conference Interval, and Z-test will be used to assess the significance of the difference between the two arms on Day 5 for each subdomain of the Long-Covid symptom questionnaire. These analyses will be conducted under each analysis datasets defined above.

The details of the above statistical analysis approaches and the analysis plan for the exploratory aims will be provided in the accompanying Statistical Analysis Plan (SAP) of the study.

9.4.4 SAFETY ANALYSES

As safety assessments are primary objectives both in Phase-IIa and IIb, the details of the safety analysis have been provided in the analysis section above in Section 9.4.2.

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9.4.5 BASELINE DESCRIPTIVE STATISTICS

The NP-101 and Placebo arms will be compared in terms of the baseline demographics and clinical characteristics. Due to randomization, no more than 1 in 20 factors and variables based on 5% Type-1 error rate is expected to be significantly different between the two arms. Any baseline markers or factor

that are significantly different at baseline between the two arms may be controlled for in the analyses for primary and secondary endpoints if they have any clinical relevance in those models.

9.4.6 PLANNED INTERIM ANALYSES

There is one interim analysis to be conducted by the DMC using the accumulated data of the initial 100 evaluable patients of the Phase IIb portion of the study. Here, evaluability of a patient will require sufficient symptom data to be able to assess the sustained clinical recovery status on Day 5. The primary statistical test for the primary efficacy endpoint in the interim analysis will be the continuity-corrected Z-test for the Sustained Clinical Recovery rate difference between the two arms as presented in Table 9.2.1. If the Z-test statistic value is greater than or equal to 2.737, the early efficacy of NP-101 will be shown significantly; on the other hand, if the Z-test statistic value is less than or equal to 0.688, the early futility of NP-101 will be shown significantly; under these conditions, the sponsor may ask for the study to be stopped early for efficacy or futility based on the evidence as the reversal of such effects with the expansion of the trial to the complete sample size is very unlikely. If the Z-test statistic value is between 0.688 and 2.737, then the enrollment will continue to the final sample size.
Secondary and exploratory objectives will not be analyzed at the time of the interim analysis until the accumulated data recommends early stopping.

This prespecified planned interim analysis for early efficacy or futility will occur once 50% of evaluable participants (n=100) in phase IIb have been followed through Day 30. These analyses will be based on the primary efficacy and safety endpoints according to pre-set-stopping rules that have been programmatically defined. The interim analysis process will be governed by the DMC.

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 regarding the effect of NP-101 on inflammatory cytokines and coagulation factors at Days 1, 5, and 14 in participants, and a plan to construct a risk prediction model for Sustained Clinical Recovery based on known risk factors as well as other patient and disease characteristics in participants. Analyses to address these 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

10.2 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 electronic and/or 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 provided to the participant electronically and documentation of the informed consent process is required prior to starting intervention/administering study intervention.

10.3 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. Electronic consent forms will be used; however, paper copies will be available for participants who require them. These will be approved by the Institutional Review Board (IRB) and the participant will be asked to read and review the document electronically . The investigator will explain the research study to the participant and answer any questions that they may have. 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.
- 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.
- A copy of the informed consent document will be given to the participants for their records

10.3.1 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 participants in each of the dose-escalation cohort during the Phase-IIa component of the trial experience Grade 3+ adverse events attributable to the study regimen and not resolved through symptomatic treatments within 48hours, 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.
- Life-threatening serious adverse events (grade 4 CTCAE) not attributable to COVID or death (grade 5 CTCAE) possibly related to study medication.
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

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).

10.3.2 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.

10.3.3 FUTURE USE OF STORED SPECIMENS AND DATA

Samples collected for this study will be analyzed and stored. 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 a participant withdraws from the study, samples collected prior to the date of withdrawal may still be analyzed, unless the participant specifically requests that the samples be destroyed, or local laws require destruction of the samples. However, if samples have been tested prior to withdrawal, results from those tests will remain as part of the overall research data. The participant may request return of his/her sample(s) at any time through official study closure.

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

10.3.4 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator (s)	**Medical Monitor**
Enrique Villa, M.D.	Hassan Benchqroun, MD

10.3.5 SAFETY OVERSIGHT

Safety oversight will be under the direction of the pharmacovigilance team, the medical monitor, the DMC and the principal investigators at the sites.

10.3.6 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 compliant 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 experienced monitors.
- 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.

10.3.7 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control (QC) procedures will be implemented from the beginning with the data entry system such as testing the database with mock data, extraction, mock analyses, etc., and data QC checks will be conducted periodically to identify data gaps and data quality issues on time and corrective action will be developed on a timelier manner. Due to these proactive steps, missing data is expected to be rare both in terms of patient-reported symptom data, as well as adverse events capturing and bio-sample collection/processing. 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, and multiple imputation approach will also be considered when appropriate. Missing data structure will be assessed as to whether the data is Missing Completely at Random (MCAR), or Missing at Random (MAR), or Not-Missing at Random (NMAR). In the event of MCAR and MAR, sensitivity analyses will be carried out by using Multiple Imputation (MI) techniques when needed. Further details of the missing data handling details will be provided in the accompanying SAP of the protocol.

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 should be conducted prior to and during the study to train examiners and maintain acceptable intra- and inter-examiner agreement.

10.4 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Each participating site will maintain appropriate medical and research records for this trial. 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.

Data should be entered directly into the database which is considered as source. If this is not possible, then 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.

If data recorded in the electronic database is derived from source documents then that data 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 activities 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 either directly or from the source documents.

10.5 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.

10.5.1 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.

10.5.2 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.

10.5.3 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.

10.5.4 ADDITIONAL CONSIDERATIONS

This section is not applicable.

10.5.5 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance

BSC	Best Supportive Care
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
DLT	Dose Limiting Toxicity
DHHS	Department of Health and Human Services
DMC	Data Monitoring Committee
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
MED	Minimum Effective Dose
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
SCR	Sustained Clinical Recovery
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	Standard of Care
SOP	Standard Operating Procedure
TDD	Total Daily Dose
UP	Unanticipated Problem
US	United States

10.5.6 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
BOSS 002-06-28-2022 V2	6-28-22	1) Clarification 9.4.4 2) Clarification of symptom assessment period 3) Various typos 4) Removal of DMC, replace with pharmacovigilance team 5) Addition of language reflecting electronic database functionality	Clarity and ease of understanding
BOSS 002-08-08-2022-V3	8-8-22	1) Addition of primary objectives for IIa 2) Additional statistical analysis information added 3) Additional language regarding recruitment through pharmacies and retailors 4) Updated TOC page numbers	Provide clarity and accuracy
BOSS 002-10-31-2022 – V4	10-31-22	1) Added BSC – Best Supportive Care 2) Removed Exclusion #2 3) Accepted antivirals for low and mod severity 4) Revise statistical strategy due to 2 and 3	Improve study design and analysis
BOSS 002-5-11-22		1)Replacement of PV team with DMC 2)Addition of a Day 30 visit 3) Addition of a conjunctivitis questionnaire 4) Addition of a long Covid questionnaire 5) Changes to statistical analysis	Improve clarity, accuracy, design and analytics

		6) Correction of typos	

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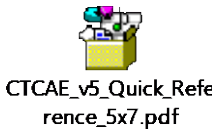
APPENDIX 1 – COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (CTCAE) V5.0

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



APPENDIX 2 – PRO – Symptom Survey- Part I

PRO – Symptom Survey Pt 1

Your Symptoms	Your Rating Please select one
1. How would you rate the severity of your stuffy or runny nose?	None Mild Moderate Severe
2. How would you rate the severity of your sore throat?	None Mild Moderate Severe
3. How would you rate the severity of your shortness of breath (difficulty breathing)	None Mild Moderate Severe
4. How would you rate the severity of your cough?	None Mild Moderate Severe
5. How would you rate the severity of your low energy or tiredness?	None Mild Moderate Severe
6. How would you rate the severity of your muscle or body aches	None Mild Moderate Severe
7. How would you rate the severity of your headache?	None Mild Moderate Severe
8. How would you rate the severity of your chills or shivering?	None Mild Moderate Severe
9. How would you rate the severity of you feeling hot or feverish?	None Mild Moderate Severe
10. How would rate the severity of your nausea? (Feeling like you wanted to throw up)	None Mild Moderate Severe
11. How many times did you vomit (throw up) in the last 24 hours?	I did not vomit at all 1 – 2 times 3 – 4 times 5 or more times

12. How many times did you have diarrhea (loose or watery stools) in the last 24 hours?	I did not have diarrhea at all 1 – 2 times 3 – 4 times 5 or more times
13. Rate your sense of smell in the last 24 hours	My sense of smell is THE SAME AS usual My sense of smell is LESS THAN usual I have NO sense of smell
14. Rate your sense of taste in the last 24 hours	My sense of taste is THE SAME AS usual My sense of taste is LESS THAN usual I have NO sense of taste
15. In the past 24 hours, have you returned to your usual health? (before your COVID – 19 illness)	Yes or No
16. In the past 24 hours, have you returned to your usual activities? (before your COVID – 19 illness)	Yes or No
17. In the past 24 hours, what was the severity of your overall COVID – 19 related symptoms at their worst?	None Mild Moderate Severe
18. It was easy to report my symptoms using this survey.	Agree Disagree_____

APPENDIX 2 b – PRO – Symptom Survey-Part II

Patient ID _____ Date of Completion _____

PT II PRO SYMPTOM SURVEY

Long COVID Symptom Scale

Since completing my last questionnaire, I am experiencing the following symptoms which are either continuing or are new to me:

Symptoms	None	Mild	Moderate	Severe
Tiredness or fatigue that interferes with daily life	☐	☐	☐	☐
Symptoms that get worse after physical or mental effort	☐	☐	☐	☐
Fever	☐	☐	☐	☐
*Difficulty breathing or shortness of breath	☐	☐	☐	☐
*Cough	☐	☐	☐	☐
Chest Pain	☐	☐	☐	☐
Fast-beating or pounding heart	☐	☐	☐	☐
Difficulty thinking or concentrating (sometimes referred to as “brain fog”)	☐	☐	☐	☐
*Headache	☐	☐	☐	☐
Sleep problems	☐	☐	☐	☐
Dizziness when you stand up (lightheadedness)	☐	☐	☐	☐
Pins-and-needles feelings	☐	☐	☐	☐
*Change in smell or taste	☐	☐	☐	☐
Depression or Anxiety	☐	☐	☐	☐
*Diarrhea	☐	☐	☐	☐
Stomach Pain	☐	☐	☐	☐
*Joint or Muscle pain	☐	☐	☐	☐
Rash	☐	☐	☐	☐
Changes in Menstrual cycle **N/A** ☐	☐	☐	☐	☐

*Questions in grey are represented in both Pt 1 and Pt II of the surey. Answers are captured in Part I only.

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APPENDIX 2c – Conjuctivitis Questionnaire

Patient ID _____ Date of Completion _____

Conjunctivitis Symptom Scale

Since becoming infected with Covid 19, I have developed the following conjunctivitis (pink eye) symptoms, which are new to me:

Symptoms	None	Mild	Moderate	Severe
Pink or red color in the white of the eye(s)	☐	☐	☐	☐
Swelling of the eye(s) and/or eyelid(s)	☐	☐	☐	☐
Increased tear production	☐	☐	☐	☐
Feeling like a foreign body is in the eye(s) or an urge to rub the eye(s)	☐	☐	☐	☐
Itching, irritation, and/or burning	☐	☐	☐	☐
Discharge of pus or mucus	☐	☐	☐	☐
Crusting of eyelids or lashes, especially in the morning	☐	☐	☐	☐
For Contact lens users only: Contact lenses that feel uncomfortable and/or do not stay in place on the eye	☐	☐	☐	☐

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.

APPENDIX 4 – PROHIBITED MEDICATIONS*

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
- EfavirenzElvitegravir
- 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.

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

Class	Anti-inflammatories	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-Hydro-xytryptamine Linoleic acid	Mestranol Fluvastatin _9-Tetrahydro-cannabinol (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.**

± It is prohibited to treat patients concomitantly with any of the medications listed under Appendix 5 while trial participants are receiving NP-101 or placebo. Thus, the use of any of these medications listed in Appendix 5 is prohibited from the first dose of NP-101 and placebo pills and until the last dose on day 14 per protocol. If the randomized participant cannot temporarily interrupt these prohibited medications during this specific time, they should not be enrolled and should be considered ineligible.