Official Title:

Clinical Study Protocol

A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study on the Safety and Efficacy of Niclosamide in Patients with COVID-19 with Gastrointestinal Infection

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Date of Document

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CLINICAL STUDY PROTOCOL

A 2-Part, 2-Arm, Phase 2, Randomized, Double-Blind, Placebo-Controlled Study on the Safety and Efficacy of Niclosamide in Patients with COVID-19 with Gastrointestinal Infection

Study Name	**RESERVOIR**: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study on the Safety and Efficacy of Niclosamide in Patients with COVID-19 with Gastrointestinal Infection
Protocol Number	AZ-NICL-COV-1
Investigational Product	Niclosamide
Phase	Phase 2
Sponsor	AzurRx BioPharma, Inc. 777 Yamato Road, Suite 502 Boca Raton, FL 33431
Protocol Date	15 December 2021
Protocol Version	3.1
Previous Versions	1.0, August 17, 2020 1.1, March 08, 2021 1.2, March 29, 2021 1.3, April 08, 2021 1.4, April 28, 2021 1.5, May 14, 2021 2.0, May 27, 2021 3.0, July 30, 2021

1 PROTOCOL APPROVAL SIGNATURES

Protocol Title: A 2-Part, 2-Arm, Phase 2, Randomized, Double-Blind, Placebo-Controlled Study on the Safety and Efficacy of Niclosamide in Patients with COVID-19 with Gastrointestinal Infection

Study Name: **RESERVOIR**: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study on the Safety and Efficacy of Niclosamide in Patients with COVID-19 with Gastrointestinal Infection

Protocol Number: AZ-NICL-COV-1

This study will be conducted in compliance with the clinical study protocol (and amendments), International Council for Harmonisation (ICH) guidelines for current Good Clinical Practice (GCP), and applicable regulatory requirements.

Sponsor Signatory

James Pennington, MD
Chief Medical Officer
AzurRx BioPharma, Inc.
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Boca Raton, FL 33431
USA

James Pennington
James Pennington (Dec 28, 2021 13:22 PST)

Signature

December 27, 2021

Date

2 DECLARATION OF INVESTIGATOR

I have read and understood all sections of the protocol entitled "A 2-Part, 2-Arm, Phase 2, Randomized, Double-Blind, Placebo-Controlled Study on the Safety and Efficacy of Niclosamide in Patients with COVID-19 with Gastrointestinal Infection" and the accompanying investigator's brochure.

I agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the Final Protocol Version 3.0, dated 30 July 2021, the International Council for Harmonisation harmonised tripartite guideline E6(R2): Good Clinical Practice and all applicable government regulations. I will not make changes to the protocol before consulting with AzurRx BioPharma, Inc. or implement protocol changes without independent ethics committee approval except to eliminate an immediate risk to participants. I agree to administer study treatment only to participants under my personal supervision or the supervision of a sub investigator.

I will not supply the investigational product to any person not authorized to receive it. Confidentiality will be protected. Participant identity will not be disclosed to third parties or appear in any study reports or publications.

I will not disclose information regarding this clinical investigation or publish results of the investigation without authorization from AzurRx BioPharma, Inc.

Signature of Principal Investigator	Date

Printed Name of Principal Investigator

3 PROTOCOL SYNOPSIS

Name of Sponsor/Company: AzurRx BioPharma, Inc.	
Name of Investigational Product: Niclosamide	
Protocol Number: AZ-NICL-COV-1 (Niclosamide in COVID-19)	
Study Name: RESERVOIR: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study on the Safety and Efficacy of Niclosamide in Patients with COVID-19 with Gastrointestinal Infection	
Study Sites: This study will be conducted at approximately 30 sites globally.	
Number of Patients: Up to approximately 168	**Phase of Development:** 2

Objectives

The primary objective for Part 1 of the study is to evaluate the safety of niclosamide administered to patients with COVID-19.

The primary objective for Part 2 of the study is to evaluate the effect of niclosamide in addition to Standard of Care (SoC) compared to placebo in addition to SoC on fecal clearance of SARS-CoV-2 RNA.

The secondary objective of the study is to evaluate the clinical efficacy, safety, and tolerability of oral niclosamide in addition to SoC compared to placebo in addition to SoC.

The exploratory objectives include characterization of the effect of niclosamide on disease progression and severity 4 and 6 months after starting treatment, and possible genotypic resistance analysis using the nasopharyngeal swabs and/or stool samples

Study Design

This is a 2-part, 2-arm, Phase 2, multicenter, randomized, double-blind, placebo-controlled study in adults with COVID-19.

Part 1 will enroll approximately 9 patients. After treatment is concluded for all 9 patients, a Data Monitoring Committee (DMC) will review the safety data and determine if the study may proceed to Part 2 or if additional patients are needed to assess tolerability of the study drug. If additional patients are needed, approximately 9 more patients will be enrolled in Part 1, and then the DMC will meet again after all patients are treated to determine if the study may proceed to Part 2.

Part 2 will enroll up to approximately 150 patients receiving at least 1 dose of study treatment.

Each part will include 2 study arms with randomized enrollment. Randomization of patients in Part 2 will be stratified by age and sex.

Inclusion Criteria

Subjects meeting all the following inclusion criteria will be considered eligible for the study:

1. Patients who give their written consent for participation in the study and for personal data processing and are willing and able to comply with all study procedures.
2. Patients of any gender who are at least 18 years of age.
3. Part 1 only: Patients with a primary diagnosis of COVID-19, with or without pneumonia, who agree to be monitored daily for at least 7 days after randomization and who accept continuing to be assessed for the study procedures.
4. Part 2 only: Patients with a primary diagnosis of COVID-19, with or without pneumonia.
5. Patients who are reasonably expected to maintain and adhere to SoC treatments prescribed by their physician.
6. Patients who had SARS-CoV-2 RNA presence in nasopharyngeal swab ≤5 days before randomization as confirmed by local or central laboratory. The assay used for testing at the laboratory should be approved or authorized for use by the FDA or local regulatory agency for diagnosing patients with COVID-19 at the time the test is used.
7. Patients who are considered reliable and capable of adhering to the protocol, according to the judgment of the investigator.

Exclusion Criteria

Patients will be excluded from the study if one or more of the following criteria are applicable:

1. At the time of randomization, patients who require intensive care unit (ICU) admission or patients with severe respiratory insufficiency who require mechanical ventilation or with rapid worsening of respiratory function leading to expectation for mechanical ventilation or ICU admission.
2. Evidence of rapid clinical deterioration or existence of any life-threatening co-morbidity or any other medical condition that, in the opinion of the investigator, makes the patient unsuitable for inclusion.
3. Patients who, at the time of enrollment, are not in a clinical condition compatible with the oral administration of the study drug.
4. Patients with serum alanine transaminase (ALT) or aspartate transaminase (AST) >3 times upper limit of normal (ULN) detected within 24 hours prior to randomization or with other evidence of severe hepatic impairment (Child-Pugh Class C).
5. Patients with an estimated GFR (eGFR) ≤30 mL/min/1.73m^2 (based on CKD-EPI formula) at screening.
6. Patients with a history of hypersensitivity or allergy to any component of the study drug.
7. Enrollment in another concurrent clinical interventional study, intake of an investigational drug within 30 days prior to randomization, or intake of an investigational drug for COVID-19 within 3 months prior to randomization. Drugs approved under a US Food and

Drug Administration Emergency Use Authorization are not considered investigational under this exclusion.

8. Pregnant or lactating women or women with a positive pregnancy test (urine or serum).

Concomitant and Prohibited Medications

Concomitant Medications:

Other than study drugs, SoC as directed by the patient's physician will be allowed. Vaccination status for COVID-19 will not influence eligibility for the study and will be considered SoC while the patient is on study. After randomization and treatment allocation, the SoC therapy can be modified by the investigator according to the patient's needs.

In case of worsening or need of mechanical ventilation or admission to ICU, patients will be treated with any medication or treatment based on the physicians' judgment, without any constraint from the sponsor, including, but not limited to, antibiotics, antimycotics, steroids, alpha interferon, and immunoglobulins.

Prohibited Medications:

Investigational drugs received through participation in another clinical trial are prohibited through the Day 43 follow-up visit.

Investigational Product, Dosage, Duration and Mode of Administration

Niclosamide (active), supplied as 400-mg tablets, or placebo will be administered orally 3 times per day (TID). Patients who are able to comply with the oral treatment must take, for each administration, 1 tablet (400-mg niclosamide or placebo) TID for 14 days. It is recommended to take the tablets after meals, with a glass of water to facilitate swallowing.
Arm A (active): Continued SoC therapy together with niclosamide 400-mg tablets TID (total daily dose 1,200 mg) for 14 days.
Arm B (placebo): Continued SoC therapy together with placebo tablets matching niclosamide TID for 14 days.

Randomization and Stratification

In Part 1: Patients will be randomized 2:1 between Arm A (active) and Arm B (placebo).

In Part 2: Patients will be randomized 1:1 between Arm A (active) and Arm B (placebo) following stratification based on age and sex.

Duration of Study

Each patient will remain in the study for approximately 6 months, including 2 weeks of treatment and approximately 5.5 months of follow-up. The recruitment period will last approximately 5 months.

The total study duration from first patient first visit (FPFV) to last patient last visit (LPLV) is expected to be approximately 11 months.

Criteria for Evaluation

The **primary endpoint of Part 1** is summarization of the safety results (adverse events [AEs], clinical laboratory results, and vital signs) comparing the niclosamide arm to the placebo arm.
The **primary endpoint in Part 2** is the time to fecal viral RNA clearance for patients with a positive Day 1 stool test for SARS-CoV-2 assessed by RT-qPCR in the niclosamide arm compared to the placebo arm.

The **secondary endpoints** will all compare the niclosamide arm to the placebo arm. These secondary endpoints will be assessed for both Part 1 and Part 2:

Gastrointestinal efficacy secondary endpoints are: (a) Part 1: time to fecal viral RNA clearance for patients with a positive Day 1 stool test for SARS-CoV-2 assessed by RT-qPCR; (b) time from the first dose of study treatment to the first formed stool (this formed stool must have been followed by a non-watery stool) in patients with loose or watery stool (Bristol Stool Scale Types 5-7) at Day 1; (c) time from the first dose of study treatment to the last loose or watery stool in patients with loose or watery stool (Bristol Stool Scale Types 5-7) at Day 1; (d) proportion of patients administered any anti-diarrheal agent from the first dose of study treatment to Day 15 and from Day 16 to 29; (e) time from first dose of study treatment to improvement of abdominal symptoms, if abdominal symptoms are present at Day 1 and (f) proportion of patients with a negative Day 1 stool test for SARS-CoV-2 who acquire SARS-CoV-2 in stool through Day 43.

Systemic and respiratory efficacy secondary endpoints are: (g) proportion of patients with each clinical severity score as recommended by the World Health Organization (WHO) for COVID-19 studies by study visit; (h) total duration, type of administration, and quantity of supplemental oxygen treatment; (i) changes in body temperature; (j) SaO2 on room air over time; (k) proportion of patients requiring ICU admission and length of ICU stay; (l) time to SARS-CoV-2 viral clearance from the nasopharynx (assessed by RT-qPCR); and (m) proportion of patients requiring hospitalization and duration of hospitalization.

Safety and tolerability secondary endpoints are: (n) all-cause mortality 6 weeks after randomization; (o) proportion of patients with treatment-emergent adverse events (TEAEs) leading to study drug discontinuation; (p) serious adverse events (SAEs); (q) clinically significant changes in laboratory measurements; and (r) clinically significant changes in vital sign measurements.

The **exploratory endpoints** will all compare the niclosamide arm to the placebo arm. These exploratory endpoints will be assessed for both Part 1 and Part 2:

(r) all-cause mortality 4 months and 6 months after randomization; (s) proportion of patients with recurrence of COVID-19 symptoms after Day 43, at 4 months and 6 months after randomization; (t) proportion of patients with persistence of COVID-19 symptoms beyond Day 43, at 4 months and 6 months after randomization (u) proportion of patients with new hospitalization or rehospitalization after Day 43, at 4 months and 6 months after randomization; and (v) summarization of genotypic resistance analysis data.

Statistical Methods

Statistical methods will be further detailed in the statistical analysis plan (SAP).
Sample size:

Part 1: The sample size was selected empirically for an initial evaluation of safety in patients with

COVID-19

Part 2: The sample size was determined by simulations based on the following assumptions:

- The time to fecal viral clearance has an exponential distribution for each treatment arm.
- The median time to fecal viral clearance is anticipated to be approximately 21 days for placebo arm and 10 days for niclosamide arm.
- Fecal viral clearance assessments are performed at Day 3, 7, 14, 28, 42 after first dose.
- Log-rank test (2-sided) is conducted to compare treatment difference.

Using a 1:1 randomization ratio, a sample size of 30 patients per treatment arm (60 patients total with a positive stool test for SARS-CoV-2 on Day 1) provides 74% power to detect a significant treatment difference for alpha=0.05 and 83% power for alpha=0.10. It is expected that approximately 40% of COVID-19 patients will be positive for SARS-CoV-2 in the stool sample. Therefore, approximately 150 patients with COVID-19 are needed to be enrolled/ randomized to ensure 60 patients have a positive stool test for SARS-CoV-2 on Day 1.

Analysis:

The primary analysis of the study will be a stratified log-rank test. Stratification will be based on strata defined for randomization. Kaplan-Meier curve figures with 95% confidence intervals (CIs) will be generated for the total population by treatment and for each stratum by treatment. Tables will present summary statistics of fraction of subjects not cleared by planned visit, and these summary tables will illustrate the total population and each stratum by treatment. Listings of all patients and the time to the primary endpoint along with stratum levels and clarifications required for sensitivity analyses will be generated.

Secondary and exploratory endpoints, along with safety and baseline characteristics, will be summarized with tables, figures, and listings appropriate for each measure.

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4 LIST OF ABBREVIATIONS

ABBREVIATIONS	DEFINITIONS
ACE2	Angiotensin-converting enzyme 2
AE	Adverse event
ALT	Alanine transaminase
AST	Aspartate transaminase
BP	Blood pressure
CI	Confidence interval
COVID-19	Coronavirus disease 2019
CRA	Clinical research associate
CRO	Contract research organization
DMC	Data Monitoring Committee
eCRF	Electronic case report form
FPFV	First patient first visit
GCP	Good Clinical Practice
GI	Gastrointestinal
GMP	Good Manufacturing Practice
ICF	Informed consent form
ICU	Intensive care unit
IMP	Investigational medical product
IWRS	Interactive web randomization system
LPLV	Last patient last visit
MedDRA	Medical Dictionary for Regulatory Activities
MERS	Middle East respiratory syndrome
PT	Preferred term
RT-qPCR	Reverse transcriptase-quantitative polymerase chain reaction
SAE	Serious adverse event
SaO2	Blood oxygen saturation by pulse oximetry
SAP	Statistical analysis plan
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SoC	Standard of care
SOC	System organ class
TEAE	Treatment-emergent adverse event
TID	3 times per day (ter in die)
ULN	Upper limit of normal
WHO	World Health Organization

5 BACKGROUND AND RATIONALE

5.1 Background

The coronavirus disease (COVID-19 2019) is a public health emergency of international concern caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. An increasing volume of convergent evidence indicates that gastrointestinal (GI) infection and fecal-oral transmission of SARS-CoV-2 are important factors in the clinical presentation, virology, and epidemiology of COVID-19. There is no etiological treatment for COVID-19 GI effects, representing a potentially unmet therapeutic need.

5.2 Background and Rationale Update

The original protocol was prepared and submitted about one year ago (August 2020). The overall rationale and objective for targeting acute GI infection with SARS-CoV-2 remains the same. The compelling set of epidemiological studies referenced in the Section below confirm that GI infection with SARS-CoV-2 remains an important COVID-19 clinical problem that deserves focused attention with a gut targeted pharmacologic agent, such as niclosamide. However, it has become apparent that the original design of the clinical trial is too restrictive. The use of overt clinical diarrhea coupled with a positive respiratory swab positive for SARS-CoV-2, which was the original plan, is likely to only capture a subset of the acute cases of GI infection.

The overall incidence of positive stool samples for SARS-CoV-2 is best estimated from two large meta-analyses. These reported that 43% (Wong et al., 2020) and 43% (van Dorn et al., 2020) of patients had stool specimens positive for virus. These reports included thousands of patients who were screened from multiple hospital centers. Wong et al., 2020 reported that among the patients with positive stool samples, only 77% reported overt GI symptoms. In separate smaller studies, prospective screening of stool specimens for SARS-CoV-2 detected virus in 55% of patients with positive respiratory disease (Wu et al., 2020) and 67% of patients with respiratory disease (Chen et al., 2020). In the latter report only 19% of patients presented with GI symptoms.

Important learnings from these reports are that: 1. In patients with SARS-CoV-2 infection of the GI tract, overt clinical signs and symptoms of GI infection, including diarrhea, are present in only a fraction of the cases. Thus, enrolling patients for treatment of GI infection based solely on diarrhea will miss a majority of infected patients; 2. In every study in which prospective monitoring of stool cultures was conducted, the presence of virus in stool lasted considerably longer than it lasted in the respiratory tract. In some cases, virus was detected in stool over one month after the respiratory episode was resolved. A reservoir of virus likely remained silently in the gut, without persistence of GI symptoms. It is also currently unknown if the GI infection results in prolonging the course and spread of the infection.

This updated (version 3.0, July 2021) protocol will therefore enroll any patient with positive respiratory swabs for SARS-CoV-2, regardless of overt GI symptoms. This will allow enrollment of patients with acute symptomatic as well as clinically silent GI infection. Stool samples from all enrolled patients will be screened just prior to beginning treatment for presence

of virus. The primary efficacy endpoint of time to resolution of stool SARS-CoV-2 positivity will remain the same and will be conducted in the subgroup with positive stools at the time of enrollment. The patients who do not have SARS-CoV-2 in stool at baseline will remain on study for the full two week treatment, and will be followed per protocol with serial stool assays. In addition to adding safety data, these tests will allow the detection of virus in stool, appearing after baseline. Since 'Long COVID Syndrome' has been reported to include 50 to 60% of patients with GI symptoms (Davis et al., 2020; Dennis et al., 2021), we will follow all enrollees for 4 and 6 month clinical follow-ups to ascertain the potential impact of our treatment on long haul COVID. The addition of clinically silent GI infections to our protocol may be of particular value for this observation.

5.3 Clinical Evidence of GI Involvement in COVID-19

The prevalence of GI signs and symptoms associated with SAR-CoV-2 infections (probably underestimated), the time course of GI symptoms often occurring early in infection, frequent liver involvement, and detection of viral RNA in stool samples and bathroom surfaces underscore the importance of the GI tract in the clinical course and epidemiology of COVID-19. Moreover, GI involvement in the clinical course and transmission of similar viruses (SARS-CoV and Middle East respiratory syndrome coronavirus [MERS-CoV]) highlight the potential significance of GI infection in COVID-19 pathogenesis.

Prevalence of GI Symptoms in COVID-19. A meta-analysis of 60 clinical studies and case series involving 4,243 patients indicates that GI manifestations, mainly diarrhea, nausea, and vomiting, affect 17.6% (95% confidence interval [CI]: 12.3 to 24.5; range: 0 to 100) of patients with diagnosis of COVID-19, with diarrhea present in 12.5% (95% CI: 9.6 to 16.0) of the reported cases (Cheung et al., 2020). Other epidemiological reviews, which were conducted on smaller, but robust, samples or observational studies not included in the above-mentioned meta-analysis, confirm these observations (Han et al., 2020; Tian et al., 2020; Jin et al., 2020; Kujawski et al., 2020; D'Amico et al., 2020). In particular, it has been reported that 28.4% of COVID-19 patients present only with GI symptoms (nausea, vomiting, and diarrhea) without respiratory symptoms (Jin et al., 2020).

The critical nature of respiratory symptoms has made them the primary focus for COVID-19 diagnosis and treatment, while the GI effects of the virus are underestimated. The progressive increase of the proportion of patients with diarrhea reported in Wuhan in the later stage of the outbreak (Tian et al., 2020; Jin et al., 2020) suggests that as a more complete understanding of the clinical features of COVID-19 has developed, GI effects are becoming better recognized. Importantly, the prevalence of GI symptoms in COVID-19 is related to severity of respiratory impairment, which is 17.1% (95% CI: 6.9 to 36.7) in patients with severe respiratory disease and 11.8% (95% CI: 4.1 to 29.1) in patients with non-severe disease (Cheung et al., 2020). This relationship suggests that possibility that GI infection with SARS-CoV-2 is a factor that determines overall disease severity.

Time Course of GI Symptoms. An average of 19.4% of the patients with COVID-19 experience diarrhea as their first symptom, even before the onset of respiratory symptoms or signs (Han et al., 2020). The importance of the GI tract in SARS-CoV-2 infection is also highlighted by several case series and case reports indicating that, in patients infected with

SARS-CoV-2, diarrhea usually precedes development of pneumonia (Pan L et al., 2020; Jin et al., 2020; Ferrey et al., 2020) or can be the only symptom reported by COVID-19 patients (Pan Q et al., 2020; Jin et al., 2020; Lo et al., 2020). In general, patients with diarrhea have longer hospital stays, longer duration of the symptoms, and longer period for complete viral clearance (44.3 vs 33.7 days; p = 0.003) compared to patients without diarrhea (Han et al., 2020; Pan L et al., 2020).

Since clinicians commonly rely on the presence of respiratory symptoms to diagnose COVID-19, patients who present with only GI symptoms may go undiagnosed and therefore may potentially remain untreated and more likely to spread the virus.

5.4 Evidence for the GI System as a Reservoir

Although GI symptoms in COVID-19 patients cannot be attributed with certainty to the presence of the virus in the GI tract, the fact that positive testing for viral RNA in stool, anal swabs, and rectal swabs and viral load are associated with a higher prevalence than GI symptoms makes it likely that a GI reservoir of infection causes the GI symptoms associated with COVID-19.

It has been demonstrated that, like SARS-CoV, SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) receptors present in the respiratory and intestinal tracts and that these receptors represent the entry point for the virus to the epithelial cells (Wan et al., 2020).

It is well documented that ACE2 is likely the receptor that allows the SARS-CoV-2 to infect the cells. The ACE2 mRNA is detectable in the entire digestive tract (Harmer et al., 2002); however, protein expression may be greatest in the brush border of the enterocytes of the small intestine (Hamming et al., 2004).

The ACE2-expressing enterocytes are invaded by SARS-CoV-2, leading to malabsorption, unbalanced intestinal secretion, and activated enteric nervous system, resulting in diarrhea (Zhang et al., 2020). Secondly, SARS-CoV-2 indirectly damages the digestive system through a chain of inflammatory responses (Pan Q et al., 2020).

The ACE2 receptors are also expressed in the esophagus and in the colon (Zhang et al., 2020; Hamming et al., 2004), but the microvilli of the small intestine amplify its surface area by 60 to 120 times; consequently, the small intestine represents approximately 92% of the entire surface of the digestive tract (Helander et al., 2014). For this reason, the target organ of the oral niclosamide can be considered the small intestine.

Intestinal Virus. As shown in another study, patients without GI symptoms seem more likely to be cured and discharged than patients with digestive symptoms (60% vs 34.3%). This could be due either to viral replication in the GI tract causing more severe disease or to patients who do not initially have typical respiratory symptoms presenting with later stages of disease (Pan Q et al., 2020).

Active viral replication was observed for SARS-CoV in biopsies obtained via colonoscopy up 10 weeks after the onset of symptoms of both small and large intestine (Leung et al., 2003). This observation fits with staining of viral nucleocapsid protein of SARS-CoV-2 visualized in the cytoplasm of the GI epithelium of patients who tested positive to viral RNA in the feces,

suggesting that the continuous positive detection of viral RNA in the feces results from secretion of the nucleic acid from infected intestinal cells (Xiao et al., 2020).

In an already mentioned meta-analysis (Section 5.3), the pooled prevalence of stool samples positive for virus RNA after hospitalization was 48.1% (95% CI: 38.3% to 57.9%) (Cheung et al., 2020). Similar data are also confirmed from other epidemiological reviews or cross-sectional studies (Tian et al., 2020; Lei et al., 2020). Remarkably, 1 study demonstrated that 70.3% (95% CI: 49.6% to 85.1%) of positive GI samples were collected after virus clearance from respiratory specimens and up to 33 days from the onset of the illness (Cheung et al., 2020). Moreover, stool viral clearance is significantly delayed ($p < 0.001$) in patients treated with steroids compared to patients not taking steroids, with mean clearance time of 20 days versus 11 days (Ling et al., 2020). These conclusions are supported by other studies (Xiao et al., 2020) and reports characterizing the disease specifically in children (Tang et al., 2020).

In a sample of 59 patients with COVID-19 in Hong Kong, the median fecal viral load was 5.1 log10 copies per mL in patients with diarrhea versus 3.9 log10 cpm in patients without diarrhea ($p = 0.06$) (Cheung et al., 2020). Similar values were reported in a European study that documented positive detection of SARS-CoV-2 in stools, with viral load of 6.8 and 8.1 log10 copies per gram of stool in 2 patients (Lescure et al., 2020).

Irrespective of whether the intestine is the primary site of infection or acts as a reservoir for viral replication, the intestine appears to be important in the pathogenesis of SARS-CoV-2.

5.5 Fecal-Oral Transmission

A number of observations support the risk of fecal-oral transmission of SARS-CoV-2 and the potentially important role that GI infection has transmissibility and epidemiology of COVID-19.

- **Family clusters.** Lin at al. identified 23 (31.08%) patients with GI symptoms who had family clustering. This percentage was significantly higher than that in patients without GI symptoms (20.45%, $p = 0.037$) (Lin et al., 2020).
- **Fecal-oral transmission in larger communities of the closely related SARS virus.** In 2003, a housing estate known as Amoy Garden, nearby Hong Kong, experienced a severe SARS outbreak affecting 329 residents (33 deaths) that was traced to poor design and faulty maintenance of stacks collecting effluent from the plumbing system in the housing systems bathrooms (Leung et al., 2003).
- **Transmission in hospitals.** Multiple SARS-CoV-2 outbreaks have been reported among healthcare workers, including physicians, nurses, and healthcare assistants, despite the use of surgical masks (Ho et al., 2020). It can be speculated that fecal-oral transmission plays a role in community hospital spread.

5.6 Niclosamide

Niclosamide is currently approved in the European Union to treat infection with cestodes and it is included on the core list of minimum medicine needs for a basic healthcare system, as

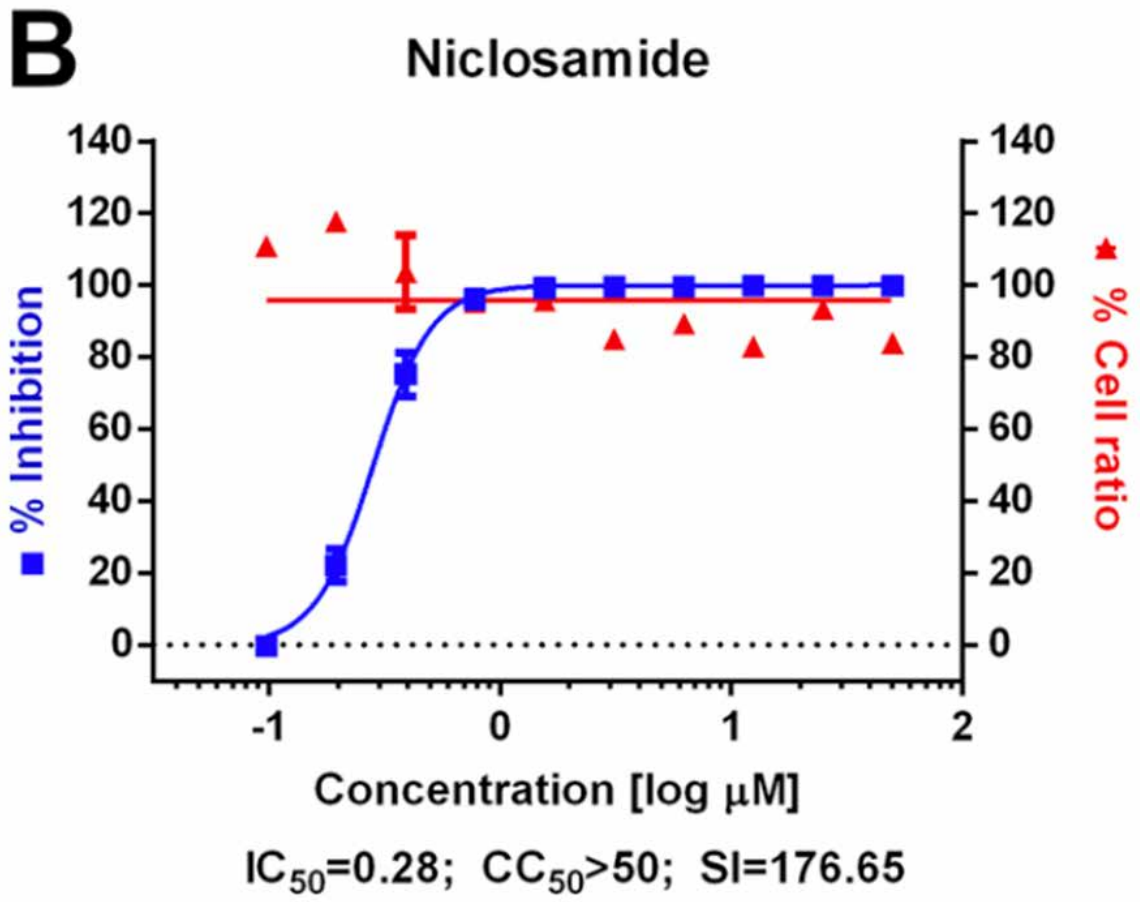
published in the most current World Health Organization (WHO) list of essential medicines (WHO 2020). Niclosamide's activity as an anti-helminthic treatment results from direct action in the intestinal lumen where it disrupts parasite oxidative metabolism, killing parasites. Niclosamide has been commercially available worldwide for more than 50 years as 500-mg tablets intended for use in pediatric and adult populations, at a dose of 2 g per adult or child over 6 years of age. No significant safety issues have been reported.

Drug repurposing/repositioning aimed at identifying new therapeutic applications for existing clinically approved drugs is a critical strategy to accelerate drug discovery for the COVID-19 pandemic. Multiple laboratories across the globe have now identified and validated niclosamide to have potent antiviral activity against SARS-CoV-2.

A small set (n = 49) of US Food and Drug Administration (FDA)-approved drugs was examined (selected based on either having known activity against SARS-CoV or being recommended by infectious disease experts) for activity against SARS-CoV-2 (Jeon et al, 2020).

Niclosamide was the most potent of all agents tested in a Vero cell cytopathic assay (half maximal inhibitory concentration [IC50] = 0.28 μM) (Figure 1). For comparison in terms of potency, niclosamide outperformed reference compounds chloroquine, lopinavir, and remdesivir with IC50 values of 7.28, 9.12, and 11.41 μM, respectively. Thus, niclosamide is approximately 25-fold more potent in vitro than remdesivir, a drug authorized for use against SARS-CoV-2 (Jeon et al., 2020).

Figure 1 Dose-Response Curve Analysis of Niclosamide by Immunofluorescence



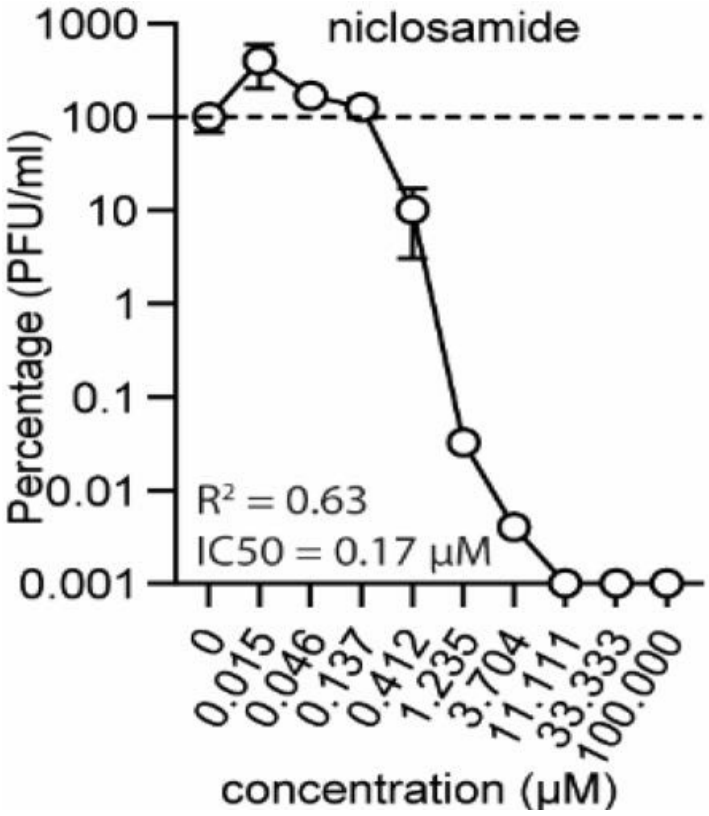
IC50 = half maximum inhibitory concentration, CC50 = half maximum cytotoxic concentration, SI = selectivity index

The blue squares represent inhibition of virus infection (%) and the red triangles represent cell viability (%). Means ± standard deviation were calculated from duplicate experiments.

Source: Jeon et al., 2020.

Gassen et al. reported studies aimed at understanding metabolic changes induced by SARS-CoV-2 infection that are required for viral propagation. They demonstrated that similar to other coronaviruses, SARS-CoV-2 inhibits autophagy as a means of survival and propagation. Niclosamide, known previously to be an inducer of autophagy, was tested and shown to reverse the effect of the virus on autophagy and, in so doing, to inhibit viral propagation with a maximal inhibition of >99% and an IC50 of 0.17 μM (Figure 2) (Gassen et al., 2020).

Figure 2 **Concentration-Dependent Inhibition of SARS-CoV-2 Growth by Niclosamide**



IC50 = half maximal inhibitory concentration, R2 = coefficient of determination, PFU = plaque forming unit, SARS-CoV-2= severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2 plaque forming units (PFU) were determined at 48 hours (after niclosamide treatment was started) by plaque assay. Data are presented as virus growth (shown as percent of untreated control) plotted against niclosamide concentration. Error bars denote standard error of mean derived from n = 3 biologically independent experiments.

Source: Gassen et al., 2020.

Zeng et al. reported on a deep-learning approach mining data from 24 million PubMed publications followed by systematic validation using transcriptomics and proteomics data generated from SARS-CoV-2–infected human cells that identified niclosamide as 1 of 41 drug candidates for further testing in clinical trials for COVID-19 (Zeng et al., 2020).

In addition to studies demonstrating antiviral effects on SARS-CoV-2, in vitro cellular assays demonstrate that niclosamide also blocks replication of the closely related coronaviruses SARS-CoV-1 and MERS-CoV; relevant published studies are summarized in Table 1. For example, the IC100 for SARS-CoV-2 antiviral activity, determined on a widely used in vitro model (Vero cell) is <3.0 µM (Figure 2), approximately the same magnitude as the IC100 of SARS-CoV, which is 94.6% similar in amino acid sequence and 80% similar in nucleotide sequence (Zhou et al., 2020). Moreover, earlier publications have also reported on niclosamide's potent and broad-spectrum antiviral properties against Zika, Ebola, Rhinovirus, Chikungunya, and Epstein-Barr virus (Xu et al., 2020).

Table 1 In Vitro Antiviral Activity of Niclosamide Against Coronaviruses That Cause SARS and MERS

Reference Year	Virus	Cell Type	Substrate or Model	EC_{50}	EC_{100}
Wu et al., 2004	SARS-CoV-1	Vero E6	Viral antigens S and N proteins (immunoblot)	0.78 µM to 1.56 µM	1.56 µM
Wu et al., 2004	SARS-CoV-1	Vero E6	Viral antigens S and N proteins (IFA)	1.56 µM to 3.12 µM	3.12 µM
Wu et al., 2004	SARS-CoV-1	Vero E6	Viral RNA replication (PCR)	1.56 µM to 3.12 µM	3.12 µM
Wen et al., 2007	SARS-CoV-1	Vero E6	Expression of S protein (ELISA)	<0.1 µM	-
Gassen et al., 2019	MERS-CoV	Vero B4	Autophagic stimulation by SKP2 inhibition	0.32 µM	3 µM*
Jeon et al., 2020	SARS-CoV-2	Vero E6	Viral N protein (IFA)	0.28 µM	-
Gassen et al., 2020	SARS-CoV-2	Vero FM	Viral RNA replication (PFU)	0.17 µM	1.24 µM**

EC_{50} = half maximal effective concentration; EC_{100} = maximal effective concentration; ELISA = enzyme-linked immunosorbent assay; IFA = immunofluorescence assay; MERS = Middle East respiratory syndrome; MERS-CoV = Middle East respiratory syndrome coronavirus; N = nucleocapsid protein; PFU = plaque-forming units; S = spike protein; SARS = severe acute respiratory syndrome; SARS-CoV = severe acute respiratory syndrome coronavirus.
* >99.99<99.999 viral growth inhibition; ** >99.99% viral growth inhibition

The mechanistic basis for activity of niclosamide against SARS-CoV-2 has been investigated. Gassen et al. show that SARS-CoV-2 viral replication is linked to the ability of SARS-CoV-2 to activate SPK2 (S-Phase Kinase-Associated Protein 2), which, in turn, induces the degradation of a critical autophagy protein, Beclin-1 (BECN1). The overall consequence of this series of steps is that the virus limits autophagy as a survival mechanism. Niclosamide treatment overcomes this by inhibiting SPK2, thereby preventing Beclin-1 degradation, which enhances autophagy and restricts viral replication (Gassen et al., 2020).

Niclosamide's antiviral activity against SARS-CoV-2 is reproducible across studies, mechanistically well-defined, and approximately 25-fold more potent than other antiviral agents (chloroquine or remdesivir) that have already been clinically tested and used against SARS-CoV-2 infection. Moreover, studies showing it inhibits replication of the related viruses SARS-CoV-1 and MERS-CoV further support activity against pathogenic coronaviruses.

At present, no animal models of SARS-CoV-2 have been validated as being able to predict clinical response of test articles in human disease. Studies of niclosamide in animal models of SARS-CoV-2, therefore, cannot be used to judge the likelihood of benefit in COVID-19 patients. This is underscored by differences in the role of the GI site of infection (target tissue for niclosamide therapy) in disease in animals versus humans that are expected based on animal-specific behaviors such as coprophagia that cause the virus in the stool to be continually and uncontrollably reintroduced through the oral route. Based on these considerations, preclinical efficacy studies of niclosamide in animal models of SARS-CoV-2 have not been performed by the sponsor.

Independent expert opinions have concluded that available evidence of niclosamide's antiviral effects is sufficient to support clinical testing in COVID-19 (Kupferschmidt, 2020). Multiple other Phase 2/3 clinical trials evaluating standard formulation (non-micronized) niclosamide in COVID-19 have been announced (NCT04644705, NCT04749173, NCT04558021, NCT04753619, NCT04603924, and NCT04399356). This further indicates that available data on niclosamide's antiviral properties against SARS-CoV-2 is widely considered sufficient to proceed with clinical testing.

Niclosamide is poorly absorbed following oral administration, leaving the majority of the dose in the GI tract. This fact, together with the use of micronized niclosamide in the drug product to accelerate dissolution, allows this drug product to achieve pharmacologically effective concentrations of niclosamide in the GI tract while having almost no bioavailability, enhancing efficacy and safety. These properties make it ideal for treating GI disease and infections, including SARS-CoV-2 infection and GI symptoms of COVID-19.

In summary, AzurRx BioPharma Inc. is developing a drug product containing micronized niclosamide in an immediate-release tablet formulation as treatment for SARS-CoV-2 intestinal infection in patients presenting with GI symptoms of COVID-19 disease. Evidence of niclosamide's antiviral properties is sufficient to expect a clinical pharmacodynamic response against viral replication and clinical benefit, justifying the proposed clinical study in COVID-19 patients and favorable benefit-risk assessment.

5.7 Rationale for Dose Selection of Niclosamide

The rationale for determining the dose of niclosamide is based on the following path and data.

The primary factor differentiating the current formulation from standard niclosamide formulations is rate of solubilization. The apparent increase in solubilization rate increases the potential for the current formulation to treat SARS-CoV-2. The rate of solubilization is low with only approximately 13% of the maximum amount entering solution in 90 minutes as reported by Devarakonda et al. (Devarakonda et al., 2005). The long time needed for solubilization is believed to be the source of differences between reports of solubility.

The upper limit of niclosamide solubility in water is reported between 15 and 24 µmol/L (Devarakonda et al., 2005), while other references report lower concentrations. Experiments suggest that a standard formulation of niclosamide may not be able to achieve the concentration at the limit of solubility within 1 hour as may be required to treat the small intestine, but the micronized formulation is able to achieve approximately the upper limit of solubility concentrations in rat colonic mucosa within 1 hour after administration in the rectum as a suspension enema, whereas the standard formulation only achieves approximately 1% of that concentration in intestinal mucosa.

The target location for prevention of SARS-CoV-2 infection in the current study is the gastric and intestinal epithelium. While the stomach, small and large intestines may be targets, based on ACE2 receptor expression levels, the small intestine is likely the most relevant target.

5.8 Dose Selection of Niclosamide in the Treatment of COVID-19

Dose selection for micronized niclosamide in the current study is made on the basis of safety and anticipated efficacy. The selected dose is 400-mg micronized niclosamide 3 times per day (TID) with meals, yielding a total daily dose of 1,200 mg (Schiller et al., 2005; Mudie et al., 2014).

Safety of niclosamide is based on the long history of administration of niclosamide at doses up to 2,000 mg/day, typically administered to adults as 2,000 mg once daily after breakfast (Bayer, Yomesan label). While the micronized formulation is anticipated to decrease the time to dissolve, it is not anticipated to significantly increase the extent of absorption. This is due to extensive first-pass metabolism in the intestine and liver in conjunction with the low solubility. Therefore, systemic toxicity is anticipated to be minimal (similar to standard formulations of niclosamide).

To maximize potential efficacy, the goal of dose selection is to maintain coverage of the stomach, small intestine, and potentially the large intestine with a ≥3-μM dissolved niclosamide concentration for complete inhibition of in vitro viral replication (Gassen et al., 2020; Jeon et al., 2020). A 400-mg niclosamide dose is approximately 1,220 μmol. When administered with a typical meal, approximately 750 mL (Schiller et al., 2005), this dose will achieve a nominal concentration of approximately 1,630 μM, but due to solubility limitations, the dissolved concentration would be approximately 20 μM (Devarakonda et al., 2005). The dynamics of transit through the small intestine (with decreasing concentrations starting approximately 2 hours after dose due to gastric half-emptying times) warrant increased concentrations relative to those required for viral inhibition. The concentration above the target allows for variability between patients along with both potential absorption and metabolism (both anticipated to be minimal) and for continued coverage during the transit of the dose from the stomach through the small intestine and to the colon. Dosing with meals is selected to increase the duration of stomach and small intestinal exposure to niclosamide.

The dosing duration of 14 days is based on typical fecal viral shedding dynamics observed in COVID-19 (He et al., 2020).

The micronized formulation is required to achieve efficacy in treatment of SARS-CoV-2 infection rather than the standard formulation to increase the rate of dissolution, as the standard formulation is not observed to achieve a significant amount of dissolution prior to entering the colon: 13% of the saturating concentration is achieved in 90 minutes (Devarakonda et al., 2005).

5.9 Other Clinical Trials on COVID-19 Using Niclosamide

Currently (as of February 2021), the ClinicalTrial.gov database reports 6 interventional studies that are recruiting:

- NCT04644705: Phase 1 study. A 3-part Study to Investigate the Safety and Pharmacokinetics of a Novel Niclosamide Solution as a Treatment Option for COVID-19 in Combination With Camostat.
- NCT04749173: Phase 1 study. A Double-blind, Randomized, Placebo-Controlled, Single-Ascending Dose Phase I Study to Evaluate the Safety, Tolerability and Pharmacokinetic

Properties of Niclosamide Injectable (DWRX2003) Following Intramuscular Administration in Healthy Volunteers. Injectable formulation of niclosamide.

- NCT04558021: Phase 3 study. A Phase III, Randomized, Placebo-Controlled, Clinical Trial to Evaluate the Efficacy and Safety of Co-administered Niclosamide in Patients Treated With an Established Regimen for Novel Coronavirus Infectious Disease (COVID-19).
- NCT04753619: Phase 2 study. Effectiveness of Niclosamide as Add-on Therapy to the Standard of Care Measures in COVID-19 Management.
- NCT04603924: Phase 2/3 study. A Phase 2/3 Randomized and Placebo-Controlled Study of ANA001 in Moderate and Severe COVID-19 Patients.
- NCT04399356. Phase 2 study. Niclosamide for Patients With Mild to Moderate Disease From Novel Coronavirus (COVID-19). Standard formulation of niclosamide.

None of these studies have intestinal viral clearance as a primary endpoint. The oral niclosamide used in these other studies is non-micronized material, whereas this study investigates micronized niclosamide that is expected to provide significant advantages by achieving greater soluble drug levels in the GI tract without significantly increasing systemic bioavailability and exposure.

5.10 Rationale for the Study

There are currently no approved or investigational treatments with demonstrated clinical efficacy for control of the intestinal SARS-CoV-2 viral shedding and fecal positivity. The evaluation of a safe and effective antiviral agent that is able to potently block SARS-CoV-2 replication in the intestine addresses serious unmet medical and epidemiological needs. Considering the objective of this protocol, the overall risks to participants are outweighed by the potential benefits of niclosamide experimental therapy. For these reasons, the benefit-risk balance for this study is considered positive.

6 RISK/BENEFIT ASSESSMENT

6.1 Risk for the Patient

A specific risk for a patient participating in this study is the exposure to the adverse reactions of niclosamide. This drug was approved for use in humans as an anti-helminthic treatment in the early 1980s and is included in the WHO's list of essential medicines (WHO, 2007).

To date, niclosamide has been administered to millions of people, including children, and appears to have a very good safety profile: GI disturbance is occasionally reported, whereas light-headedness and pruritus have been reported less frequently (Sweetman, 2012). Nausea, retching, and abdominal pain are also reported (WHO, 2008).

Other reported AEs (1%-4% of patients) are vomiting, abdominal discomfort possibly associated with anorexia, diarrhea, drowsiness, dizziness, and headache. Less frequently reported AEs (<1% of patients) are rash, oral irritation, fever, rectal bleeding, weakness, bad taste in mouth, sweating, palpitation, constipation, alopecia, edema of an arm, backache, and irritability. A transient increase in serum AST has reportedly occurred in at least 1 patient who was physically dependent on opiate agonists. Urticaria, which may be caused by the presence of breakdown products of the dead or dying worms, has occurred rarely (McEvoy et al., 1995).

6.2 Other Risks

The examinations and procedures required by the study do not expose the investigator or healthcare professionals to risks other than those that would normally involve the management of COVID-19 patients, assuming that the collection and treatment of biological samples (about 9 pharyngeal swabs, 9 stool samples, and 6 blood draws per patient over 43 days) are performed using the standard safety procedures.

6.3 Potential Benefit for the Patient

If the gut-selective antiviral activity of niclosamide were confirmed, the potential therapeutic or prophylactic return for the patient would be:

a. Effects on GI symptoms, and diarrhea in particular, due to the control of the viral replication within the epithelial cells and reduced interaction with ACE2 receptors.
b. Effects on liver complications, likely as a consequence of the spread of the virus through the enterohepatic circulation.
c. Possible effects on respiratory and systemic symptoms due to the reduction of circulating viral load of enteric origin.
d. Potential systemic effects due to, even if limited, intestinal absorption and potential model for the development of formulations acting at systemic level.
e. No expected pharmacokinetic drug interactions when used with other systemic treatments for COVID-19, as niclosamide acts locally at the intestinal level and has poor systemic absorption.

6.4 Potential Benefit for the Community

If the gut-selective antiviral activity of niclosamide were confirmed, the potential returns for the community, from a prophylactic point of view, would be:

a. Reduction of the viral load of enteric origin and of the probability of fecal-oral transmission, with repercussions for the risk for health personnel during hospitalization periods, staff in the GI department during the flares, and for members of the family after hospital discharge.
b. Potentially greater control of infections in the event of outbreaks that affect restricted communities.
c. Possible reduction, especially in those at risk, of relapses if these were sustained by self-reinfection.
d. Reduction of the number of viral replications and, consequently, lower probability of introducing new mutations and potential effects on virulence and clinical presentation (Jin et al., 2020).

6.5 Risk Mitigation

The risk mitigation strategy for this study includes:

a. Reduction of the total standard daily dose in adults. Niclosamide is typically administered to adults as 2,000 mg once daily. Even if the available data are only in vitro and in rats, the micronized formulation is anticipated to decrease the time to dissolve and not significantly increase the extent of absorption. However, for precaution micronized formulation of niclosamide will be administered at a total daily dose of 1,200 mg (400 mg TID).
b. Restriction of the study population to those patients without a history or evidence of acute and chronic hepatic impairment or failure, history of significant renal dysfunction, or patients in critical condition (see Section 9.6).
c. No limitation in the treatments, including antiviral drugs, that the doctor decides to use in relation to the clinical conditions of the patients (see Section 9.8).
d. An independent Data Monitoring Committee (DMC), including 2 physicians, will be appointed to provide recommendations about the individual safety assessment, early closures, or protocol modifications. Due to the double-blind design of the study, the members of the DMC will have unblinded access to the data.

6.6 Regulatory Compliance

This study will be conducted in compliance with this protocol, Good Clinical Practice (ICH GCP), and all applicable national and local regulatory requirements and in accordance with the ethical principles that have their origin in the Declaration of Helsinki and following revisions.

The investigator is responsible for ensuring that the study is conducted in accordance with the procedures described in this protocol.

7 OBJECTIVES

7.1 Primary Objective

The primary objective for Part 1 of the study is to evaluate the safety of niclosamide administered to patients with COVID-19.

The primary objective for Part 2 of the study is to evaluate the effect of niclosamide in addition to standard of care (SoC) compared to placebo in addition to SoC on fecal clearance of SARS-CoV-2 RNA.

7.2 Secondary Objective

The secondary objective of the study is to evaluate the clinical efficacy, safety, and tolerability of oral niclosamide in addition to SoC compared to placebo in addition to SoC.

7.3 Exploratory Objective

The exploratory objectives include characterization of the effect of niclosamide on disease progression and severity 4 and 6 months after starting treatment, and possible genotypic resistance analysis using the nasopharyngeal swabs and/or stool samples.

8 ENDPOINTS

8.1 Primary Endpoint

The primary endpoint of Part 1 is summarization of safety (AEs, clinical laboratory results, and vital signs) comparing the niclosamide arm to the placebo arm.

The primary endpoint of Part 2 is the time to fecal viral RNA clearance for patients with a positive Day 1 stool test for SARS-CoV-2 assessed by RT-qPCR in the niclosamide arm compared to the placebo arm.

8.2 Secondary Endpoints

The secondary endpoints will all compare the niclosamide arm to the placebo arm. These secondary endpoints will be assessed for both Part 1 and Part 2:

- **Gastrointestinal Efficacy Secondary Endpoints**

  a. Part 1: time to fecal viral RNA clearance for patients with a positive Day 1 stool test for SARS-CoV-2 assessed by RT-qPCR.

  b. Time from the first dose of study treatment to the first formed stool (this formed stool must have been followed by a non-watery stool) in patients with loose or watery stool (Bristol Stool Scale Types 5-7) at Day 1.

  c. Time from the first dose of study treatment to the last loose or watery stool in patients with loose or watery stool (Bristol Stool Scale Types 5-7) at Day 1.

  d. Proportion of patients administered any anti-diarrheal agent from the first dose of study treatment to Day 15 and from Day 16 to 29.

  e. Time from first dose of study treatment to improvement of abdominal symptoms in patients with abdominal symptoms at Day 1.

  f. Proportion of patients with a negative Day 1 stool test for SARS-CoV-2 who acquire SARS-CoV-2 in stool through Day 43.

- **Systemic and Respiratory Efficacy Secondary Endpoints**

  a. Proportion of patients with each clinical severity score as recommended by the WHO for COVID-19 studies (Appendix C) by study visit.

  b. Total duration, type of administration (eg, mean increased room oxygen, nasal tubes, ventilator, or ECMO), and quantity of supplemental oxygen treatment, whenever possible.

  c. Body temperature and proportion of patients with normal body temperature by study day. Criteria for normalization was temperature: ≤36.0°C axillary, ≤36.6°C oral, ≤37.0°C rectal, and ≤36.6°C tympanic (Geneva et al., 2019).

d. Proportion of patients with normal blood oxygen saturation by pulse oximeter (SaO2) >90% on room air by study day.

e. Proportion of patients admitted to the intensive care unit (ICU) and length of ICU stay.

f. Time to nasopharyngeal SARS-CoV-2 virus clearance from the nasopharynx (assessed by RT-qPCR).

g. Proportion of patients requiring hospitalization and duration of hospitalization.

- **Safety and Tolerability Secondary Endpoints**

a. All-cause mortality 6 weeks after randomization.

b. Proportion of patients with treatment-emergent adverse events (TEAEs) leading to study drug discontinuation.

c. Serious adverse events (SAEs) coded by system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA).

d. Clinically significant changes in laboratory measurements.

e. Clinically significant changes in vital sign measurements.

8.3 Exploratory Endpoints

The exploratory endpoints will all compare the niclosamide arm to the placebo arm. These exploratory endpoints will be assessed for both Part 1 and Part 2:

a. All-cause mortality 4 months and 6 months after randomization.

b. Proportion of patients with recurrence of COVID-19 symptoms after Day 43, at 4 months and 6 months after randomization.

c. Proportion of patients with persistence of COVID-19 symptoms beyond Day 43, at 4 months and 6 months after randomization.

d. Proportion of patients with new hospitalization or rehospitalization after Day 43, at 4 months and 6 months after randomization.

e. Summarization of genotypic resistance analysis data.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan:

Patients will be recruited and randomized to niclosamide oral formulation plus SoC or placebo matching niclosamide tablets plus SoC.

This is a 2-part, 2-arm, Phase 2, multicenter, randomized, double-blind, placebo-controlled study in adults with COVID-19.

The study will initiate with Part 1 and, upon safety review, the study may proceed to Part 2.

9.1.1 Study Design, Part 1

Part 1 will enroll approximately 9 patients. After treatment is concluded for all 9 patients, a DMC will review the safety data and determine if the study may proceed to Part 2 or if additional patients are needed to assess tolerability of the study drug. If additional patients are needed, approximately 9 more patients will be enrolled in Part 1, and then the DMC will meet again after all patients are treated to determine if the study may proceed to Part 2.

Arm A and Arm B will be randomized in a 2:1 ratio for active treatment versus placebo using an interactive web-based randomization system (IWRS).

9.1.2 Study Design, Part 2

Part 2 will enroll up to approximately 150 patients receiving at least 1 dose of study treatment. Arm A and Arm B will be randomized in a 1:1 ratio using an IWRS and balanced and stratified by age and sex:

1. Age:
   a. <65 years old
   b. ≥65 years old
2. Sex:
   a. Female
   b. Male

9.2 Study Treatment and Its Duration

Patients will be randomized into 1 of the following 2 treatment arms:

Arm A (active): Continued SoC therapy together with niclosamide 400-mg tablets TID, equivalent to a total daily dose 1,200 mg, for 14 days.

Arm B (placebo): Continued SoC therapy together with placebo tablets matching niclosamide TID for 14 days.

Treatment may begin at any time of day; if only 1 or 2 doses are administered on Day 1, the planned total number of doses should be administered (42 total doses) and the final doses may be administered on Day 15. The total study duration for a patient will not be extended if the final dose occurs on Day 15 for this reason.

The long-term care of the participant will remain the responsibility of their primary treating physician and there is no provision for post-study availability of niclosamide.

9.3 Study Duration and Recruitment Period

Each patient will remain in the study for approximately 6 months, including 2 weeks of treatment and approximately 5.5 months of follow-up. The recruitment period will last approximately 5 months.

The total study duration from first patient first visit (FPFV) to last patient last visit (LPLV) is expected to be approximately 11 months.

9.4 Study Population

Both Parts:

Eligible patients will have a diagnosis of COVID-19 with or without respiratory symptoms. No gender and/or ethnicity restrictions will apply. Each patient should meet all the inclusion and none of the exclusion criteria in order to be eligible for the study.

Part 1:

The study population will include 1 or 2 cohorts of approximately 9 patients (up to approximately 18 patients) randomized in a ratio of 2 active to 1 placebo. Patients will have a primary diagnosis of COVID-19. Patients may be hospitalized, inpatient in a research unit, or seen in an outpatient clinic or in a home care setting. At-home assessments may be performed using a combination of remote digital monitoring of patient-reported information, home visits by qualified research team staff (include nurses or medical assistants), and telehealth evaluation by study investigators. Implementation of the home-based outpatient assessment, including remote digital monitoring and telehealth evaluation, may include use of a digital application.

Part 2:

Part 2 will enroll up to approximately 150 patients receiving at least one dose of study treatment. Patients may be hospitalized, inpatient in a research unit, or seen in an outpatient clinic or in a home care setting.

If a patient begins treatment but is not confirmed to have a positive SARS-CoV-2 result from the pre-treatment stool sample by the central lab, the patient will remain on randomized treatment and will continue study assessments.

9.5 Inclusion Criteria

Subjects meeting all the following inclusion criteria will be considered eligible for the study:

1. Patients who give their written consent for participation in the study and for personal data processing and are willing and able to comply with all study procedures.
2. Patients of any gender who are at least 18 years of age.
3. Part 1 only: Patients with a primary diagnosis of COVID-19, with or without pneumonia, who agree to be monitored daily for at least 7 days after randomization and who accept continuing to be assessed for the study procedures.
4. Part 2 only: Patients with a primary diagnosis of COVID-19, with or without pneumonia.
5. Patients who are reasonably expected to maintain and adhere to SoC treatments prescribed by their physician.
6. Patients who had SARS-CoV-2 RNA presence in nasopharyngeal swab ≤5 days before randomization as confirmed by local or central laboratory. The assay used for testing at the laboratory should be approved or authorized for use by the FDA or local regulatory agency for diagnosing patients with COVID-19 at the time the test is used.
7. Patients who are considered reliable and capable of adhering to the protocol, according to the judgment of the investigator.

9.6 Exclusion Criteria

Patients will be excluded from the study if one or more of the following criteria are applicable:

1. At the time of randomization, patients requiring ICU admission or patients with severe respiratory insufficiency requiring mechanical ventilation or with rapid worsening of respiratory function leading to expectation for mechanical ventilation or ICU admission.
2. Evidence of rapid clinical deterioration or existence of any life-threatening co-morbidity or any other medical condition that, in the opinion of the investigator, makes the patient unsuitable for inclusion.
3. Patients who, at the time of enrollment, are not in a clinical condition compatible with the oral administration of the study drug.
4. Patients with serum alanine transaminase (ALT) or aspartate transaminase (AST) >3 times upper limit of normal (ULN) detected within 24 hours prior to randomization or other evidence of severe hepatic impairment (Child-Pugh Class C).
5. Patients with an estimated GFR (eGFR) ≤30 mL/min/1.73m^2 (based on CKD-EPI formula) at screening.
6. Patients with a history of hypersensitivity or allergy to any component of the study drug.
7. Enrollment in another concurrent clinical interventional study, intake of an investigational drug within 30 days prior to randomization, or intake of an investigational drug for COVID-19 within 3 months prior to randomization. Drugs approved under a US Food and Drug Administration Emergency Use Authorization are not considered investigational under this exclusion.
8. Pregnant or lactating women or women with a positive pregnancy test (urine or serum).

9.7 Investigational Medicinal Products (IMPs)

Niclosamide and Placebo

The chemical name of niclosamide is 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide. The IMPs are in the form of white yellowish oval 400-mg uncoated immediate-release (IR) tablets containing the active ingredient micronized niclosamide or a matched placebo.

The IMPs will be supplied by the sponsor for use in the protocol and are limited to investigational use only. Please refer to the current investigator's brochure for additional information.

9.7.1 Formulation and Packaging

The IMPs (active and matched placebo) are manufactured according to applicable Good Manufacturing Practice (GMP) rules. The primary packaging consists of high-density polypropylene white bottles of 75-mL capacity with polyethylene (PE) white screw caps (child-proof and safety closure).

Clinical kits will be identified by means of a label, as required by current GMPs (Annex 13) and law on IMP locally in force.

9.7.2 Allocation to Study Treatment

Patients will be randomized to the niclosamide and placebo arms using a computer-generated randomization list generated by the sponsor or designee and using an IWRS.

The treatment assignment is performed by pre-assigning the subject's number to the treatment kits corresponding to the randomization list.

9.7.3 Product Shipment, Storage, Distribution, and Accountability

The IMPs will be supplied in bottles to the sites by the sponsor, in accordance with local requirements. The IMPs will be shipped at room temperature.

The IMPs must be stored in a secured limited-access area and maintained at room temperature ≤25°C. The IMPs will be stored according to the storage conditions identified on the drug label.

The investigator, or pharmacist as applicable, is responsible for receipt, proper storage, and usage of the study drug. On receipt, the pharmacist or delegated site staff will record the date, details of the bottle, and quantity of tablets.

The investigator, or pharmacist as applicable, will keep a cumulative inventory and dispensing records and will maintain all supplies under adequate security. Adequate record of receipt, use, or loss of study drug will be retained.

The IMP inventory and accountability records will be kept by the investigator or designee using an IMP Accountability Log and will include details of IMP received and a clear record of when it was dispensed and to which subjects. The investigator or designee will perform the drug

accountability to calculate the number of tablets remaining after the returns the study drug bottle. The investigator agrees not to supply IMPs to any person except subjects enrolled in this study.

9.7.4 IMP Resupply

The investigational sites will be re-supplied with IMPs according to their respective recruitment rates. The pharmacist or delegated site staff will be provided with specific forms for accountability of the IMP (including the returned bottles). Records will be kept up to date throughout the study and must be complete and accurate.

Used and unused IMPs must be made available to the monitor or sponsor designee, who will verify the IMP accountability and cross-check pharmacy and investigator records for compliance with the protocol requirements. Any discrepancy must be accounted for and documented.

9.7.5 IMP Misuse/Overdose

Any IMP misuse or overdose associated or not associated with any AE should be reported to the sponsor or designee. Overdose is considered as dose taken above the prescribed daily dose for the current dosing phase.

9.7.6 IMP Administration

Patients who are able to comply with the oral treatment must take, for each administration, 1 tablet (400-mg niclosamide or placebo) TID for 14 days. It is recommended to take the tablets after meals, with a glass of water to facilitate swallowing.

Treatment may begin at any time of day; if only 1 or 2 doses are administered on Day 1, the planned total number of doses should be administered (42 total doses) and the final doses may be administered on Day 15. The total study duration for a patient will not be extended if the final dose occurs on Day 15 for this reason.

Management of patient who discontinues therapy. Patients may discontinue therapy due to progression to more severe illness if they are unable to tolerate oral niclosamide or placebo. If the patient recovers and is willing and able to resume treatment, the patient will be allowed to resume and complete the course of treatment. The duration when the patient could not tolerate study drug will be considered a dose delay and only the planned number of doses will be administered; it will not count as a restart of dosing.

Management of vomiting after dose administration. If vomiting occurs within 1 hour after dosing, the dose should be re-administered. The re-administration should only occur once per planned dose; if vomiting occurs a second time after the same planned dose, it should not be administered a third time. The times and dose amount below are selected based on published gastric half-emptying times (Maes et al., 1994). In case of re-administration, the next dose should still be taken on schedule.

Diarrhea. No dose adjustment is required in the case of diarrhea, as diarrhea primarily affects large intestinal transit, which is less sensitive to transient dose effects (Read et al., 1980).

Inability to tolerate oral tablet administration. For patients who are unable to tolerate or swallow tablets, it is possible, based on the judgment of the investigator, to administer the IMP through a nasogastric tube dispersing, for each administration, one 400-mg tablet in 50 mL of drinking water in a suitable glass container and to administer the mixture to the patient through a nasogastric tube using a needle-free syringe.

9.7.7 IMP Return, Destruction, and Recall

Return of IMP:

Unused, partially used, or empty bottles will be returned by the patient to the site at the end of each cycle.

At the end of the study, the sponsor will conduct a final reconciliation between delivered, dispensed, and used/unused IMPs.

Destruction of IMP:

Unused, partially used, or empty bottles must not be destroyed at the investigative sites without written authorization from the sponsor.

Recall:

If an IMP batch is suspected to be defective, then the sponsor will immediately inform the investigator and the hospital pharmacist.

The monitor will coordinate with the investigative site staff for the return of the concerned batches as per the return procedure. Depending on the study status, new batches may be sent to the investigational site.

9.8 Concomitant Medications and Prohibited Treatments

Other than study drugs, SoC as directed by the patient's physician will be allowed. Vaccination status for COVID-19 will not influence eligibility for the study and will be considered SoC while the patient is on study. After randomization and treatment allocation, the SoC therapy can be modified by the investigator according to the patient's needs.

In case of worsening or need of mechanical ventilation or admission to ICU, patients will be treated with any medication or treatment based on the physicians' judgment, without any constraint from the sponsor, including, but not limited to, antibiotics, antimycotics, steroids, alpha interferon, and immunoglobulins.

All prior and concomitant medication and treatments taken or received during the study, from screening visit to discharge, must be recorded. For concomitant or rescue medication: dose, posology, frequency of administration, start and end dates, and reason for use will be required and collected.

Investigational drugs received through participation in another clinical trial are prohibited through the Day 43 follow-up visit.

10 STUDY PROCEDURES

Clinical or laboratory procedures for the assessment of the inclusion and exclusion criteria will be performed after the patient provides informed consent. Procedures may be conducted in a hospital, in a clinic, or at home.

The Schedule of Assessments of the study is in Appendix A.

10.1 Day -3, -2, or -1: Screening

The following procedures and tests will be performed at the screening/baseline visit (V1):

- Informed consent/privacy (it must be signed prior to the initiation of any study-related activities).
- Evaluation of inclusion/exclusion criteria
- Demography
- Significant medical history, including primary diagnosis
  - Initial medical history will collect date of initial COVID-19 symptom onset.
  - Documentation of concomitant and previous medications or treatments.
- Pregnancy test (urine or serum)
- Nasopharyngeal swab for SARS-CoV-2 viral RNA analysis to determine eligibility at the local laboratory. Only one screening sample is required and can be determined by local requirements. If the local laboratory is not able to run the analysis, the sample or samples may be sent to the central laboratory to confirm eligibility as long as the central laboratory can perform the analysis before the screening period ends. The sampling method used for testing at the laboratory should be approved or authorized for use by the FDA or local regulatory agency for diagnosing patients with COVID-19 at the time the test is used.
- Clinical assessment of diarrhea (including anti-diarrheal agents)
- Stool sample for infection analysis at the local laboratory. Results are not required prior to randomization.
- Laboratory assessments:
  - Central: None required
  - Local: At least AST, ALT, and serum creatinine to determine eligibility. Any other laboratory tests may be conducted for the physician's safety assessment according to the local practice and patient's clinical needs.
- Overall clinical assessment
- AE monitoring after the patient has signed the informed consent form (ICF)

Rescreening may be considered. The investigator should discuss the case with the medical monitor before rescreening a patient.

10.2 Day 1: Treatment

The following procedures and tests will be performed on Day 1. If screening and Day 1 occur on the same day, assessments completed for screening are not required to be repeated on Day 1.

- Pre-dose
  - Confirmation of eligibility
  - Changes in the medical history and concomitant medications
  - Stool sample for the central laboratory
  - Clinical assessment of diarrhea (including anti-diarrheal agents)
  - Nasopharyngeal swab for the central laboratory
  - Laboratory assessments:
    - Central: Obtain samples for central laboratory.
    - Local: Any other laboratory tests may be conducted for the physician's safety assessment according to the local practice and patient's clinical needs.
  - Overall clinical assessment
- Randomization by means of an IWRS (randomization should occur after eligibility is confirmed, including all required laboratory tests)
- Administration of first dose and other TID doses, as they apply for the time of day
- Part 1 patients, who are not hospitalized, will be observed for approximately 30 minutes after receiving their first dose.
- Daily diary
- AE monitoring

10.3 Part 1 Only: Days 2, 3, 5, 6, and 7: Safety Monitoring

- Vital signs (For safety monitoring only. Vital signs do not need to be entered into the CRF, though detected adverse events should be entered in the CRF.)
- AE monitoring
- Concomitant medications

10.4 Days 4 (±1 day), 8 (±1 day), and 15 (±2 day): Treatment

- Changes in the medical history and concomitant medications since previous visit
- Pregnancy test (urine or serum) on Day 15
- Stool sample for the central laboratory
- Clinical assessment of diarrhea (including anti-diarrheal agents) since previous visit
- Nasopharyngeal swab for the central laboratory
- Laboratory assessments:
  - Central: Obtain samples for central laboratory at Day 8 and Day 15.

- Local: Any other laboratory tests may be conducted for the physician's safety assessment according to the local practice and patient's clinical needs.

- Overall clinical assessment
- Continued TID daily dosing
- Daily diary
- AE monitoring
- Concomitant medications

10.5 Days 22 (±2 days), 29 (±2 days), 36 (±3 days), and 43 (±3 days): Short-Term Follow-Up

The following procedures and tests will be performed at the days noted above or upon early termination if the patient discontinues the study less than 4 weeks after the last dose of study drug.

- Changes in the medical history and concomitant medications since previous visit
- Stool sample for the central laboratory
- Clinical assessment of diarrhea (including anti-diarrheal agents) since previous visit
- Nasopharyngeal swab for the central laboratory
- Laboratory assessments:
  - Central: Obtain samples for central laboratory at Day 29 and 43.
  - Local: Any other laboratory tests may be conducted for the physician's safety assessment according to the local practice and patient's clinical needs.
- Overall clinical assessment
- Daily diary, with diary collection on Day 43
- AE monitoring
- Concomitant medications

10.6 Four and Six Months After First Dose (±1 week): Long-Term Follow-Up

The patient will be contacted 4 months and 6 months after the first dose of study drug (±1 week) to collect:

- Survival status
- Persistence of COVID-19 symptoms beyond Day 43
- Recurrence of COVID-19 symptoms after Day 43
- Hospitalization or rehospitalization due to COVID-19 after Day 43

10.7 Assessments

Patients may be hospitalized, inpatient in a research unit, or seen in an outpatient clinic or in a home care setting. At-home assessments may be performed using a combination of remote

digital monitoring of patient-reported information, home visits by qualified research team staff (include nurses or medical assistants), and telehealth evaluation by study investigators. Implementation of the home-based outpatient assessment, including remote digital monitoring and telehealth evaluation, may include use of a digital application.

10.7.1 Assessments for Diarrhea

Assessments for the evaluation of diarrhea will be performed at all the study time points (except the long-term follow-up visits at months 4 and 6) and include:

- Number of watery episodes per day according to Type 6 or 7 of the Bristol Stool Scale, as reported in Appendix B (Lewis at al., 1997)
- Number of evacuations with formed stool
- Use of anti-diarrheal agents
- Daily presence of other GI signs and symptoms: vomiting, nausea, anorexia, gastralgia, abdominal pain or discomfort, flatulence

In the hospital or inpatient setting, the clinical assessment of diarrhea will be performed by the investigator, whereas after discharge or in an outpatient setting, it will be assessed daily and at defined study time points based on information entered by the patient using either a digital application or paper home diary.

10.7.2 Overall Clinical Assessment

The following information and clinical data will be captured as appropriate by the investigator, the study nurse, or a digital application daily and at defined study time points:

- Death
- ICU admission and discharge
- Hospitalization admission and discharge dates, including hospital readmission
- WHO severity score as reported in Appendix C
- Duration, type of administration, and quantity of supplemental oxygen treatment
- SaO2 on room air (if available, fingertip pulse oximeter)
- Vital signs, including blood pressure (BP), pulse, SaO2, and temperature, with temperature including method of measurement (axillary, oral, rectal, or tympanic)

10.7.3 Stool Samples and Nasopharyngeal Swabs

Stool samples and nasopharyngeal swabs will be collected in the hospital or clinic or at patient's home, according to written instructions using a pre-labeled patient's kit and shipped to a central laboratory for RT-qPCR analysis of the endpoints related to viral RNA. At screening, the sample will be collected for testing at the local laboratory of viral RNA to determine eligibility. Samples that are obtained in the outpatient setting will be collected by appropriately trained and qualified personnel, for example, a home-visiting medical assistant, or by the patient after the patient receives adequate training that has been documented by qualified personnel.

10.7.4 Safety and Laboratory Assessments

- Stool infection analysis at the local laboratory at screening. Clostridium difficile toxin, Salmonella, Shigella, Yersinia, Plesiomonas Campylobacter, and intestinal parasites are recommended tests, but the specific bacteria or parasites to be tested can be based on local standard of care testing.
- Safety (SAEs and AEs)
- Central laboratory testing will include complete blood count (CBC), blood urea nitrogen (BUN), blood sugar, electrolytes (sodium, chloride, potassium, and bicarbonate [HCO3]), alkaline phosphatase, AST, ALT, lactate dehydrogenase (LDH), total bilirubin, serum creatinine, C-reactive protein, and fecal calprotectin.
- Any other laboratory assessment may be conducted at the local laboratory for the physician's safety assessment according to the local practice and patient's clinical needs.

10.8 Patient Withdrawal

10.8.1 Individual Treatment Discontinuation

The assigned study treatment might be permanently discontinued at any time by the investigator for safety or medically justified reasons.

There are several other reasons the patient may withdraw from treatment, including, but not limited to, patient withdrawal of consent to treatment, the sponsor terminating the study, or the patient being lost to follow-up. The reason for early discontinuation of treatment will be documented in the patient's medical records and the electronic case report form (eCRF). Patients who discontinue treatment early will continue to be followed through the end of the study.

10.8.2 Participant Withdrawal from the Study

There are several reasons the patient may withdraw from the study, including, but not limited to, patient withdrawal of consent for participation in the study, the sponsor terminating the study, or the patient being lost to follow-up. The reason for early discontinuation of treatment will be documented in the patient's medical records and the eCRF.

10.8.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he/she fails to provide data or biologic samples after the discharge at the time points described in the protocol before the completion of the study.

The site should attempt to contact the patient (eg, by phone or last known mailing address) in order to:

a. Determine if the patient is deceased and the reason of the death;

b. Reschedule, if still alive, the missed hospital or home visit as soon as possible; counsel the participant on the importance of maintaining the assigned visit schedule; and ascertain whether the participant wishes to and/or should continue in the study.

Should the participant continue to be unreachable, he/she will be considered lost to follow-up.

11 DATA MANAGEMENT

11.1 Data Sources

Data required by the protocol will be collected both at the hospital or at the subject's home, as applicable.

11.2 Data Collection

Data collection is the responsibility of the clinical study staff at the site, under the supervision of the principal investigator. The study eCRF is the primary data collection instrument for the study. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported in the eCRFs and all other required reports. The investigator must ensure that the clinical data required by the study protocol are carefully reported in the subject's source documents detailing the unique identification number and date and time of the study procedures performed. Data reported in the eCRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained. Any correction to the source data entries must be carried out by the investigator or a designated member of the staff. Incorrect entries must not be covered with correcting fluid, obliterated, or made illegible in any way.

All data requested on the eCRF must be recorded. Any missing data must be explained. An audit trail will be maintained by the eCRF system.

An internet-based, study-specific eCRF will be designed, validated (FDA CRF 21 Part 11 compliant), and set-up for data storage and remote data entry by the principal investigator. All the information included into the eCRF should accurately reflect the source documents. Patient-reported data captured by a digital application and self-reported by study patients may be organized into reports that can be used as source data for entry into the eCRF, or the application may be linked directly to the eCRF. The investigator is responsible for the management and accuracy of the information collected in the eCRF.

Access to, consultation with, and use of the eCRF will be password protected, with restrictions tailored for the function of the users (eg, read-only, read and write). The eCRF will be designed to generate automatic alerts and queries in case of inconsistencies or errors during data entry. Any changes in the dataset will be tracked (eg, user, date and time, old entry, new entry).

11.3 Data Verification and Data Cleaning

The sponsor or designee will review the data over the course of the study to identify data inconsistencies and prepare the queries to resolve such inconsistencies. The investigator will be asked to resolve queries by making changes directly in the eCRF.

11.4 Data Coding

Concomitant diseases and events in the medical history, such as safety-related events, and relevant additional records related to the medical history will be coded using MedDRA (latest version in use).

Medications (products under study and concomitant medications) will be entered in the eCRF by the investigator, and they will be coded using the WHO-DRUG dictionary (latest version in use).

11.5 Database Closure

Prior to study database lock, all tasks or processes described in relevant study plans, such as data entry and check of all data, resolution of all queries, coding validation, and data review, must be completed and documented. The study database must be locked before generation of any results. The database lock will be documented and approved by relevant study personnel and all edit accesses will be removed. Following database closure, the study database can only be unlocked in case critical errors that affect the main conclusions of the study are discovered. The unlock justification and process must be documented.

11.6 Data Storage and Retention

Investigators are reminded that all sponsor representatives keep professional confidentiality with regards to the patient data. Source documents along with all other trial-related documents (copies of all ICFs and personal data processing consent, product inventories, and any correspondence related to the study) must be kept in the investigator's file throughout the study and then must be stored for a period of at least 15 years or as per local legal requirements on force, whichever is longer.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator as indicated in the contract. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

12 STATISTICAL ANALYSIS PLAN AND STATISTICAL ANALYSIS

12.1 Statistical Analysis Plan

All statistical methodology will be described in detail in the statistical analysis plan (SAP) which will be finalized prior to database lock. All variables collected in the eCRF and/or other recordings (if applicable) and all derived parameters will be used in the statistical analysis.

12.2 Sample Size Calculation

Part 1:

The sample size was selected empirically for an initial evaluation of safety in patients with COVID-19.

Part 2:

The sample size for Part 2 of the study was determined by simulations based on the following assumptions:

- The time to fecal viral clearance has an exponential distribution for each treatment arm.
- The median time to fecal viral clearance is anticipated to be approximately 21 days for placebo arm and 10 days for niclosamide arm.
- Fecal viral clearance assessments are performed at Day 3, 7, 14, 28, 42 after first dose.
- Log-rank test (2-sided) is conducted to compare treatment difference.

Using a 1:1 randomization ratio, a sample size of 30 patients per treatment arm (60 patients total with a positive stool test for SARS-CoV-2 on Day 1) provides 74% power to detect a significant treatment difference for alpha=0.05 and 83% power for alpha=0.10. It is expected that approximately 40% of COVID-19 patients will be positive for SARS-CoV-2 in the stool sample. Therefore, approximately 150 patients with COVID-19 are needed to be enrolled/randomized to ensure 60 patients have a positive stool test for SARS-CoV-2 on Day 1.

12.3 Analysis Sets

The following analysis sets will be defined:

Safety Set will consist of all randomized patients who received at least 1 dose of the IMP and will be used to present results on safety data. The Safety Set will be used for safety summaries and will be analyzed according to the study treatment actually received.

Full Analysis Set will consist of all randomized patients who received at least 1 dose of the IMP. The Full Analysis Set will be used for systemic and respiratory efficacy secondary endpoints and will be analyzed according to the study treatment as randomized.

Efficacy Evaluable Set will consist of all randomized patients who received at least 1 dose of the IMP, and who had a positive stool test for SARS-CoV-2 on Day 1 assessed by RT-PCR, and who did not test positive for bacteria or parasites in the screening stool analysis. The Efficacy Evaluable Set will be used for the primary efficacy endpoint and relevant gastrointestinal efficacy secondary endpoints and will be analyzed according to the study treatment as randomized.

Per-Protocol Set will consist of patients in the Efficacy Evaluable Set who did not have major protocol deviations affecting the primary efficacy endpoint. The Per-Protocol Set will be used as a supportive analysis set for gastrointestinal efficacy analyses and will be analyzed according to the study treatment as randomized.

12.4 Analysis of Efficacy Variables

The primary endpoint for Part 2, as well as the key secondary efficacy endpoint for Part 1, will be the time from first drug administration to the first fecal RT-qPCR sample negative for SARS-CoV-2 as defined as a quantitative test either below the limit of quantification or above a number of cycles equivalent to a limit of quantification. Secondary efficacy endpoints for Part 1 and Part 2 will be analysed separately.

The primary analysis of the study will be a stratified log-rank test. Stratification will be based on strata defined for randomization. Kaplan-Meier curve figures with 95% CIs will be generated for the total population by treatment and for each stratum by treatment. Tables will present summary statistics of fraction of subjects not cleared by planned visit, and these summary tables will illustrate the total population and each stratum by treatment. Listings of all patients and the time to the primary endpoint along with stratum levels and clarifications required for sensitivity analyses will be generated.

Secondary and exploratory endpoints, along with safety and baseline characteristics, will be summarized with tables, figures, and listings appropriate for each measure.

Full details for all analyses will be provided in the SAP.

12.5 Analysis of Safety Variables

The primary endpoint of Part 1 is summarization of safety (AEs, clinical laboratory results, and vital signs) comparing the niclosamide arm to the placebo arm.

The TEAEs, SAEs, and AEs will be presented for each treatment arm in terms of number of AEs and their incidence by SOC and preferred term (PT) using MedDRA. Analyses will be provided also by severity and relationship to the treatment.

Laboratory tests will be presented using descriptive statistics at each available visit.

Additionally, the frequency of subjects reporting an abnormal or abnormal clinically significant laboratory value at each available visit will be presented for each laboratory parameter.

12.6 Interim Safety Analysis for Study Continue/Pause Decision Part 1

The interim safety analysis for Part 1 will be performed by the DMC (see Section 12.9 below). Due to the relatively small number of subjects in Part 1, the decision for DMC review of Part 1 will be based on medical judgment of niclosamide tolerability compared to placebo and will not require specific statistics.

12.7 Interim Safety Analysis Part 2

The interim safety analysis for Part 2 will be performed by the DMC (see Section 12.9 below). The DMC will be unblinded to study data and will operate under a DMC charter. The planned interim analysis will occur when 75 patients have been enrolled and completed the 2-week treatment period. The outcome from the DMC to be delivered to the sponsor will be: (1) continue as planned; (2) pause enrollment and undertake further evaluation of safety; or (3) recommend that the study be terminated due to safety concerns. No rigid statistical stopping rules will be used to make these decisions. While the DMC statistician may perform statistical analyses to examine adverse trends in safety, such as excess deaths and SAEs in the active arm, the decision to recommend a pause in enrollment or termination of the study will be reached by clinical consensus of the DMC.

The chairperson of the DMC will be apprised of SAEs and other important safety events in real time and may elect to convene an ad hoc DMC meeting at any time.

12.8 Handling of Missing Data

All reasonable efforts will be made to reduce the rate of missing data. Investigators will be trained about the importance of patient retention and full data capture. In addition, reasonable attempts should be made by the investigators to emphasize continued subject's participation for the full duration of the trial.

Missing data for the primary endpoint will be handled as follows:

1. In the case of death of any cause, the patient will be considered to have the worst outcome with censoring at the planned follow-up visit (Day 43).
2. In the case of patients who are hospitalized for any reason and withdraw consent, the patient will be considered to have the worst outcome with censoring at the planned follow-up visit (Day 43).
3. In the case of loss to follow-up or other reasons unrelated to COVID-19 for discontinuing the study, the data will be considered missing at random and will be censored at the last available visit if the patient does not have fecal viral RNA clearance before discontinuation from the study.

12.9 Data Monitoring Committee

An independent DMC, including 2 physicians and a statistician, will be appointed to provide recommendations about the individual safety assessments, early closures, or protocol modifications to the sponsor.

Due to the double-blind design of the study, the members of the DMC will have unblinded access to the data. The members of the DMC will not be allowed to be in contact with the study staff at the sites.

12.10 Planned Analyses and Unblinding

12.10.1 Day 43 Final Analysis

This analysis will be carried out for the main clinical study report (CSR) when the last patient has completed the Day 43 follow-up visit. This CSR will contain all clean efficacy data (primary and secondary) and safety data through Day 43 for all randomized patients. At the time of the Day 43 analysis for writing the main CSR, the randomization will be unblinded to the central study team. However, the investigators, other site study staff, and patients will remain blinded to their treatment for as long as they are participating in the study.

12.10.2 End of Study Final Analysis

This analysis will be provided in an addendum to the main CSR. It will include limited exploratory data collected at the Month 4 and Month 6 long-term follow-up visits.

13 SAFETY REPORTING

13.1 Definitions of Adverse Events and Serious Adverse Events

13.1.1 Evaluation of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or study patient administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not it is related to the investigational product.

The period of observation for AEs extends from the time the patient gives informed consent until the patient completes the study. Adverse events that are still present after the patient's last scheduled visit will be followed up by means of a phone call or visit, as considered appropriate. After that time point, the need for additional follow-up of ongoing AEs/SAEs will be discussed between the investigator and the sponsor, although in the event of discrepancies, the investigator's criteria will prevail. Adverse events occurring after the end of the clinical trial must be reported if the investigator considers there is a causal relationship with the investigational product.

The investigator will be responsible for the necessary acute medical treatment of any AEs required during the study and will ensure that appropriate medical care will be maintained thereafter, if necessary.

All patients experiencing AEs, whether considered related to the use of the IMP or not, will be monitored periodically until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report will be supplied, if possible.

All AEs, including intercurrent illnesses, will be reported and documented as described below.

Adverse events are divided into the categories "serious" and "nonserious." This determines the procedure that will be used to report/document the AE (see below).

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical trial protocol and the condition(s) leading to these measures are not AEs if the condition(s) were known before the start of treatment with investigational product. In the latter case, the condition should be reported as medical history.

13.1.2 Definition of Serious and Nonserious Adverse Events

An SAE is any untoward medical occurrence that at any dose:

- results in death or is life-threatening.

- results in permanent or significant disability/incapacity.
- requires inpatient hospitalization or prolongation of hospitalization.
- results in a congenital abnormality/birth defect.

Hospitalization solely for the purpose of diagnostic tests, even if related to an AE, elective hospitalization for an intervention that was already planned before the inclusion of the patient in the clinical trial, and admission to a daycare facility may not themselves constitute sufficient grounds to be considered an SAE.

Medical and scientific judgment will be exercised in the classification of other important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Adverse events that do not fall into these categories are defined as nonserious.

13.2 Reporting/Documentation of Adverse Events

The investigator or qualified sub-investigator is responsible for assessing AEs and SAEs for causality and severity and for final review and confirmation of accuracy of event information and assessments.

Adverse events either reported by the patient or observed by the investigator must be recorded on the AE section of the eCRF and should be described in the following manner:

The **nature** of the event will be described in precise, standard medical terminology. If known, a specific diagnosis should be stated (eg, allergic contact dermatitis).

The **severity** of the AE will be described in terms of mild, moderate, or severe according to the investigator's clinical judgment.

Mild: The AE does not interfere in a significant manner with the patient's normal functioning level but may be an annoyance.

Moderate: The AE produces some impairment of functioning but is not hazardous to health but is uncomfortable and/or an embarrassment. These events are usually ameliorated by simple therapeutic measures.

Severe: The AE produces significant impairment of functioning or incapacitation and is a hazard to the patient.

The **duration** of the event will be described by the start date and end date.

The **location** for cutaneous AEs will be described as at or just around the application area (≤2 cm from the application area) or distant (>2 cm from the application area).

For the **causal relationship** of the event to the use of the IMP, the investigator or qualified sub-investigator is responsible for assessing the relationship to study drug using clinical judgment and the following considerations:

- Certain: The AE occurs in a plausible time relationship to IMP administration, cannot be explained by concurrent disease or other drugs or chemicals, follows a clinically plausible response to withdrawal of the IMP, is definitive based on recognized pharmacological or other parameter associated with the IMP, and is confirmed by re-challenge procedure, if performed.
- Probable: The AE follows a reasonable temporal sequence from administration of the IMP, is unlikely to be attributed to a disease or other drug/s and disappears or decreases upon withdrawal of the IMP.
- Possible: The AE follows a reasonable temporal sequence from administration of the IMP but can also be explained by disease or other drugs, and information on drug withdrawal may be lacking or unclear.
- Unlikely: The AE does not follow a reasonable temporal sequence from administration of the IMP, can be reasonably explained by disease or other drugs, does not follow a known pattern of response to the IMP, and does not reappear or worsen upon re-challenge, if performed.
- Not related: The AE occurs prior to IMP administration.

The **outcome** of the event will be described in terms of (a) Recovered/resolved; (b) Recovering/resolving; (c) Recovered/resolved with sequelae; (d) Not recovered/not resolved; (e) Fatal; and (f) Unknown. It will also be recorded if the study product use is continued, interrupted, or discontinued.

13.3 Reporting Requirements

All AEs, SAEs, and clinically significant laboratory abnormalities will be recorded in the eCRF in a timely manner and will be followed until resolution or stability of the AE, SAE, or laboratory abnormality has been demonstrated, whenever possible. Laboratory abnormalities without clinical significance are not recorded as AEs or SAEs.

Site personnel record all SAE data in the applicable eCRFs and, from there, transmit the SAE information to the sponsor-designated pharmacovigilance unit within 24 hours of the investigator's knowledge of the event.

Transmission of such documents should occur without personal subject identification, maintaining the traceability of a document to the subject identifiers.

13.4 Special Situations

Special situation reports include all reports of medication error, abuse, misuse, overdose, occupational exposure, drug interactions, unexpected benefit, transmission of infectious agents via the product, and pregnancy, regardless of an associated AE.

- **Medication error** is any unintentional error in the prescribing, dispensing, preparation for administration, or administration of an investigational product while the medication is in the control of a healthcare professional, patient, or consumer. Medication errors may be classified as a medication error without an AE, which includes situations of missed dose, medication error with an AE, intercepted medication error, and potential medication error.
- **Abuse** is defined as persistent or sporadic intentional excessive use of an investigational product by a subject.
- **Misuse** is defined as any intentional and inappropriate use of an investigational product that is not in accordance with the protocol instructions or the local prescribing information.
- **Overdose** is defined as an accidental or intentional administration of a quantity of an investigational product given per administration or cumulatively, which is above the maximum recommended dose as per protocol or in the product labeling (as it applies to the daily dose of the subject in question). In cases of a discrepancy in drug accountability, overdose will be established only when it is clear that the subject has taken the excess dose(s). Overdose cannot be established when the subject cannot account for the discrepancy, except in cases in which the investigator has reason to suspect that the subject has taken the additional dose(s).
- **Occupational exposure** is defined as exposure to an investigational product as a result of one's professional or non-professional occupation.
- **Drug interaction** is defined as any drug-drug, drug-food, or drug-device interaction.
- **Unexpected benefit** is defined as an unintended therapeutic effect where the results are judged to be desirable and beneficial.
- **Transmission of infectious agents** is defined as any suspected transmission of an infected agent through the IMP.
- **Pregnancy** itself is not considered an AE, nor is an induced elective abortion to terminate a pregnancy without medical reasons. Any premature termination of pregnancy such as a spontaneous abortion, an induced therapeutic abortion due to complications, or other medical reasons should be considered an SAE.

The investigator should report special situations, pregnancies and premature termination of pregnancies, study subjects who are identified after initiation of study drug and throughout the study, including the post-study drug follow-up period, to the sponsor.

14 OTHER ASPECTS

14.1 Quality Assurance and Quality Control Procedures

The strategy to ensure data quality and integrity will include remote and/or on-site monitoring and procedures for data management and may include a Quality Assurance (QA) audit.

14.1.1 Site Monitoring

The clinical research associate (CRA) is responsible for routine review of the eCRF at regular intervals during the study in order to verify adherence to the protocol and the completeness, accuracy, and consistency of the data collected by the investigator or his/her staff and entered by them in the eCRF. The CRA should have access directly or remotely, as appropriate, to any subject records needed to verify the entries in the eCRF.

The investigator agrees to cooperate with the CRA to ensure that any problems detected through remote or on-site monitoring are resolved.

14.1.2 Quality Assurance Audits

Quality Assurance audits might be performed by 1 or more auditors if considered necessary by the sponsor or by the contract research organization (CRO). The QA audits will imply the examination and evaluation of the accuracy of information obtained and collected by the investigator or managed by the CRO. Study facilities and equipment might be also subjected to QA audits.

14.2 Ethical Considerations

The study will be conducted in accordance with the Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects, and applicable GCP principles.

14.3 Regulatory and Ethical Compliance

This protocol, subject information, and ICF, and personal data processing consent, if applicable, will be submitted to an Institutional Review Board (IRB) along with a notification to regulatory authorities according to the local regulations. Notification in writing of ethical approval must be obtained from the IRB by the investigator before study initiation.

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the sponsor, IRB, and/or health authority(ies) according to applicable regulations.

The study will be conducted at each approved site under the scientific, clinical, and operative responsibility and supervision of the principal investigator by the medically and scientifically trained qualified person appointed by him/her.

14.4 Subject Information and Consent

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations (CFR) for Protection of Human Subjects (21 CFR 50.26[a,b], CFR 50.27, and CFR Part 56, Subpart A), and the Health Insurance Portability and Accountability Act.

Before entering the study, written informed consent will be obtained from all subjects.

Investigators are responsible for the correctness of the recruitment procedure and for using comprehensible verbal communication when providing information to subjects.

The investigators will also clearly inform the patient that he/she can leave the study at any time and for any reason without giving an explanation, and that this discontinuation would not in any case deteriorate the patient's relationship with the physician.

This information is exhaustively reported in the information sheet enclosed with the study ICF.

A copy of the ICF, including the information sheet, will be given to the patient, together with any needed clarification. Sufficient time will be given to enable the patient to take a decision whether to participate in the study.

14.5 Subject Confidentiality

Before entering the study, the written informed consent will be obtained from the subjects.

Patient confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor and their agents. This confidentiality is extended to cover testing of samples 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.

Patient will be assigned a unique identifier by the sponsor. Any patient's record or dataset that is transferred to the sponsor will contain the identifier only; the patient's name or any information that would make the participant identifiable will not be transferred.

The patient will be informed that his/her personal study-related data will be used by the sponsor. The level of disclosure will also be explained to the patient. The patient will be informed on the nature of the data, processing mechanisms, period of data retention, and exercising of their rights.

The patient will be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

A copy of the ICF will be given to the patient, together with any needed clarification. The patient's data will be collected only if informed consent has been obtained in writing.

14.6 Protocol Amendments

Any amendment to this protocol must be approved by AzurRx. Amendments will be submitted to the IRB for written approval. Written approval must be obtained before implementation of the amended version occurs. The written signed approval from the IRB should specifically reference the investigator name, protocol number, study title, and amendment number(s) that is applicable. Amendments that are administrative in nature do not require IRB approval but will be submitted to the IRB for information purposes.

14.7 Registration of the Protocol

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable law, regulation, and guidance, the protocol will be registered in and publicly disclosed on ClinicalTrials.gov.

14.8 Study Report and Communication of Results

A clinical study report will be prepared according to standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3) and provided to the regulatory agency(ies), regardless of the use or otherwise of these results for regulatory purposes.

AzurRx is committed to report at scientific meetings or to publish the results of the study in a complete and transparent manner, following the recommendations of the International Committee of Medical Journal Editors.

The results of the study, or any part thereof, shall not be published without the prior review or written consent and approval of AzurRx, and such consent and approval is not to be unreasonably withheld. However, no communication or publication will include AzurRx's confidential information.

AzurRx will register the study on a publicly accessible website (eg, ClinicalTrials.gov or EudraCT) in accordance with applicable laws and regulations.

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16 APPENDICES

Appendix A - Schedule of Assessments

Appendix B - Bristol Stool Scale

Appendix C - WHO Ordinal Scale for Clinical Improvement

16.1 Appendix A - Schedule of Assessments

Visit[1]	Screening[2]	Treatment				Follow-up					
						Short-term				Long-term	
Timing (D=day, M=month)	**D -3 to -1**	**D1**	**D4**	**D8**	**D15**	**D22**	**D29**	**D36**	**D43**	**M4**	**M6**
Deviation vs randomization day			±1 day	±1 day	±2 days	±2 days	±2 days	±3 days	±3 days	±1 week	±1 week
Informed consent	X										
Eligibility (inclusion/exclusion criteria)	X	X									
Demography	X										
Medical history (or changes)	X	X	X	X	X	X	X	X	X		
Pregnancy test (urine or serum)	X				X						
Stool analysis	X										
Stool sample for SARS-CoV-2 RNA[3]	X	X	X	X	X	X	X	X	X		
Diarrhea clinical assessment	X	X	X	X	X	X	X	X	X		
Nasopharyngeal swab for SARS-CoV-2 RNA[3]	X	X	X	X	X	X	X	X	X		
Laboratory assessments[4](L=local, C=central)	L	C		C	C		C		C		
Overall clinical assessment[5]	X	X	X	X	X	X	X	X	X		
Randomization		X									
Dosing study drug		Daily TID, with last dose on D14 or 15									
Daily diary		Daily									
Adverse events	X	X	X	X	X	X	X	X	X		
Concomitant medications		X	X	X	X	X	X	X	X		
Survival status										X	X
Persistence of COVID-19 symptoms										X	X
Recurrence of COVID-19 symptoms										X	X
(Re)Hospitalization due to COVID-19										X	X

[1] Patients may be hospitalized, inpatient in a research unit, or seen in an outpatient clinic or in a home care setting.

[2] Screening may occur on the same day as randomization, if all procedures may be completed. If they occur on the same day, procedures scheduled for both visits are not required to be duplicated.

[3] At screening, eligibility can be determined by local SARS-CoV-2 viral RNA analysis on a nasopharyngeal swab. Only one screening sample is required and can be determined by local requirements. Eligibility can be determined at a local laboratory at screening; however, a central laboratory may be used at screening if needed. On Days 1 to 43, the nasopharyngeal swab and stool sample will be collected and sent to the central lab.

[4] Local laboratory testing at screening should include at least AST, ALT, and serum creatinine to determine eligibility. Central laboratory testing will include CBC, BUN, blood sugar, electrolytes (sodium, chloride, potassium, and bicarbonate [HCO3]), alkaline phosphatase, AST, ALT, LDH, total bilirubin, serum creatinine, C-reactive protein, and fecal calprotectin. When only central testing is required, any other laboratory assessment may be conducted at the local laboratory for the physician's safety assessment according to the local practice and patient's clinical needs

[5] Including: mortality; hospital admission and discharge; ICU admission and discharge; WHO severity score and duration, type of administration, vital signs, and quantity of supplemental oxygen treatment. Vital signs should be completed on Days 1-8, 10, and 12 for Part 1 patients, but only need to be recorded in the CRF when the full Overall Clinical Assessment is due at the study visits above.

* Initial medical history will collect date of initial symptom onset. If date is uncertain, verbatim response from patient will be collected.

16.2 Appendix B - Bristol Stool Scale

Type	Descriptor
Type 1	Separate hard lumps, like nuts (difficult to pass and can be black)
Type 2	Sausage-shaped, but lumpy
Type 3	Like a sausage but with cracks on its surface (can be black)
Type 4	Like a sausage or snake, smooth and soft (average stool)
Type 5	Soft blobs with clear-cut edges
Type 6	Fluffy pieces with ragged edges, a mushy stool (diarrhea)
Type 7	Watery, no solid pieces, entirely liquid (diarrhea)

Source: Lewis et al., 1997.

16.3 Appendix C - WHO Ordinal Scale for Clinical Improvement

Patient State	Descriptor	Score
Uninfected	No clinical or virologic evidence of infection	0
Ambulatory	No limitation of activities	1
Ambulatory	Limitation of activities	2
Hospitalized, Mild Disease	Hospitalized, no oxygen therapy	3
Hospitalized, Mild Disease	Oxygen by mask or nasal prongs	4
Hospitalized, Severe Disease	Non-invasive ventilation or high-flow oxygen	5
Hospitalized, Severe Disease	Intubation and mechanical ventilation	6
Hospitalized, Severe Disease	Ventilation + additional organ support – pressors, RRT, ECMO	7
Dead	Dead	8

A special WHO (2020) committee arrived at the ordinal scale that measures illness severity over time.