Statistical analysis plan -- Niclosamide for Patients with Mild to Moderate Disease from Novel Coronavirus (COVID-19)

Clinicaltrials.gov NCT04399356

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Background and rationale

Therapeutic approaches are needed to improve outcomes in patients with COVID-19. Niclosamide is an oral anthelminthic drug primarily used to treat parasitic infections. However niclosamide may have broad clinical applications to treat diseases other than those caused by parasites. Niclosamide has potent antiviral activity against single-stranded RNA viruses including coronaviruses. It was proposed as an antiviral during the SARS outbreak in 2002. It was found to inhibit SARS coronavirus, SARS-CoV, in *in vitro* studies and similarly structured RNA viruses (both *in vitro* and *in vivo)*. Niclosamide has antiviral properties for similarly structured pathogenic viruses, including Zika virus, adenovirus, dengue, and chikungunya virus.

Because the drug is inexpensive and has few if any side effects, taking Niclosamide prophylactically might help to prevent COVID-19 spreading. Even if this treatment does not completely eradicate infection, niclosamide treatment may help to decrease viral load, thereby allowing the host immune system to better combat the disease.

Objectives

Primary Objective: To evaluate the efficacy of niclosamide in shortening contagious period as determined by time to viral clearance.

Secondary Objectives: To evaluate the efficacy of niclosamide on symptoms and progression to severe COVID-19.

Study Methods

Trial design
The trial is a single-center, randomized, parallel-group, placebo-controlled trial. Treatment allocation was a 1:1 ratio. Patients were randomized to either niclosamide (2 grams orally once daily for 7 days) or matched placebo control.

Randomization
The random allocation sequence was computer-generated by a biostatistician. Randomization was stratified into three strata: Tufts lab and non-Tufts lab. We used blocking (block size 4). The random allocation sequence was implemented into the Redcap Electronic Data Capture (EDC) system which guaranteed concealment of the sequence until treatments were assigned.

Sample size
For the primary efficacy endpoint of respiratory virologic clearance at Day 3 measured by oropharyngeal viral shedding, 40 participants in each group achieve 89.1% power to detect a difference between the group proportions of 35%. We assumed that 50% and 15% of participants in the niclosamide and placebo groups would have a negative test on Day 3. The calculation was under a two-sided Fisher's Exact Test and a significance level of 0.05.

Framework
For all objectives, we test for superiority of niclosamide against placebo.

Statistical interim analyses and stopping guidance
We did not plan or perform any interim analyses.

The trial was closed to recruitment on June 22, 2021 due to the effective ending of the COVID-19 epidemic in Massachusetts resulting in lack of available candidates.

Timing of final analysis
Final analysis will take place in one stage, when every patient has reached 30-day follow-up and data for the primary and secondary endpoints have been received and cleaned.

Timing of outcome assessments, including visit windows
Primary efficacy endpoint: respiratory viral clearance at Day 3.

Secondary efficacy endpoints

- Fecal viral clearance at Day 14.
- Reduction in viral shedding as measured by oropharyngeal swab on days, 3, 7, 10, 14.
- Reduction in fecal viral shedding as measured by fecal PCR on days, 3, 7, 10, 14 and 21.
- Progression to severe COVID.
- Resolution of symptoms (including but not limited to fever, cough, fatigue).

Statistical principles

Confidence intervals and P values
All statistical tests will be 2-sided and will be performed using a 5% significance level. We will derive 95% two-sided confidence intervals.

Adherence and protocol deviations
In this study, four pills are to be taken daily for 7 days. Compliance is assessed by the percentage of subjects who have taken the scheduled number of pills:
% compliance = 100x (number of pills taken / 28 pills supposed to have been taken).
We will summarize compliance by randomization group: mean % compliance as well as number and percentage of participants with more than 80% compliance (24 pills out of 28).
Similarly, we will assess compliance with oropharyngeal and fecal sampling.

Protocol deviations will be classified into:

- PCR > 3 days before randomization
- taking the first dose before the first telehealth visit
- missing at least 1 telehealth visit
- collecting at least one oropharyngeal sample outside of window (day 1, 3, 7, 10, 14 ±1 day)
- collecting at least one fecal sample outside of window (day, 1, 3, 7, 10, 14, 21 ±1 day)
- collecting less than 5 oropharyngeal samples
- collecting less than 6 fecal samples
- taking the wrong dose at least once
- taking the wrong dose at least once
- missing a dose at least once
- expected assessments not completed during at least one telehealth visit (e.g., physician disconnected)

Protocol deviations were classified prior to unblinding of treatment assignment. We will report detailed protocol deviations per participant. We will summarize the number and percentage of participants with protocol deviations by treatment group with details of type of deviation.

Analysis populations
We will exclude participants who withdrew consent after randomization but before taking any sample or pill.

The intention-to-treat (ITT) population will include all randomized patients according to the treatment they were randomized to receive.

The per-protocol population will a subset of the participants in the full analysis (ITT) set who took at least 80% of study intervention.

The modified ITT population will include participants who took at least one pill, have a positive oropharyngeal test result on Day 1 and have Day 3 oropharyngeal sample results available for analysis.

The safety population will include participants who took at least one pill.

Trial population

Screening, eligibility, recruitment, and withdrawal/follow-up
We will use a CONSORT flow diagram to summarize the number of participants who were:

- assessed for eligibility at screening
- eligible at screening
- eligible and randomized
- withdrew prior to received first dose
- randomized and included in the primary analysis

The flow diagram will also show the numbers who were eligible but not randomized, who did not receive the randomized allocation, who were lost to follow-up, who discontinued the intervention, and we will describe the reasons.

Baseline patient characteristics
Participants will be described at the time of randomization with respect to age, sex, race/ethnicity, COVID-19 symptoms, smoking, obesity/overweight, asthma, COPD, cancer, cerebrovascular disease, CKD, cystic fibrosis, heart conditions, hypertension, immunocompromised state, liver disease, neurologic conditions, pulmonary fibrosis, sickle cell disease, thalassemia, diabetes, both overall and separately for the two randomization groups. We will summarize categorical data by numbers and percentages. We will summarize continuous data by mean, SD (or median, Q1-Q3 if data are skewed). We will not undertake tests of statistical significance. We will note the clinical importance of any imbalance.

Analysis

Outcome definitions

Viral clearance
Respiratory viral clearance is defined as the first day a participant's oropharyngeal sample result is negative, provided that none of the subsequent oropharyngeal sample results are positive. In primary analyses, we will use sample results (positive or negative) as returned by Diagnostic Solutions Laboratory. We will use a similar definition for fecal viral clearance. We will calculate the time to clearance since Day 1.

If a participant has several samples on the same day, we will consider the result to be positive if at least one of the sample results is positive, and negative otherwise.

In table 1, we illustrate possible patterns of time to viral clearance and time post-viral clearance. We illustrate samples available on days per protocol. But, according to our definition of clearance, time to clearance and time post-clearance are identifiable even if samples taken on different days.

Table 1: Patterns of sample results and time to viral clearance

Day 1	Day 3	Day 7	Day 10	Day 14	Clearance vs. censored	Time to clearance	Time post-clearance

+	+	+	-	-	1	10	4
-	-	-	-	-	1	1	13
-	+	-	+	+	0	14	0
-	+	-	-	-	1	7	7

In absence of deviations from protocol, we expect oropharyngeal sample results on 5 distinct days (day 1, 3, 7, 10, 14) and fecal sample on 6 distinct days (day 1, 3, 7, 10, 14, 21). Some participants may have samples on less than 5 or 6 distinct days. We will identify the occurrence of viral clearance as defined above based on available samples. If clearance is not observed, we will censor participants at their last available sample. In Table 2, we illustrate possible patterns of non-available sample results.

Table 2: Patterns of days without sample result available and time to viral clearance

Day 1	Day 3	Day 7	Day 10	Day 14	Clearance vs. censored	Time to clearance	Time post-clearance
+	+	NA	-	-	1	10	4
-	-	-	NA	NA	1	1	6
-	+	-	+	NA	0	10	0
-	+	NA	-	-	1	10	4

NA: not available.

Analysis methods

Primary analysis

The primary endpoint is viral clearance in respiratory samples at day 3. The primary analysis will be based on the ITT population (i.e., all randomized participants). We will estimate the cumulative probability of being in clearance in each randomization group by using the Kaplan-Meier estimator. We will compare the cumulative probability of clearance at day 3 between the two groups by using a chi-square test based on a log(− log(·)) transformation for the survival function.[Klein et al. Statist. Med. 2007; 26:4505-4519].

$$\chi^2 = \frac{\left\{\log\left(-\log\left(\hat{S}_1(t)\right)\right) - \log\left(-\log\left(\hat{S}_0(t)\right)\right)\right\}^2}{\frac{\hat{\sigma}_1(t)^2}{\log\left(\hat{S}_1(t)\right)^2} + \frac{\hat{\sigma}_0(t)^2}{\log\left(\hat{S}_0(t)\right)^2}}$$

Under the null hypothesis of no difference in probability of being in clearance between the two groups, the statistic of test is asymptotically χ_1^2-distributed. We will provide the between-group difference in probability of viral clearance at day 3 and the associated 95% confidence interval based on the cloglog transformation of the survival function.

Secondary analyses

In secondary analyses,

1) We will compare the respiratory clearance probability functions from day 1 to 14 between the two groups by using a log-rank test.
2) We will calculate the area under each respiratory clearance-free probability curve which gives the mean time to viral clearance up to day 14. We will estimate the mean difference in time post-viral clearance between groups and the associated 95% confidence interval.
3) We will compare how the proportion of negative oropharyngeal sample results evolved over time between treatment groups by using random-intercepts logistic regression models for longitudinal binary outcome data.
4) We will repeat the primary analysis for fecal viral clearance at day 14.
5) We will repeat the secondary analyses 1)-3) above for fecal viral clearance.
6) We will repeat the primary and secondary analysis by considering the combined oropharyngeal and/or fecal sample results. If both types of samples are available on a given day, we will consider the result to be positive if at least one of the sample results is positive, and negative otherwise. If only one type of sample is available on a given day and it is positive (respectively negative), the result will be positive (negative) for that day.
7) We will compare the proportion of participants who progressed to severe COVID disease between groups.
8) We will compare the proportions of participants free of symptoms. We will include participants who reported symptoms on Day 1 and we will assess the time to symptom resolution (symptom no longer reported). We will analyze 8 symptom categories described in Table 3.
9) We will repeat the primary and secondary analyses for viral clearance in the mITT population.
10) We will repeat the primary and secondary analyses for viral clearance in the per protocol population.

Subgroup analyses

We pre-specified subgroup analyses of viral clearance and symptom resolution according to BMI (<25 kg/m^2 vs. ≥25 kg/m^2) and according to diabetes.

Exploratory analyses

In exploratory analyses, we will repeat the primary and secondary analyses of viral clearance by calculating the time to viral clearance from the confirmatory PCR before randomization.

Moreover, in an analysis blinded to random allocation, we will analyze the trajectories of oropharyngeal and fecal sample results. In particular, we will identify participants with a negative test result followed by at least one positive test result (as defined by the DSL COVID-19 Assay); we will examine the Ct values of the positive test results following a negative test result. We will repeat the primary and secondary analyses by considering alternative thresholds to define a positive result, based on the blinded review of data.

We also will perform longitudinal analyses to assess the between-group difference in gene expression over time. For each participant and each sample, we will calculate the difference in expression (in terms of quantification cycle) between the target gene (SARS-CoV-2 specific nucleocapsid N1; panspecific CoV nucleocapsid N3; or SARS-CoV-2 spike) and a reference gene (RNase P): $\Delta Cq = Cq(\text{target gene}) - Cq(\text{reference gene})$. We will create spaghetti plots in each randomization group with lowess smoother curve superimposed. We will create heatmaps of ΔCq values scaled to the mean and standard deviation within each participant. We will use linear mixed models, including a term for the group, a term for time, and group x time interaction term and a subject-level random intercept, to compare the trajectories in normalized expression between groups over time.

Data will include a number of non-detects, i.e. reactions lacking a Cq value. Missing Cq value can correspond to a true Cq value above the Cq threshold. Alternatively it can correspond to zero expression (no amplification above the Cq threshold). Or it can correspond to a failure to detect a true Cq value below the Cq threshold. We will account for non-detects by setting undetermined Cq values at 40. In sensitivity analyses, we will account for non-detects by using hot deck imputation.

We will also consider the following modified ITT analyses of viral clearance:

- for the analysis based on oropharyngeal samples, fecal samples, and both types, we will include participants who took at least one pill, have a positive test result at any time during the trial and have Day 3 sample results available for analysis.

- for the analysis based on fecal samples, we will include participants who took at least one pill, have a positive oropharyngeal test result and have Day 3 fecal sample results available for analysis.

Missing data
For the longitudinal analyses, we will perform sensitivity analyses by imputing data according to sequential multiple imputation

Harms
The number (and percentage) of patients experiencing each adverse event will be presented, both for the overall safety population and in each randomization group. We will not perform statistical testing. We will assess the clinical significance of the differences. Adverse events will include abdominal pain, congestion or runny nose, cough, diarrhea, dizziness, dyspnea, fatigue, fever or chills, headaches, hypoxia, loss of appetite, muscle or body aches, nausea, new loss of taste or smell, pruritus, shortness of breath/difficulty breathing, skin rash, sore throat, vomiting, and other.

Statistical software
The analysis will be carried out by using SAS and R.

Table 3: Classification of symptoms into 8 categories

1.Central nervous system
Dizziness Fatigue Headache
2.Upper gastrointestinal
Appetite change Nausea Vomiting
3.Lower gastrointestinal
Abdominal pain Diarrhea
4.Ear, nose, throat
Sore throat Congestion Loss of taste/smell
5.Respiratory/Pulmonary
Cough Dyspnea/Shortness of breath Hypoxia
6.Musculoskeletal
Muscle aches
Dermatologic
Rash Pruritus
7.Systemic
Fever/Chills
8.Other