TITLE

A Randomized Double-Blind Phase 2 Study Comparing the Efficacy, Safety, and Tolerability of Nitazoxanide Versus Placebo in Addition to Standard Care for the Treatment of Hospitalized Subjects with Severe Acute Respiratory Illness

Sponsored by:
Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Division of Clinical Research (DCR)
National Institute of Allergy and Infectious Diseases (NIAID)
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LIST OF ABBREVIATIONS

AE	Adverse event
ARDS	Acute Respiratory Distress Syndrome
ARI	Acute Respiratory Illnesses
CAP	College of American Pathologists
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Act
CSO	Clinical Safety Office
DSMB	Data and Safety Monitoring Board
eCRF	electronic Case Report Form
FDA	Food and Drug Administration
GCP	Guidelines for Good Clinical Practice
GI	Gastrointestinal
h	Hours
H1N1	Influenza A H1N1, swine flu
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IND	Investigational New Drug Application
ILI	Influenza Like Illness
IQR	Interquartile Range
IRB	Institutional Review Board
ITT	Intention to treat
NIAID	National Institute of Allergy and Infectious Diseases
NP(S)	Nasopharyngeal (swab)
NTZ	Nitazoxanide
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OTC	Over the counter
PCR	Polymerase chain reaction
PCB	Placebo
RVI	Respiratory Viral Infection
SAE	Serious Adverse Event
SARI	Severe Acute Respiratory Illness
SOC	Standard of Care
SOFA	Sequential Organ Failure Assessment
ULN	Upper limit of normal
VRI	Viral Respiratory Illness
WHO	World Health Organization

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PROTOCOL SUMMARY

Full Title: A Randomized Double-Blind Phase 2 Study Comparing the Efficacy, Safety, and Tolerability of Nitazoxanide Versus Placebo in Addition to Standard Care for the Treatment of Hospitalized Subjects with Severe Acute Respiratory Illness

Short Title: NTZ-SARI

Clinical Phase: 2

IND Sponsor: Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
National Institute of Allergy and Infectious Diseases (NIAID)

Conducted by: Mexican Emerging Infectious Diseases Clinical Research Network (LaRed)

Sample Size: N= 290 subjects

Accrual Ceiling: Up to 500 subjects will be screened to randomize 290 subjects

Study Population: Subjects who are hospitalized in Mexico with Severe Acute Respiratory Illness [SARI] (using the World Health Organization [WHO] case definition)

Accrual Period: 3 years

Background: Respiratory viruses are a significant cause of hospitalization for respiratory tract infections. Etiologic agents include influenza, parainfluenza virus, rhinovirus, adenovirus, metapneumovirus, respiratory syncytial virus (RSV), bocavirus and coronavirus. Of these, only influenza has a licensed treatment. Nitazoxanide (NTZ) is a licensed anti-infective agent with activity against many of these respiratory viruses. In a pediatric Phase 2 study of outpatient ILI, the NTZ cohort showed symptom resolution in 4 days versus 7 days in the placebo group. In a 624 person Phase 3 study of ILI, the NTZ cohort showed symptom resolution in 94 h compared to 108 h in the placebo group (p=0.0026). These benefits were observed in the entire cohort, not just those infected with influenza. If similar efficacy can be seen in the inpatient ILI population, there could be reductions in duration of hospitalization, morbidity, and costs.

LaRed is a collaboration between the Mexican Ministry of Health and the US NIAID. In a period of 4 years, LaRed has enrolled over 5600 subjects (of which over 2000 were hospitalized) into an observational ILI study at 6 sites in Mexico. Viral etiologies are found in approximately 60% of subjects enrolled.

Study Design: The study will be a multicenter, randomized, double-blind, placebo controlled trial to evaluate the efficacy of NTZ + standard of care (SOC) compared to placebo (PCB) + SOC in treating acute viral respiratory infections in adults and children ≥ 12 months old with SARI (hospitalized ILI). It is anticipated that SOC would likely include antibiotics and may include treatment for influenza. The study is designed to enroll a total of 290 subjects who will be randomized in a 1:1 ratio. Subjects will be followed on Days 3, 7, 14, and 28. The subject will

be discharged from the hospital when clinically indicated as determined by the treating physician. Follow-up visits may occur as inpatients or as outpatients. Subjects still hospitalized on Day 28 will be followed until discharged from the hospital.

Study Duration:
Enrollment start date: February 2014
Anticipated enrollment end date: March 2017
Anticipated final analysis: December 2017

Study Agent: Nitazoxanide

Primary Objective: Evaluate the effect of NTZ in addition to SOC as compared to PCB + SOC in the treatment of SARI as measured by the time to hospital discharge

Secondary Objectives:
1. Evaluate the clinical efficacy of NTZ + SOC vs. PCB + SOC with regards to:
    - Percent hospitalized at Study Day 3, 7, 14 and 28
    - Death by Study Day 28
    - Duration of clinical symptoms (defined in Section 7.3.1.2)
    - Duration of fever
    - Use of supplemental oxygen (or increase greater than baseline oxygen requirement)
    - Admission to ICU
    - Intubation/mechanical ventilation
    - Proportion of subjects who develop complications including pneumonia, respiratory failure requiring mechanical ventilation, acute respiratory distress syndrome (ARDS), sepsis, or bronchiolitis.
    - Time to resumption of normal activity
    - Antibiotic/antiviral use during hospitalization
    - Systemic corticosteroid use during hospitalization
    - Re-hospitalization within 28 days

2. Evaluate the virologic efficacy of NTZ + SOC vs. PCB + SOC with regards to:
    - Percent cessation of viral shedding at Study Day 3

3. Evaluate the safety and tolerability of NTZ assessed by:
    - Cumulative incidence and severity of adverse events (AEs)
    - Cumulative incidence of serious AEs (SAEs)
    - Cumulative incidence of laboratory abnormalities on Study Days 3, 7 and 28

Endpoints:
<u>Clinical Endpoints:</u>
- Hospitalization on Study Days 3, 7, 14, and 28
- Date and time of hospital discharge
- Date of death, if applicable

- Clinical symptoms (measured daily through Study Day 14 and then again on Study Day 28)
- Temperature (measured daily through Study Day 14 and then again on Study Day 28)
- Oxygen use
- Date of admission to ICU and discharge from ICU, if applicable
- Date of intubation/extubation, if applicable
- Presence of complications during study (pneumonia, respiratory failure requiring mechanical ventilation, ARDS, sepsis, or bronchiolitis)
- Global assessment (measured daily through Study Day 14 and then again on Study Day 28) (defined in Section 7.3.1.3)
- Antibiotic/antiviral use during hospitalization (number and duration)
- Re-hospitalization within 28 days
- Use of systemic corticosteroids

Virologic Endpoints:

- Presence of virus on nasopharyngeal (NP) swab (or aspirate) at Study Day 3 (same virus as Day 0)

Safety Endpoints:

- AEs
- SAEs
- Chemistry and hematologic laboratory assessments on Study Days 3, 7 and 28

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background

1.1.1 Burden of Influenza-Like Illness

Respiratory viral infections (RVI) are one of the most common health conditions in the world and have enormous but under-recognized impacts on public health. According to the World Health Organization (WHO) estimates, 450 million cases of pneumonia are recorded every year[1]. In addition to their high frequency, RVIs are major causes of severe acute respiratory tract infection (SARI) that can lead to severe outcomes including hospitalization and death. Evidence of viral infection have been detected in 22% of cases in adults, [1-6] and 49% (range 43–67) of cases in children[1,7-11]. Etiologic agents of influenza-like illness (ILI) include several respiratory viruses, such as influenza virus, parainfluenza virus, rhinovirus, adenovirus, metapneumovirus, respiratory syncytial virus (RSV), bocavirus and coronavirus[12].

Acute respiratory illnesses (ARIs) impose an enormous burden on communities both directly in terms of medical care and indirectly on the whole of society. Besides causing severe complications for patients, ARIs have huge impacts on outpatient care and hospital services, resulting in high costs for patients, families and society in general. In addition to direct costs of care, ARIs are responsible each year for major losses in productivity in part due to absenteeism.

1.1.2 Existing Treatments

Of the viruses causing ILI, only influenza has an effective treatment. Four medications are licensed for the treatment of influenza: oseltamivir (OST), zanamivir, amantadine, and rimantadine. Parainfluenza virus, rhinovirus, adenovirus, metapneumovirus, respiratory syncytial virus (RSV), bocavirus and coronavirus have no effective therapy.

1.1.3 WHO's Battle Against Respiratory Viruses (BRaVe) Initiative

In July and November 2012 the World Health Organization (WHO) held 2 informal consultations to introduce a new initiative. "Battle against respiratory viruses" (BRaVe) is highlighting the need of effective treatment options for non-influenza respiratory viruses. From these consultations, the WHO released a Call to Action, specifically stating "cost-effective antiviral agents and other therapies for other (non-influenza) VRIs are not currently available, and an urgent need exists to support research for new safe and effective therapeutics to target specific respiratory viruses but also, if possible, to develop antivirals with broad spectrum activity."

1.1.4 Severe Acute Respiratory Illness

The WHO has created a case definition for SARI. This case definition was created for outbreaks such as severe acute respiratory syndrome (SARS) and pandemic influenza.

The WHO 2004 SARI case definition was:

> *Meets ILI case definition (sudden onset of fever ≥38°C and cough or sore throat in the absence of other diagnosis) AND shortness of breath or difficulty breathing AND requiring hospital admission.*

The WHO[22] SARI case definition was revised in 2014 to read:

An acute respiratory infection with:

- history of fever or measured fever of ≥ 38 C°;
- and cough;
- with onset within the last 10 days;
- and requires hospitalization.

This case definition is the basis for defining a severe respiratory illness in the selection of participants in this protocol.

1.2 Nitazoxanide

1.2.1 History and Significance

Nitazoxanide (NTZ) is an investigational new drug for the treatment of acute influenza that has also demonstrated efficacy against other respiratory illnesses. NTZ is the approved generic name for 2-acetyloxy-N-(5-nitro-2-thiazolyl)-benzamide, also known as PH-5776, or Alinia®. The drug is already marketed in the United States as an oral suspension for children ≥1 year of age and as tablets for patients ≥12 years for treatment of *Giardia lamblia* or *Cryptosporidium parvum.* In Mexico, NTZ is licensed for use in patients ≥2 years of age.

1.2.2 Mechanism of Action and Pre-Clinical Findings

Anti-influenza activity of NTZ and its circulating metabolite, tizoxanide, has been demonstrated in several human and canine cell lines after infection with 4 different strains of influenza A virus (including 2 mammalian H1N1 strains, 1 H3N2 strain, and the H5N9 low pathogenicity avian strain) and 1 strain of influenza B virus with EC50s between 0.3 and 1 μg/mL.

In cell culture, tizoxanide has antiviral activity against other RNA and DNA viruses. It inhibits replication of parainfluenza (Sendai virus, IC50 = 1 μg/mL), coronavirus (canine strain S-378, IC50 = 1 μg/mL), adenovirus type 5, and respiratory syncytial virus A2 [redacted]. Rhinovirus is not thought to be inhibited by NTZ.

The wide spectrum of antiviral activity suggests a cell-mediated effect rather than a specific viral target. Tizoxanide inhibits the replication of H1N1 and other strains of influenza A virus by a novel mechanism, acting at the post-translational level by selectively blocking the maturation of the viral hemagglutinin at a stage preceding resistance to endoglycosidase H digestion, thus impairing hemagglutinin intracellular trafficking and insertion into the host plasma membrane, a key step for correct assembly and exit of the virus from the host cell .[13]

The antiviral activity of NTZ alone or in combination with the neuraminidase inhibitors, OST phosphate and zanamivir, was studied in MDCK cells infected with 2 different strains of influenza A virus. NTZ exhibited synergism when combined with OST or zanamivir during

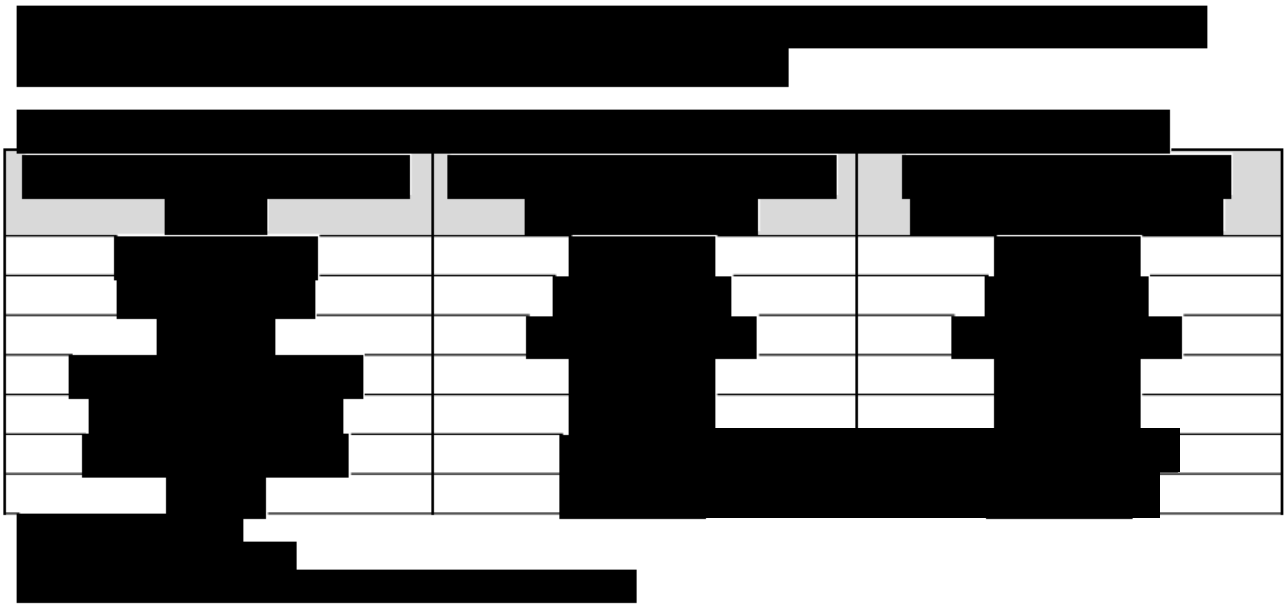
infections with the H1N1 A/PR/8/34 (PR8) influenza A strain. NTZ also exhibited synergism when combined with OST during infections with the avian A/Ck/Italy/9097/97 (A/Ck) influenza A strain. No cell toxicity was detected at the highest doses in the combination studies in uninfected cells [redacted].

1.2.3 Pharmacokinetics of Nitazoxanide and Tizoxanide in Humans

NTZ is not detectable in the plasma following oral administration of the drug. The main metabolites of NTZ in man are tizoxanide and tizoxanide glucuronide. Tizoxanide is highly bound to plasma proteins (>99%). The absorption of NTZ in immediate release tablets is significantly enhanced (Cmax and AUC of tizoxanide and tizoxanide glucuronide in plasma are almost doubled) when it is administered with food. In fasted human volunteers receiving a single 500 mg dose of ^{14C}NTZ, approximately one-third of the dose was excreted in urine as tizoxanide and tizoxanide glucuronide, and two-thirds was excreted in feces as tizoxanide. No other significant metabolites were detected [redacted].

During a Phase 2b/3 clinical trial for treatment of acute uncomplicated influenza, pharmacokinetics was studied in 6 subjects after receiving 300 mg NTZ with food and 10 subjects after receiving 600 mg NTZ with food. The study medication was administered twice daily for 5 days, and the blood samples for pharmacokinetics were collected over a 12-hour period [redacted]

[redacted]



1.2.4 Phase 2 Clinical Trials of NTZ in Subjects with Viral Respiratory Infection

In children age 1-11 years, 100 subjects (50 NZT/50 placebo [PCB]) with viral respiratory infection for less than 72 hours duration were included and given an age-based dose of NTZ 100-200 mg twice daily (BID) or PCB for 5 days. Criteria for respiratory infection were met by fever, at least 1 respiratory symptom (cough, nasal discharge or congestion, sneezing, or sore throat), and at least 1 constitutional symptom (myalgia, malaise, fatigue, headache, chills/sweat). The NTZ cohort showed symptom resolution in 4 days (median, IQR 2-7) versus >7 days (median, IQR3->7) in the placebo group ($p<0.0001$, log rank test). Furthermore, analysis of secondary endpoints showed that the pediatric patients randomized to placebo were more likely to experience at least 1 illness-related physical exam abnormality at Day 7 follow up (81% vs. 28%, $p<0.0001$) and that they were also more likely to require post-study antibiotics (64% vs. 9%, $p<0.0001$). Lastly, treatment with NTZ was associated with a significant decline in the amount of nasal discharge (as measured by daily weighing of used facial tissues) during the 5-day treatment period ($p=0.0347$).

Using the same design and endpoints as the pediatric Phase 2 study, a second Phase 2 study was conducted in adults and adolescents (age ≥12 years). There were 86 subjects included (43 NTZ/43 PCB) with viral respiratory infection. Subjects were randomized to either NZT 500 mg BID or PCB for 5 days. Time to resolution of symptoms was a median of 4 days (IQR >3-5) in the treatment arm versus 7 days (IQR 3->7) in the placebo group ($p=0.0365$, log rank test). Secondary efficacy analysis also showed that patients randomized to the placebo treatment group were more likely to have at least 1 illness-related physical exam abnormality on Day 7 follow up (71% vs. 37%, $p=0.0032$). At Day 7 follow-up, the placebo group was twice as likely to require antibiotics (36% vs. 18%), but this finding was not statistically significant ($p=0.132$). The mean weight of tissue collected over the treatment period was also higher for patients in the placebo group, but this was also a statistically insignificant result ($p=0.20$).

1.2.5 Phase 2b-3 Clinical Trial of NTZ in Acute Uncomplicated Influenza

During the 2010-2011 flu season, a Phase 2b-3 trial in 74 outpatient centers enrolled 624 subjects between the ages of 12 and 65 years who presented with acute uncomplicated influenza (symptoms onset <48 h).[23] At baseline, 41% of patients had confirmed (RT-PCR) influenza infection (50% AH1N1, 30% Influenza B, 20% AH3N2). Rhinovirus was the second-most detected pathogen (14%) of enrolled patients, followed by coronavirus (3%). Over one third of patients (37%) had no pathogen detected by RT-PCR.

Randomized subjects were given the extended release formula of NTZ 600 mg BID for 5 days. In a population infected with influenza, NTZ reduced time from illness onset to alleviation of flu symptoms (95.5 h in 600 mg dose [IQR 71-126] and 116.7 h in PCB [IQR 91-144] respectively, p=0.0084). The median duration of symptoms for the 300 mg cohort (109.1 h in 300 mg dose [IQR 82-152]) was not statistically more significant than placebo (p=0.5208).

Analysis of all patients treated (i.e., all ILI, not just those with influenza) also showed a statistically significant reduction of times from first dose to resolution of symptoms for the 600 mg NTZ treatment group (median 94.9 h, IQR: 62-126) compared to the placebo group (median 108.2 h, IQR: 82-152, p=0.0026).

An analysis of the subset of 37% of subjects with no virus identified at baseline also showed a dose-dependent reduction in duration of symptoms with a statistically significant difference (p=0.0208) observed between the 600 mg NTZ treatment group (median 88.4 h, IQR: 60-120) and the placebo group (median 105.7 h, IQR: 76.5-160). The numbers of subjects with other laboratory-documented respiratory virus infections at baseline were too small to draw meaningful interpretations, although the data suggested that there was no apparent treatment effect in subjects infected with rhinovirus.

There are no data on the use of NTZ in a hospitalized population with influenza or other causes of ILI.

1.3 Etiology and Burden of ILIs in Mexico

During 2009-2010, a new influenza virus (A/California/07/2009 H1N1) spread worldwide causing the first flu pandemic in more than 40 years. Because of the significant fatality rates and the fact that Mexico City was the epicenter of the earliest reported cases, the Mexican Ministry of Health and NIAID collaborated to establish the LaRed network. LaRed's observational cohort study of ILIs in Mexican adults and children has over 5600 subjects over 4 years (data as of July 2014). Analysis of the first years data found that, in addition to influenza which accounted for 24% of viral isolates, rhinovirus, coronavirus, RSV, and adenovirus contributed significantly to the burden of disease, accounting for 25%, 14%, 10%, and 9% of all viral isolates respectively.[12] Similar results are seen in the 3 year data (unpublished). For severe (hospitalized) disease, similar viruses caused disease (see Table 2). While NTZ is not thought to treat rhinovirus, it is thought to be efficacious in treating adenovirus, coronavirus, influenza, parainfluenza, and RSV, and likely efficacious in treating mixed infections. Additionally, in the Phase 2b-3 data there was efficacy in the "negatives" (pointing to likely infectious agent). Taken together, these categories make up 76% of hospitalized ILI (i.e., SARI) patients that LaRed has been able to enroll previously.

Table 2: Pathogen distribution in hospitalized ILI cases in LaRed over 3 years, by age group

Pathogen	Children ≥2 to <12 years old	Adults ≥12 years old	Total	%Total
Adenovirus	**6**	**24**	**30**	**2.2**
Bordetella pertussis	**2**	**5**	**7**	**0.5**
Coronavirus	**9**	**55**	**64**	**4.7**
Coronavirus 229E	*2*	*18*	*20*	
Coronavirus NL63	*3*	*20*	*23*	
Coronavirus OC43	*4*	*17*	*21*	
Chlamydophila pneumoniae	**0**	**0**	**0**	**0.0**
Influenza	**21**	**112**	**133**	**9.7**
Influenza A	*2*	*10*	*12*	
Influenza A H1N1	*7*	*34*	*41*	
Influenza A H3N2	*5*	*44*	*49*	
Influenza B	*7*	*24*	*31*	
Legionella pneumophila	**0**	**0**	**0**	**0.0**
Metapneumoviurs	**17**	**34**	**51**	**3.7**
Mycoplasma pneumoniae	**2**	**18**	**20**	**1.5**
Parinfluenza	**15**	**29**	**44**	**3.2**
Parainfluenza I	*4*	*2*	*6*	
Parainfluenza 2	*5*	*8*	*13*	
Parainfluenza 3	*4*	*9*	*13*	
Parainfluenza 4	*2*	*10*	*12*	
Rhinovirus	**40**	**207**	**247**	**18.0**
RSV	**44**	**35**	**79**	**5.7**
RSV A	*37*	*31*	*68*	
RSV B	*7*	*4*	*11*	
Mixed	**43**	**107**	**150**	**10.9**
Negative	**54**	**497**	**551**	**40.0**
Total	**253**	**1,123**	**1,376**	

Given the potential efficacy in influenza and other respiratory viruses as described above (section 1.2.4 and 1.2.5), the hospitalizations and morbidity associated with these viruses described in this section, and the lack of data of NTZ in this hospitalized SARI population, LaRed would like to study the use of NTZ for the treatment of hospitalized subjects with SARI.

1.4 Risks and Benefits

1.4.1 Risks of NTZ

NTZ has been marketed in the United States since 2003 and in Latin America since 1996. It is estimated that more than 50 million patients have been exposed to NTZ post-marketing worldwide. No drug-related serious adverse events (SAEs) have been reported during post-marketing experience with NTZ.

Phase 2 and 3 clinical studies have been conducted in approximately 4,000 patients to evaluate the safety and efficacy of NTZ in treating parasitic, bacterial, and viral infections. During these studies, no drug-related SAEs were observed. The side effects have been usually of a mild transient nature, and less than 1% of patients have discontinued therapy because of an AE. The most common AEs reported in clinical trials include abdominal pain, diarrhea, headache, nausea, and vomiting and did not differ significantly from those of placebo. Clinical chemistry and hematology obtained before and after treatment have not revealed any abnormalities attributable to the test drug.

Nitazoxanide is US FDA Pregnancy Category B: No impaired fertility or harm to the fetus in animal studies was noted, but there are no adequate and well-controlled studies in pregnant women.

1.4.2 Risk of Phlebotomy

The primary risks of phlebotomy include local discomfort, occasional bleeding or bruising of the skin at the site of needle puncture, and rarely hematoma, infection, or fainting. At the time of enrollment and during study visits, each subject will be asked about participation in other research studies to ensure that blood draws do not exceed each institution's limits of blood drawn.

In children, the volume of blood samples for this study required for each visit represents 0.4% of circulating blood volume in well-nourished children, up to 1% of the circulating volume in malnourished children. Therefore, on a month-long study, the total volume of blood required is 1.2% to 3% of circulating blood volume, depending on the constitution of each child.

1.4.3 Risk of Nasopharyngeal Swab

The primary risk of a nasopharyngeal swab is local discomfort. Rarely, there can be local bleeding from the nasal mucosa, which is controlled with local measures such as pressure or packing with gauze. Additionally the swab can cause coughing, gagging or infrequently vomiting.

1.4.4 Risk of Nasopharyngeal Aspirate

The primary risk of a nasopharyngeal aspirate is local discomfort. Rarely, this procedure can cause bleeding. Also, this procedure cause coughing, gagging, or vomiting.

1.4.5 Benefits

There may be no benefits to individual subjects for participation in this study. There may be earlier resolution of symptoms or fewer complications from SARI.

1.4.6 Alternatives

The alternative to participation in this study is routine standard of care (SOC). For suspected or confirmed severe influenza this will generally include an antiviral (likely oseltamivir or zanamivir based largely on availability and susceptibility).

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective is to evaluate the effect of NTZ in addition to SOC as compared to PCB + SOC in the treatment of SARI as measured by the time to hospital discharge.

2.2 Secondary Objectives

1. Evaluate the clinical efficacy of NTZ + SOC vs. PCB + SOC with regards to:
   - Percent hospitalized at Study Day 3, 7, 14 and 28
   - Death by Study Day 28
   - Duration of clinical symptoms (defined in Section 7.3.1.2)
   - Duration of fever
   - Use of supplemental oxygen (or increase greater than baseline oxygen requirement)
   - Admission to ICU
   - Intubation/mechanical ventilation
   - Proportion of subjects who develop complications including pneumonia, respiratory failure requiring mechanical ventilation, acute respiratory distress syndrome (ARDS), sepsis, or bronchiolitis.
   - Time to resumption of normal activity
   - Antibiotic/antiviral use during hospitalization
   - Systemic corticosteroid use during hospitalization
   - Re-hospitalization within 28 days

2. Evaluate the virologic efficacy of NTZ + SOC vs. PCB + SOC with regards to:
   - Percent cessation of viral shedding at Study Day 3

3. Evaluate the safety and tolerability of NTZ assessed by:
   - Cumulative incidence and severity of AEs
   - Cumulative incidence of SAEs
   - Cumulative incidence of laboratory abnormalities on Study Days 3, 7 and 28

3 INVESTIGATIONAL PLAN

3.1 Research question

Does treatment with NTZ improve the outcome of subjects with SARI caused by respiratory viruses?

3.2 Hypothesis

NTZ improves the outcome of subjects with SARI caused by respiratory viruses, as measured by (duration of hospitalization, duration of symptoms, ICU requirements, O_2 requirements, use of antibiotics, etc).

3.3 Justification for the research

As respiratory viruses are major causes of ILI and SARI that can lead to severe outcomes including hospitalization and death, and the majority of these viruses have no available treatment, potential treatments of respiratory viruses causing SARI are needed.

3.4 Overview of Study Design

The study will be a multicenter, randomized, double-blind, placebo controlled trial to evaluate the efficacy of NTZ+ SOC compared to PCB + SOC in treating acute viral respiratory infections in adults and children ≥ 12 months old with SARI. The study will be conducted in up to 8 sites within Mexico, at sites that are part of the LaRed network. Subjects will be selected according to the inclusion and exclusion criteria listed below (Sections 4.3 and 4.4). The study is designed to enroll a total of 290 subjects who will be randomized in a 1:1 ratio. Enrollment will continue until 290 subjects are randomized, or March 2017, whichever comes first.

Prior to screening for inclusion in the study, informed consent (and assent for pediatric subjects as applicable) will be obtained. After completion of informed consent, screening for the study will occur. This involves review of the subjects' symptoms, medical conditions, physical exam, and pregnancy test for females able to become pregnant. All patients will initially be hospitalized. If the subject qualifies for the study, baseline assessments will occur including baseline vitals, safety labs, and nasopharyngeal swab or aspirate for PCR. Subjects will be randomized to a treatment kit and will begin treatment immediately. They will receive instruction on completion of their diaries, concomitant medications, and attending follow-up visits. Subjects will be followed on Study Days 3, 7, 14, and 28. The subject will be discharged from the hospital when clinically indicated as determined by the treating physician – follow-up visits may occur as inpatients or as outpatients.

On Study Days 3 (+/- 1) and 7 (+/- 1) a clinical assessment will be performed, diary cards collected, and safety labs will be obtained. Subjects that are hospitalized on Day 14 will have an in person visit. For subjects that are no longer hospitalized, the Day 14 visit may occur in person or by telephone. This visit is primarily for the collection of Day 7-14 diary card information. Study Day 28 will mark the end of study where subjects return for final clinical assessment, safety labs, and convalescent serologies.

Subjects that are still hospitalized after Study Day 28 will continue to be followed through the date of discharge.

3.5 Definitions for the Purpose of this Study

Enrolled

For the purpose of collecting data and samples and reporting AEs, a subject will be considered enrolled beginning from the time the informed consent form is signed until the subject is considered "screen failure", "discontinued", or "completed".

Randomized

Subjects are considered randomized when they meet all of the following criteria:

- Confirmation that the inclusion and exclusion criteria are met
- Consent is given and signed by the subject for the study
- Randomization number is assigned

Screen Failures

Subjects are considered screen failures when they meet 1 of the following criteria after signing consent:

- Screening tests reveal that the subject is ineligible
- Subject withdraws consent before being randomized

Discontinued

Subjects are considered discontinued when they meet 1 or more of the following criteria:

- Subject withdraws consent after being randomized (see Section 4.5)
- Subject is withdrawn after enrollment by investigator (see Section 4.7) including lost to follow-up

Completed

Subjects are considered completed when they are followed through Study Day 28 and complete the final study follow-up visit (Study Day 28) or die prior to this study visit.

3.6 Study Endpoints

3.6.1 Primary Endpoint

Duration of hospitalization (days and hours)

3.6.2 Secondary Endpoints

Clinical Endpoints:

- Hospitalization on Study Days 3, 7, 14, and 28
- Date and time of hospital discharge
- Date of death, if applicable
- Clinical symptoms (measured daily through Study Day 14 and then again on Study Day 28)
- Temperature (measured daily through Study Day 14 and then again on Study Day 28)
- Oxygen use
- Date of admission to ICU and discharge from ICU, if applicable
- Date of intubation/extubation, if applicable
- Presence of complications during study (pneumonia, respiratory failure requiring mechanical ventilation, ARDS, sepsis, or bronchiolitis)

- Global assessment (measured daily through Study Day 14 and then again on Study Day 28) (defined in Section 7.3.1.3)
- Antibiotic/antiviral use during hospitalization (number and duration)
- Re-hospitalization within 28 days
- Use of systemic corticosteroids

Virologic Endpoints:

- Presence of virus on nasopharyngeal (NP) swab (or aspirate) on Study Day 3 (same virus as Day 0)

Safety Endpoints:

- AEs
- SAEs
- Chemistry and hematologic laboratory assessments on Study Days 3, 7 and 28

4 STUDY POPULATION

4.1 Rationale for Research Subject Selection

The inclusion criteria define those subjects with SARI. In an ongoing LaRed study, similar ILI criteria have been successful in identifying a population whose respiratory illness is caused by a virus 70% of the time.

4.1.1 Justification for the Inclusion of Children ≥ 12 Months Old

In Mexico, nitazoxanide is licensed by COFEPRIS from 2 years old indicated for the treatment of diarrhea caused by *Giardia lamblia* and by *Cryptosporidium parvum*. In the US Nitazoxanide is approved by FDA for the management of diarrhea caused by *G. lamblia* or *C. parvum* in pediatric Subjects 1 year of age and older. Nitazoxanide pediatric dosage recommendations are summarized in Table 3.

Table 3: Pediatric Nitazoxanide Dosage Recommendations

Disease	Maximum dosage
Diarrhea caused by *G. lamblia* or *C. parvum*	• 1-3 years of age: 100 mg (or 5 mL as oral suspension) every 12 hours (with food) for 3 days • 4-11 years of age: 200 mg (or 10 mL as oral suspension) every 12 hours (with food) for 3 days • ≥ 12 years of age: 500 mg (or 25 mL as oral suspension) every 12 hours (with food) for 3 days

** Tablets are only approved for use in children 12 years of age and older.*[19]

However, there is evidence in papers of nitazoxanide administered in children less than 2 years old. One study was conducted to assess the activity of nitazoxanide in hospitalized pediatrics Subjects with severe gastroenteritis due to Rotavirus. In that trial drug has been used in children from 5 months old. The blinded study medication was administrated as: children 12-47 months of age 5mL (100 mg) twice a day for 3 days, and 7.5 mg/kg twice a day in subjects younger than 12 months. They found no adverse experiences associated with the use of nitazoxanide. There

were no significant adverse events, only two events (one case of mild otitis media and one case of mild bronchitis) both these subjects were in the placebo treatment group.[20]

In another large study in Egypt nitazoxanide there were included 546 subjects. They took medication with food at 12-hour intervals over 3 days; those older than 12 years received 500 mg of nitazoxanide, children aged 4 to 11 years received 200 mg of drug, and children aged 1 to 3 years received 5 mL of a 100 mg per 5 mL suspension. Their results indicate that nitazoxanide is safe and effective for treating intestinal protozoan and helminthic infections with eradication rates of 81% for intestinal amebiasis, 94% for giardiasis, and 77% for balantidiasis.[21]

4.1.2 Justification for the Exclusion of Pregnant Women and Children < 6 Months Old

Given the lack of known efficacy of the combination and the potential toxicities including teratogenicity, pregnant women are excluded from this study.

NTZ is licensed in the US and Mexico for adults and children (1 year of age and older in the US and 2 years and older in Mexico) for gastrointestinal parasitic diseases (*Giardia lamblia* or *Cryptosporidium parvum*). Given the clinical impact of respiratory viruses and SARI in children (as previously described), children (greater than or equal to 12 months) are eligible for inclusion in this study. The use in children under 2 years of age in Mexico, and the use for respiratory infections are investigational.

As the safety of nitazoxanide in children < 12 months of age has not been established, these children will be excluded.

4.2 Recruitment

Subjects will be recruited through the hospital sites in Mexico that comprise LaRed. Information about this study will be disseminated to health care providers in these settings. Subjects that have been recently hospitalized with ILI, or that are in the emergency department or clinic that are going to be hospitalized with ILI will be evaluated for potential participation in this study. Additionally, direct-to-subject recruitment (using posters and/or other informational items) may occur in these hospital settings.

Any direct-to-subject recruitment documents/materials will be submitted to the reviewing Institutional Review Board (IRB)/Independent Ethics Committee (IEC) for approval. Any flyers and advertisements that will be viewed directly by potential study subjects will be submitted to the site IRB/IEC for approval.

Informational items about the study distributed to the health care staff only, including but not limited to the manual of procedures are not required to be reviewed by the IRB/IEC.

4.3 Inclusion Criteria for Enrollment

1. Signed informed consent prior to performance or initiation of any study procedures.
2. Age ≥ 12 months of age (no upper age limit).
3. One of the following constitutional signs or symptoms within 7 days of screening:
   - Temperature of ≥ 38° C

- Temperature ≤ or 36° C
- Myalgia
- Headache
- Malaise
- Irritability (children < 5 years of age only)
- Decreased appetite (children < 5 years of age only)

4. One of the following lower respiratory sign/symptom within 7 days of screening:
   - Cough
   - Hypoxia (SaO2 < 95%)
   - Tachypnea (age adjusted, see section 7.3.4)
   - Dyspnea
   - Nasal flaring (children <2 only)
   - Chest retractions (children <2 only)
5. Hospitalization for ILI (decision for hospitalization will be up to the individual treating clinician), with anticipated hospitalization for more than 24 hours.
6. One of the following to avoid pregnancy:
   - Females who are able to become pregnant (i.e., are not postmenopausal, have not undergone surgical sterilization, and are sexually active with men) must agree to use at least 1 effective form of contraception from the date of informed consent through Day 28 of study. For hospitalized subjects, this can begin after hospital discharge.
   - Males who have not undergone surgical sterilization and are sexually active with women must agree to use condoms or have a partner use at least 1 effective form of contraception through Day 28 of study. For hospitalized subjects, this can begin after hospital discharge.

4.4 Exclusion Criteria

1. Women who are pregnant or breast-feeding.
2. Clinical suspicion that etiology of illness is primarily bacterial in origin.
3. Prior treatment with antivirals (e.g. oseltamivir) for the current illness for more than 24 hours.
4. Subjects unable to take enteral medications (adults must tolerate tablets by mouth as they cannot be crushed, children must be able to swallow pediatric suspension or receive drug by nasogastric tube).
5. Prior treatment with any investigational drug therapy within 30 days prior to screening.
6. Known sensitivity to NTZ or any of the excipients comprising the NTZ tablets.
7. Prior NTZ use within 1 week.
8. Self-reported history of impaired renal function (no blood or urine kidney function laboratory testing will be done prior to enrollment, but intent is to exclude disease severe enough to cause estimated CrCl <30 mL/min).
9. Self-reported history of liver disease (no blood laboratory testing will be done prior to enrollment, but intent is to exclude disease severe enough to cause cirrhosis or total bilirubin > 1.5×ULN, AST/ALT > 3×ULN, based on labs obtained previously for clinical diagnosis).

10. Presence of any pre-existing medical condition not listed above that, in the opinion of the investigator, would place the subject at an unreasonably increased risk through participation in this study.
11. Subjects who, in the judgment of the investigator, will be unlikely to comply with the requirements of this protocol.
12. The onset of respiratory symptoms occurs after hospitalization (to exclude nosocomial infections).
13. Hospitalized for any reason for greater than 60 hours prior to enrollment.
14. Participants previously enrolled in this study.
15. Known chronic respiratory infection (e.g., tuberculosis, atypical mycobacterial infections).

4.5 Subject Withdrawal

Subjects (or their parents or legal surrogates if subjects become unable to make informed decisions) can terminate study participation at any time without prejudice. If a subject terminates participation before completing the study, the reason for this decision will be recorded in the case report form (CRF).

Subjects who indicate interest in withdrawing from the study should also be asked permission to be contacted at Study Day 28 by telephone for vital status (any AEs that occurred during the study). This is not considered full withdrawal of consent, and the agreement for limited follow-up should be noted in the medical record.

Subjects who fully withdraw consent will not be contacted further.

Subjects who withdraw from the study will not be replaced.

4.6 Discontinuation of Study Medication

Subjects will discontinue study medication for any of the following reasons:

- Laboratory results from baseline or subsequent visits that show any of the following:
  - Estimated CrCl <30 mL/min
  - Total bilirubin >2×ULN
  - AST/ALT >3×ULN
- The subject becomes unable to take oral medications (study drug cannot be given by nasogastric tube without unblinding)
- Clinically significant grade 3 or 4 AEs (including safety laboratory results) that in the judgment of the site investigator are causally related to study medication
- Any other AE or clinically significant laboratory result for which the investigator believes that continuation of the study medication would be detrimental to the subject
- Positive pregnancy test

Subjects who discontinue study drug should still be treated with standard care treatments (Section 5.6).

The reason for discontinuation of the study drug is to be recorded on the CRF. All subjects who discontinue study drug will be encouraged to comply with the study visit schedule including

clinical and virologic assessment through Day 28.

4.7 Discontinuation of Subject by Investigator

The investigator has the right to withdraw subjects from the study. Subjects may be withdrawn from the study for any of the following reasons:

- The subject is lost to follow-up
- The subject experiences an AE and the investigator believes that continuation in the study would be detrimental to the subject (in contrast to discontinuing the study medication and continuing follow-up as discussed in Section 4.6)

The reason for withdrawal from the study is to be recorded on the CRF. If a non-serious AE is unresolved at the time of discontinuation, efforts should be made to follow-up until the event resolves or stabilizes, the subject is lost to follow-up, or there is some other resolution of the event. The investigator is to make every attempt to follow all SAEs to resolution.

Subjects who miss a study visit should be contacted to reschedule. If subjects cannot be contacted immediately, periodic attempts should continue until study completion (Study Day 28). Lost to follow-up is defined as unsuccessful contact after at least 2 documented telephone calls.

Any subject withdrawn from the study will not be replaced.

4.8 Discontinuation of Study

NIAID as the study sponsor, the reviewing IRB/IEC, the US Food and Drug Administration (FDA), and the Mexico Federal Commission for the Protection against Sanitary Risk (COFEPRIS) have the right to terminate the study at the sites they are responsible for at any time.

4.9 Participation in Other Protocols

Subjects in this protocol may participate in other research protocols, as long as the other research does not require more than 30 mL of blood to be given in any 4-week period of time and does not administer any unlicensed drug, unlicensed vaccine, or any treatment (licensed or unlicensed) for influenza or influenza like illness within the 30 days or 5 half-lives (whichever is longer) prior to enrollment or during the duration of this study.

LaRed has an ongoing ILI/SARI surveillance study and it is anticipated many subjects may be co-enrolled in this study.

4.10 Emergency Unblinding

Any pregnancy occurring on study will be immediately unblinded (regardless of known AE or known fetal toxicities). As nitazoxanide is US FDA pregnancy category B, this unblinding only applies to subjects and not partners. The participant will be told if they were receiving nitazoxanide or placebo.

At any point, if AEs occur and investigators are concerned about the treatment allocation, the treatment can be discontinued as discussed in Section 4.6. However, if all of the following 3 criterion are met, the subject's randomization may be unblinded regarding treatment:

1. The AE must be a SAE as also defined in Section 8.2
2. The AE must be thought to be probably or definitely related to the study drug.
3. The treating clinician believes that knowledge of the treatment arm may change the therapy provided to the patient.

The procedure for unblinding will be further detailed in the Manual of Operations.

5 TREATMENT

Subjects will be randomized to 1 of the following 2 arms in a 1:1 treatment allocation ratio (Table 4):

Table 4: Treatment Allocation

Arm	Drug	Allocation Ratio
1	Nitazoxanide	1
2	Placebo	1

5.1 Blinding, Randomization, Stratification, and Treatment Assignment

Treatment kits will be blinded and numbered. The two treatment arms are not distinguishable at the site. Therefore the study team, the pharmacy, and the monitors will be blinded.

The randomization scheme will be prepared by the sponsor. Those unblinded are limited to the statistician not involved with the study who generated the randomization code, and the pharmaceutical group that packaged the study drug. The Data and Safety Monitoring Board (DSMB) will have access to the unblinding code in a sealed envelope.

The study will remain blinded until after the last subject enrolled has completed the last follow-up visit and all data queries have been resolved. The study will then be unblinded for analysis.

Randomization will be built into the treatment kit order at each site (i.e., kit #101 may be NTZ or PCB depending on the randomization scheme).

Randomization will be stratified by sites and by adult and pediatric (<12 years). Randomization will occur in blocks of 4. Each site will receive study drug in multiples of 4, thus ensuring randomization is balanced by site.

Subjects who meet all criteria for enrollment will be assigned the next study kit at a site. Kits will be assigned in a consecutive manner starting with the first kit number in the block at each site.

5.2 Study Regimens and Administration

The sponsor will supply each site with study agent near the time of site activation. Each randomized subject will receive a study drug kit consisting of 5 days of treatment. The site pharmacy will distribute the study kits. The method of supplying study agent to hospitalized subjects will be customized at each site.

Subjects take the medication twice daily, with food, and each dose will consist of the following:

Adults and Children 12 years and older:
The active formulation for this study is a yellow, film-coated tablet that contains 300 mg NTZ plus standard excipients. This is an extended release formulation of licensed NTZ. This formula provides a mean AUC_t 29 μg*hr/mL and mean C_{min} (12 h) 0.8 μg/mL.

The placebo tablet will have the same appearance and inactive ingredients as the active tablet.

Group 1 (NTZ): 2 NT-300 mg tablets orally BID x 5 days.
Group 2 (Placebo): 2 placebo tablets orally BID x 5 days.

Children younger than 12 years:
The formulation for children younger than 12 years is the currently licensed suspension of nitazoxanide (Alinia®), or matching placebo.

Group 1 (NTZ):
4-11 years: 10 mL oral suspension (200 mg NTZ) every 12 hours x 5 days
1-3 years: 5 mL oral suspension (100 mg NTZ) every 12 hours x 5 days

Group 2 (Placebo):
4-11 years: 10 mL oral suspension (placebo) every 12 hours x 5 days
1-3 years: 5 mL oral suspension (placebo) every 12 hours x 5 days

5.3 Justification of Dose

The pediatric dose of NTZ for subjects 1-11 years or age is the same as the licensed dose.

The adult dose (600 mg) is slightly higher than the licensed adult dose (500 mg) but is equivalent to the dose used in previous influenza treatment studies where efficacy was demonstrated (as discussed in the background section). This higher dose is needed to provide favorable pharmacokinetics thought to be needed for the treatment of ILI.

5.4 Study Drug Acquisition

Study drug will be stored in a central repository. Sites will be given an initial supply of study drug after the local IRB/IEC has approved the study, the US FDA and Mexico COFEPRIS have granted the study safe to proceed, the sponsor has approved shipment of study agent, and after all import licenses have been obtained, as applicable.

5.5 Study Drug Accountability

The investigator or his/her designee is required to maintain accurate drug accountability records. A binder containing instructions and the required accountability documentation will be provided to the investigator or his/her designee. When the study is completed, copies of the study drug accountability records will be maintained at the study site and all originals returned to the sponsor. Copies of the drug accountability records must be maintained with the rest of the documentation for the study. All unused study drug must be disposed of upon authorization by NIAID or its designee. All standard operating procedures and records regarding the disposition of study drug must be available for inspection by the study monitors and regulatory authorities.

5.6 Standard of Care Treatment

It is anticipated that subjects will receive concomitant medications for likely treatable pathogens that may cause SARI. These would likely include antibiotics and may include other treatments for influenza such as oseltamivir. As treatments will be different due to age, medical conditions, exposures, clinical presentation, etc., the treating physician will make all decisions about standard of care treatments, and no specific treatment regimen is mandated by this protocol.

5.7 Concomitant Medications

Subjects will be monitored throughout the study for use of concomitant medications. Any prescription medications, and/or over-the-counter preparations (OTC) taken during the study period must be recorded on the source documents and in the designated CRF.

5.8 Prohibited Medications

Subjects may not receive investigational medications (other than the study drug provided) at any time during the study. Their use would not be considered SOC and therefore withholding these compounds would still be consistent with best medical practice.

5.9 Treatment Compliance

Treatment compliance during hospitalization will be documented by the hospital record. Compliance after discharge will be assessed by examination of subject's remaining study product on each study visit.

6 STUDY PROCEDURES

The following table (Table 5) outlines all of the assessments to be conducted during the study. A detailed presentation of assessments immediately follows the table. The day when the subject is randomized is denoted as Study Day 0, the first day after enrollment is Study Day 1, etc.

Table 5: Schedule of Assessments

	Screen	*Baseline*	*Follow-up*						
Window (+/- Days) - applies only to evaluations, not study drug)	0		1	2	3 ± 1	4	7 ± 1	14 ± 2	28 ± 3
Evaluation/Procedure									
ELIGIBILTY									
Informed consent	X								
Demographics	X								
Medical history	X								
RANDOMIZATION/ STUDY DRUG									
Randomize subject		X							
Dispense Study Treatment Kit		X							
Study Treatment Administration		X	X	X	X	X			
STUDY PROCEDURES									
Review and collect diary card *(see Section 7.3.1)*		X[1]			X		X	X	X
Assess for complications of influenza *(see Section 7.3.2)*		X			X		X	X	X
Vital signs, including SaO2 *(see Section 7.3.3)*		X			X		X		X
Review of concomitant medications	X				X		X	X	X
Physical exam[2] *(see Section 7.1.2)*	X				X		X		X
Adverse events		X			X		X	X	X
SAFETY LABORATORY									
Hematology Panel *(see Section 7.1.1)*		X			X		X		X
Chemistry Panel *(see Section 7.1.1)*		X			X		X		X
Urine βHCG (for females of childbearing potential)	X								
REFERENCE PROCEDURE									
Nasopharyngeal swab for virus isolation		X			X				

Notes: 1. On Day 0, subject will be instructed on how to complete the diary card, and baseline assessment will be collected.

2. Brief physical examinations will be conducted at screening to ensure there are not medical conditions (either chronic or acute) that would increase a subject's risk for participation in this study. Symptom-targeted physical examinations will be conducted at all other visits as needed to evaluate new complaints and possible adverse events.

6.1 Personnel and Location for Study Procedures

All study assessments should be performed by members of the investigative team that are specifically designated to perform such activities (according to site practices, local law, and as designated on the appropriate study documents).

The initial screening evaluation and baseline visits will occur during hospitalization (as per inclusion criteria). Subsequent visits may occur either during hospitalization or as outpatients. Outpatient visits may occur in a health care facility or at home according to site practices and local law. Any sites performing home visits should have:

- IRB approval for home visits
- Written study specific procedures including obtaining samples with the storage condition, and timeframe of this protocol that are approved by the study team.
- Documentation of institutional permission or policies permitting home visits
- Adequate staff and resources to perform home visits

6.2 Detailed Description of Assessments

6.2.1 Study Day 0: Screening

Screening evaluations must be completed before randomization. As screening does not require any laboratory results, screening should be completed within 12 hours of enrollment.

Subjects will be identified from subjects that are in the emergency room or have been recently hospitalized, or may be referred from other health care workers within the institution. As early treatment is likely to provide the best treatment effect (as seen in influenza), subjects should be identified, and if appropriate, approached, enrolled, and if enrolled, administered baseline evaluation and given first does as soon as possible, but no longer than 24 hours.

6.2.1.1 Informed Consent

The site investigator will review the informed consent document with the subject or legal representative. Informed consent should be obtained from the subject, or if a subject is judged by the investigator to be unable to provide informed consent, consent should be obtained from his/her legally authorized representative. The use of a surrogate for consent may only be permitted if allowed by local regulation and must be in accordance with local requirements.

Children should be presented the study assent (in addition to consent being obtained from his/her legally authorized representative). The age cutoff for assent will follow institutional or site IRB policies/stipulations.

A single informed consent form will be used for both screening and enrollment into this protocol.

6.2.1.2 Demographics

The following information should be recorded:

- Age
- Sex
- Ethnicity
- Race

6.2.1.3 Medical History

The Investigator (or designee) will take a medical history at the time of consent and conduct a complete physical examination. Information about the ILI symptoms, tolerating oral diet, medical history, and other research protocols must be obtained from the subject if possible, other information can be abstracted from the chart if needed. The following information should be recorded:

- Day of onset of ILI symptoms
- Ability to tolerate oral medications and food/fluids
- Date and hour of hospital admission
- Influenza vaccination history
- History of chronic medical conditions (including, but not limited to, kidney and liver disease). This includes calculation of Charlson comorbidity index.
- Current use of prescription and OTC medications within the last 7 days
- Chronic oxygen use
- History of allergies
- History of exposure to NTZ products
- Participation in any recent research protocols (within the last 3 months)
- Smoking history

6.2.1.4 Physical Exam

A brief physical exam including vital signs and SaO_2 should be performed and will be used to ensure there are no medical conditions (either chronic or acute) that would increase a subject's risk for participation in this study.

6.2.1.5 Laboratory

Female subjects of child bearing potential will have a urine pregnancy test performed at screening (serum pregnancy test may be substituted as needed).

6.2.2 Determination of Eligibility and Randomization

6.2.2.1 Determination of Eligibility

After the previously mentioned evaluations have been completed (i.e., medical history), the investigator is to review the inclusion/exclusion criteria and determine the subject's eligibility for study participation. Any laboratory obtained as part of the subject routine care should be reviewed for clinically significant findings and taken into account to determine study eligibility.

6.2.2.2 Randomization

The study team will assign the subject the next study kit in sequence that is available at the site. This study drug kit number will be conveyed to the pharmacy and the kit will be distributed from the pharmacy.

6.2.2.3 Screening Failures

The following information will be collected on screening failures (as subjects have already signed consent): demographics (age, screen number, sex, birth date, ethnicity, and race) and reason for ineligibility. Subjects who are found to be ineligible will be told the reason for ineligibility and may not be re-screened for the study.

6.2.3 Study Day 0: Baseline Evaluation

Baseline evaluation must be completed prior to the first dose of study medication.

6.2.3.1 Clinical Data on Day 0

- Assessment for presence of complications (see Section 7.3.2)
- Vital signs, including SaO_2 (see Section 7.3.3)
- Assessment for AEs occurring after consent and prior to administration of study drug
- Severity of illness score (Sequential Organ Failure Assessment (SOFA) score or if < 24 months of age use Tal bronchiolitis score)

6.2.3.2 Laboratory Data

The following laboratory test will be performed:

- CBC with differential white cell count (to include neutrophil, lymphocyte, and eosinophil percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, ALT/GPT, AST/GOT, total bilirubin, LDH, CRP)

If these labs were performed for clinical care on the day of enrollment (or within 24 hours prior to enrollment), these labs do not need to be repeated and can be obtained from the clinical record. These tests are performed as a baseline for safety monitoring, and results do not need to be obtained before starting study drug.

If laboratory tests are drawn after enrollment, within 24 hours of enrollment these labs should be reviewed. If laboratory results that show estimated CrCl < 30 mL/min, total bilirubin >2×ULN, or AST/ALT >3×ULN, the study drug should will be stopped (per section 4.6)).

6.2.3.3 Research Procedures on Day 0

At baseline, a NP swab will be sent for multiplex PCR. If a NP swab cannot be obtained for medical reasons, an NP aspirate can be substituted. See Section 7.2

If this testing is performed for clinical care or other protocols on Day 0, and the results can be used for this study, the testing does not need to be repeated.

6.2.3.4 Study Day 0: Study Drug Distribution

While the patient is hospitalized, the study drug will be distributed from the pharmacy per local standards.

6.2.3.5 Distribution of Diary Cards

A subject diary card booklet assessing fever, symptoms and functional assessment, will be distributed to subjects. Collection of baseline assessments and subject recalled pre-illness condition(s) (See Section 7.3.1) will be completed by the study team and instructions will be given to the subject on the completion of the diary card.

6.2.4 Study Day 3 (+/- 1 day)

6.2.4.1 Clinical Data on Day 3

- Review and collection of diary cards since last visit (i.e. Days 1-3). The diary card documents fever, symptoms and functional assessment (See Section 7.3.1)
- Assessment for presence of complications (See Section 7.3.2)
- If discharged – complete discharge assessment and reinforcement of study procedures post discharge will be performed.
- Vital signs, including SaO_2 (See section 7.3.3)
- Review of study drug and assessment of compliance (see Section 7.4)
- Review of concomitant medications
- Symptom targeted physical exam (for safety assessment, see Section 7.1.2)
- Assessment for AEs

- Severity of illness score (SOFA score or if < 24 months of age use Tal bronchiolitis score) (see Section 7.5.1)

6.2.4.2 Clinical Laboratory Tests on Day 3(+/- 1 day)

The following laboratory test will be performed:

- CBC with differential white cell count (to include neutrophil, lymphocyte, and eosinophil percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, ALT/GPT, AST/GOT, total bilirubin, LDH, CRP)

If these labs were performed for clinical care on this study day (or within the window), these labs do not need to be repeated and can be obtained from the clinical record.

6.2.4.3 Research Procedures on Day 3 (+/- 1 day)

A follow-up NP swab will be sent for multiplex PCR. If a NP swab cannot be obtained for medical reasons, an NP aspirate can be substituted. The method of specimen collection on Day 0 should be the same method used on Day 3. See Section 7.2

6.2.5 Study Day 7 (-1 Day /+2 Days)

For subjects that are hospitalized and subjects no longer hospitalized, this visit will occur in person.

6.2.5.1 Clinical Data on Day 7

- Review and collection of diary cards since last visit (i.e. Days 3-7). The diary card documents fever, symptoms and functional assessment (See Section 7.3.1)
- Assessment for presence of complications (See Section 7.3.2)
- If discharged – complete discharge assessment. If still hospitalized – assess if medically able to be discharged from hospital.
- Vital signs, including SaO_2 (See Section 7.3.3)
- Review of concomitant medications
- Symptom targeted physical exam (for safety assessment, see Section 7.1.2)
- Assessment for AEs

6.2.5.2 Clinical Laboratory Tests on Day 7 (+/- 1 day)

The following laboratory test will be performed:

- CBC with differential white cell count (to include neutrophil, lymphocyte, and eosinophil percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, ALT/GPT, AST/GOT, total bilirubin, LDH, CRP)

If these labs were performed for clinical care on this study day (or within the window), these labs do not need to be repeated and can be obtained from the clinical record.

6.2.6 Study Day 14 (+/- 2 Days)

For subjects that are hospitalized, this visit will occur in person. For subjects that are no longer hospitalized, this visit may occur in person or by telephone. However, if telephone visits are used, all study endpoint information must still be collected.

6.2.6.1 Clinical Data on Day 14

- Review and collection of diary cards since last visit (i.e. Days 8-14). The diary card documents fever, symptoms and functional assessment (See Section 7.3.1). If this visit occurs by telephone, all diary card data is collected by phone, and subjects are instructed to bring the diary card at Day 28 visit.
- Assessment for presence of complications (See Section 7.3.2)
- If discharged – complete discharge assessment. If still hospitalized – assess if medically able to be discharged from hospital.
- Review of concomitant medications
- Assessment for AEs

6.2.7 Study Day 28 (+/- 3 Days)

6.2.7.1 Clinical Data on Day 28

- Review symptoms and functional assessment (See Section 7.3.1)
- If Day 14 was a telephone visit contact – the diary cards for Day 7 – 14 are collected, and verified against the data obtained by telephone by the study staff.
- Assessment for presence of complications (See Section 7.3.2)
- If discharged – complete discharge assessment. If still hospitalized – assess if medically able to be discharged from hospital.
- Vital signs, including SaO_2 (See section 7.3.3)
- Review of concomitant medications
- Symptom targeted physical exam (for safety assessment, see Section 7.1.2)
- Assessment for AEs

6.2.7.2 Clinical Laboratory Tests on Day 28

The following laboratory test will be performed:

- CBC with differential white cell count (to include neutrophil, lymphocyte, and eosinophil percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, ALT/GPT, AST/GOT, total bilirubin, LDH, CRP)

If these labs were performed for clinical care on this study day (or within the window), these labs do not need to be repeated and can be obtained from the clinical record.

6.2.8 Hospital Discharge Evaluation

Near the time of hospital discharge, data pertaining to the hospitalization will be collected. If subjects are still hospitalized after Day 28, they will be followed through hospital discharge.

A discharge source document and CRF should be completed by reviewing the medical chart within 4 days of discharge (if subjects are hospitalized for over 28 days, this evaluation should be done within 10 days of the day of discharge). This will document:

- Date and hour of discharge
- Date of death (if applicable)
- Oxygen use during hospitalization
- Dates of admission to ICU and discharge from ICU (if applicable)
- Dates of intubation/extubation (if applicable)

- Antibiotic/antiviral use during hospitalization (number and duration)

7 MEASURES OF SAFETY, EFFICACY,AND COMPLIANCE

7.1 Safety Evaluations

7.1.1 Laboratory Evaluations

All laboratory evaluations (except reference endpoint assays) will be performed at the local certified (e.g., Clinical Laboratory Improvement Amendments [CLIA], College of American Pathologists [CAP], or comparable certification) clinical laboratory. The same laboratory should be used throughout the study for any given patient. Blood samples will be collected from subjects at baseline (Day 0) and on Study Days 3, 7, and 28 for evaluation of routine laboratory safety (chemistry, complete blood count with differential).

Abnormal labs, if thought to be erroneous, should be repeated. Not all abnormal labs need to be repeated. Abnormal labs, if thought to be erroneous, which will cause cessation of study medication, should be confirmed by repeating the lab test as soon as possible.

On the designated days, the following laboratory test will be performed:

- CBC with differential white cell count (to include neutrophil, lymphocyte, and eosinophil percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, ALT/GPT, AST/GOT, total bilirubin, LDH, CRP)

7.1.2 Other Safety Examinations

A brief physical examination will be conducted at screening to ensure there are not medical conditions (either chronic or acute) that would increase a subject's risk for participation in this study. Symptom-targeted physical examinations will be conducted at all other visits as needed to evaluate new complaints and possible AEs. Due to the variability and the difficulty of standardization, the physical exam will not be used as efficacy data.

7.2 Viral Diagnostics

The NP swab on Days 0 and 3 will be tested by a central lab using a multiplex PCR for 22 respiratory pathogens and influenza types. The platform, Respifinder®, will be used according to manufacturer instructions. This multiplex PCR test can detect and differentiate 18 viruses (Coronavirus NL63, OC43, 229E, HKU1, Human metapneumovirus, Influenza A, Influenza A H1N1v, Influenza B, Parainfluenza 1 to 4, Respiratory syncytial virus (RSV) A and B, Rhinovirus/Enterovirus, Adenovirus, Bocavirus) as well as 4 bacteria (*Bordetella pertussis, Chlamydophila pneumoniae, Legionella pneumophila, Mycoplasma pneumoniae*).

7.3 Efficacy Evaluations

7.3.1 Diary Card for Fever, Symptoms, and Functional Assessment

Subjects will maintain a diary card, and will be asked to make a daily entry in the evening. On Day 0, subjects will be instructed on the diary card and the initial (baseline) assessment will be

completed by the subject and collected. The subject will make daily entries through Day 14. The subject completes the final diary card at the time of the final visit on Day 28 to represent end of study functional assessment and symptoms.

Completion of subsequent diary cards should be done by the subject if possible. For children unable to complete the card, it may be completed by a parent or close relative (and documented accordingly). For those subjects too ill to complete the diary card, it may be completed by the study team or treating team (physicians or nurses) and this will be documented accordingly.

At each subsequent study visit, the diary card for the day(s) since the last visit will be collected. This diary will contain the information listed below (Section 7.3.1.1 – 7.3.1.3).

7.3.1.1 Fever

Subjects will be asked to measure temperature orally at the time of completing the diary card and at any time they exhibit symptoms of feverishness, and will be asked to record the maximal temperature since the prior diary card).

7.3.1.2 Symptoms

Using a 4-point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe), each symptom will be assessed for severity.

The symptoms assessed are:

- Cough
- Sore throat
- Fatigue
- Headache
- Myalgia
- Rhinorrhea
- Nausea
- Vomiting
- Diarrhea
- Difficulty breathing/shortness of breath

7.3.1.3 Global Assessment

Subjects will be asked 2 global assessment questions:

- "Are you feeling as good as you did before you had the respiratory illness?" with a yes/no response, referring to the status immediately prior to onset of ILI symptoms.
- "Are you functioning as well as you were before you had the respiratory illness?" with a yes/no response, referring to the status immediately prior to onset of ILI symptoms.

Pediatric subjects that are not capable of answering the above questions will be assessed by a parent or close relative regarding:

- "Is your child as active as they were before they had the respiratory illness?" with a yes/no response, referring to the status immediately prior to onset of ILI symptoms.
- "Is your child eating as much as they were before they had the respiratory illness?" with a yes/no response, referring to the status immediately prior to onset of ILI symptoms.

7.3.2 Assessment for Presence of Complications

Subjects will be assessed for the signs/symptoms of the syndromes listed below, at baseline (Day 0) in order to know the presence of these prior to treatment, and on each study day to evaluate for the development of complications while participating in the study. In order to establish the listed diagnosis, the expected information to be in the source documentation follows each diagnosis in parentheses.

- Pneumonia (clinical diagnosis supported by radiographic data)
- Respiratory failure requiring mechanical ventilation (documentation of mechanical ventilation)
- ARDS (clinical diagnosis supported by radiographic data)
- Sepsis (clinical diagnosis)
- Bronchiolitis (clinical diagnosis)

7.3.3 Vital Signs, Including SaO_2

Vital signs assessments (temperature, blood pressure, pulse, respiration rate, and SaO_2) will be taken on Study Days 0, 3, 7, and 28. SaO_2 will be measured by clinically available transcutaneous pulse-oximeters.

7.3.4 Tachycardia

The respiratory rate should be measured while the subject is breathing room air and at rest. If the respiratory rate is high while breathing a FiO2 greater than room air, the measurement does not need to occur on room air.

Table 6: Respiratory Status

Age (in years)	Normal Respiratory Rate (breaths/min)	*Ref*	Protocol Defined Normal Respiratory Rate	Protocol Defined Tachypnea
<1	24–38	*1*	<=38	>38
1–3	22–30	*1*	<=30	>30
4–6	20–24	*1*	<=24	>24
7–9	18–24	*1*	<=24	>24
10–13	16–22	*1*	<=22	>22
14–17	14–20	*1*	<=20	>20
18 and older	12-20	*2*	<=20	>20

Ref: 1. Johns Hopkins: The Harriet Lane Handbook, 18th ed
2. Goldman: Cecil Medicine, 23rd ed.

Endotracheal intubation (both at enrollment and subsequent evaluation) will be considered as meeting the criteria for tachypnea.

7.4 Compliance Measures

Treatment compliance will be assessed by examination of subject's remaining study product at each study visit, and by asking subjects to document study drug compliance on the diary cards. No pharmacokinetics or other drug exposure testing is planned for this study.

7.5 Severity of Illness Scores

7.5.1 SOFA Score

The SOFA score is a scoring system to determine the extent of a person's organ function or rate of failure[15, 16] and is based on 6 different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal, and neurological systems.

Respiratory System
PaO2/FiO2 (mmHg)

- ≥ 400, 0 points
- < 400, 1 point
- < 300, 2 points
- < 200 and mechanically ventilated, 3 points
- < 100 and mechanically ventilated, 4 points

If PaO2 is not available, the following table can be used to generate the SOFA score with SaO2[17]:

Table 7: Sofa Score

SpO2/FiO2 Ratio				
SOFA Score	PaO2/FiO2 Ratio	PEEP< 8 or not intubated	PEEP 8-12	PEEP>12
0	≥ 400	≥ 457	≥ 515	≥ 425
1	< 400	< 457	< 515	< 425
2	< 300	< 370	< 387	< 332
3	< 200	< 240	< 259	< 234
4	< 100	< 115	< 130	< 129

Note: The original SpO2/FiO2 Ratio as published would require a SaO2 > 110% to achieve a SOFA score of 0. Therefore, this score has been modified to accept a SaO2 of 96% or greater on room air as normal (i.e. a SpO2/FiO2 Ratio of ≥ 457 would be a SOFA score = 0).

Nervous System
Glasgow coma score

- 15, 0 points
- 13 – 14, 1 point
- 10 – 12, 2 points
- 6 – 9, 3 points
- < 6, 4 points

Cardiovascular System
Mean arterial pressure OR administration of vasopressors required (vasopressor drug doses are in mcg/kg/min)

- No hypotension, 0 points
- MAP < 70 mm/Hg, 1 point

- dopamine ≤ 5 or dobutamine (any dose), 2 points
- dopamine > 5 OR epinephrine ≤ 0.1 OR norepinephrine ≤ 0.1, 3 points
- dopamine > 15 OR epinephrine > 0.1 OR norepinephrine > 0.1, 4 points

Liver

Total Bilirubin (mg/dL)

- <1.2, 0 points
- 1.2 – 1.9, 1 point
- 2.0 – 5.9, 2 points
- 6.0 – 11.9, 3 points
- > 12.0, 4 points

Coagulation

Platelets×10^3/μL

- ≥ 150 , 0 points
- < 150, 1 point
- < 100, 2 points
- < 50, 3 points
- < 20, 4 points

Renal System

Creatinine (mg/dL) (or urine output)

- <1.2, 0 points
- 1.2 – 1.9, 1 point
- 2.0 – 3.4, 2 points
- 3.5 – 4.9 (or < 500 mL/d), 3 points
- ≥ 5.0 (or < 200 mL/d) or dialysis requirement, 4 points

7.5.2 Tal Bronchiolitis Score

The modified Tal bronchiolitis score should be used for all children < 24 months of age at the time of enrollment.[18] Each of 4 categories are evaluated, and assigned a score of 0-3 per category (total score will be 0-12).

Table 8: Tal Bronchiolitis Score

Score	Respiratory Rate (per min)	Wheezing	Cyanosis	Accessory muscle use
0	30	None	None	None
1	31-45	End expiration with stethoscope	Perioral with crying	+
2	46-60	Inspiration and expiration with stethoscope	Perioral at rest	++
3	> 60	Audible without stethoscope	Generalized at rest	+++

8 ASSESSMENT OF SAFETY

8.1 Definitions

Adverse Event (AE)
An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not it is considered related to the research.

Adverse Reaction (AR)
An AE that is caused by an investigational agent (drug or biologic)

Suspected Adverse Reaction (SAR)
An AE for which there is a reasonable possibility that the investigational agent caused the AE. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE)
A SAE is an AE that results in 1 or more of the following outcomes:

- death
- a life threatening (i.e., an immediate threat to life) event
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event*

** Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but that may jeopardize the subject or may require intervention to prevent 1 of the other outcomes listed above.*

Unexpected Adverse Event
An AE is unexpected if it is not listed in the IB or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected.

Unanticipated Problem (UP)
An Unanticipated Problem is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to

a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
b. the characteristics of the subject population being studied; and

2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk.)

Unanticipated Problem that is not an Adverse Event (UPnonAE)
An incident, experience or outcome that is not associated with an AE which meets the 3 criteria of a UP. Examples include occurrences of breaches of confidentiality, accidental destruction of study records, and unaccounted-for study drug.

Protocol Violation
A Protocol Violation is any change, divergence, or departure from the study design or procedures in an IRB-approved research protocol that has a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy or reliability of the study data.

Protocol Deviation
A Protocol Deviation is any change, divergence, or departure from the study design or procedures of an IRB-approved research protocol that does not have a major impact on the subject's rights, safety or well-being, and/or the completeness, accuracy and reliability of the study data.

8.2 Investigator Assessment of Adverse Events

8.2.1 Determination of Adverse Event

All worsening symptoms should be evaluated as potential adverse events:

- If a diagnosis is clinically evident (or subsequently determined), the diagnosis, rather than the individual signs and symptoms or lab abnormalities, will be recorded as the AE.
- Gastrointestinal tract SARI symptoms such as nausea, vomiting, or diarrhea, can be either related to SARI or the study drug. For this reason, if these gastrointestinal symptoms are new from baseline or worsen, they should be graded per the DAIDS toxicity table, and if they are new or represent a worsening grade then they should be reported as adverse events.
- Upper respiratory tract SARI symptoms (such as cough, sore throat, fatigue, headache, myalgia, rhinorrhea) can worsen due to the underlying disease. As these are unlikely to be related to the study drug, these do not need to be reported as Adverse Events.
- Any other new symptoms should be recorded as AEs.
- Symptoms present at baseline that resolve should have an end date (unless likely flu related). If they recur, the recurrence should be reported as an adverse event. Intermittent symptoms (such as cough, headache, rhinorrhea) would not be AEs if likely influenza related.

All worsening laboratory values should be evaluated as potential adverse events:

- If an abnormal laboratory finding is captured as a component of a diagnosis AE term that has already been recorded as a pre-study condition, the individual abnormal laboratory finding is **NOT** recorded as a separate AE.
- A laboratory abnormality should be reported as an AE only **if** it requires an intervention. Interventions include, but are not limited to, discontinuation of treatment, dose reduction/delay, additional assessments (including additional lab assessments), or concomitant treatment.
- In addition, any medically important laboratory abnormality may be reported as an AE at the discretion of the investigator who considers the test result clinically significant. This could include a laboratory result for which there is no intervention but the abnormal value is of clinical concern or suggests disease or organ toxicity. Grading should be based on the toxicity table where applicable.

8.2.2 Period of Assessment

All AEs that occur from the time the informed consent is signed through Day 28 will be documented, recorded, and reported. After the end of the protocol-defined AE reporting period, sites must report to the safety office serious, unexpected, clinically suspected adverse drug reactions if the study site staff becomes aware of the event on a passive basis.

8.2.3 Assessment of Adverse Event

The Investigator will evaluate all AEs with respect to **Seriousness** (criteria listed above), **Severity** (grading), and **Causality** (relationship to study agent and relationship to research) according to the following guidelines.

8.2.3.1 Severity

The Investigator will grade the severity of each AE according to the "DAIDS (Division of AIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 1.0, December, 2004: (Clarification August 2009) which can be found at: http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf

8.2.3.2 Causality of Adverse Event to the Intervention

Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship

- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related
- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related
- does not have a reasonable temporal relationship
- OR
- good evidence for a more likely alternative etiology

Not Related
- does not have a temporal relationship
- OR
- definitely due to an alternative etiology

Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

8.2.4 Investigator Documentation of Adverse Event

The severity of the AE will be recorded in an appropriate source document. AEs (per the definition above) and SAEs will be recorded in the appropriate section of the AE electronic case report forms (eCRF) and entered into a database for IRB/IEC review and FDA/COFEPRIS reporting requirements.

8.3 Investigator Reporting Responsibilities to the Sponsor

8.3.1 Serious Adverse Events

SAEs (whether or not they are also UPs) must be reported on the SAE/UP Report Form and sent to the Sponsor Clinical Safety Office (CSO) by fax or e-mail attachment. Deaths and immediately life threatening SAEs must be reported within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

SPONSOR CLINICAL SAFETY OFFICE CONTACT INFORMATION:

OCRPRO Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704

Phone 301-846-5301
Fax 301-846-6224

E-mail: rchspsafety@mail.nih.gov

8.3.2 Unanticipated Problems

Non-Serious AEs that are UPs must also be reported on the SAE/UP Report Form and sent to the CSO by fax or e-mail attachment no later than 7 calendar days of site awareness of the event. The UPs that are not AEs are not reported to the Sponsor CSO.

8.3.3 Pregnancy

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Pertinent obstetrical information for all pregnancies will be reported to the CSO via fax or email within 3 business days from site awareness of the pregnancy.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness on a protocol-specified form.

The procedures that should be followed in the event of a pregnancy include:

- Discontinuation of the study agent
- Unblind per the unblinding section of the protocol
- Pregnancies that do not result in SAEs will be reported to the IRB/IEC in summary format with the continuing review or according to the local IRB/IEC requirements
- Advise research subject to notify the obstetrician of study agent exposure

Additionally the protocol team will report the pregnancy to the DSMB.

8.4 Investigator Reporting Responsibilities to the Site IRB/EC

8.4.1 IND safety reports

Investigators are responsible for submitting IND Safety Reports and UP summaries that are received from the IND Sponsor to their local IRB/Ethics Committee. Investigators must also comply with all local IRB/Ethics Committee reporting requirements.

8.4.2 Expedited Reporting to the Site IRB/EC

Unanticipated problems that are either AEs or non-AEs, (as defined above) and protocol violations which do not meet the definition of a UP will be reported within 7 calendar days of investigator awareness (or as required by the site IRB/EC).

8.4.3 Annual Reporting to the IRB/EC

In addition to each IRB/EC yearly reporting requirements, the following items will be reported to the IRB/EC in summary at the time of Continuing Review (or more frequently as required by the site IRB/ECs):

- All unanticipated problems

- All protocol deviations which in the opinion of the investigator should be reported according to the site's IRB/EC reporting guidelines
- Summaries of all AEs

8.5 Follow-up of Adverse Events and Serious Adverse Events

AEs that occur following enrollment of the subject (by signing the informed consent) are followed until the final outcome is known or until the end of the study follow-up period (Study Day 28).

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF (if the CRF is still open) and the SAE/UP Report Form.

SAEs that occur after the study follow-up period that are reported to and are assessed by the Investigator to be possibly, probably, or definitely related must be reported to the CSO, as described above.

8.6 Sponsor's Reporting Responsibilities

Serious and unexpected suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to US FDA and all participating Investigators as IND Safety Reports.

The IND Sponsor will submit an Annual Report of the progress of the investigation to the US FDA and Mexico COFEPRIS as required.

AEs that are also UPs will be summarized by the IND Sponsor and distributed to Investigators.

8.7 Safety Monitoring

8.7.1 Investigator Safety Monitoring

Investigators participating in this clinical trial are responsible for:

- Protecting the safety and welfare of subjects
- Evaluating subject safety, including physician assessment of AEs for seriousness, severity, and causality
- Notifying the sponsor of SAEs and immediately-reportable events (per 8.3)
- Providing detailed written reports, including confirmatory tests promptly following immediate initial reports
- Informing the IRB/IEC of SAEs as required by applicable regulatory requirements

8.7.2 Protocol Team Monitoring

The protocol team will review the enrollment and study-related safety data (AEs and SAEs) from all sites periodically during the study. These reviews will occur at a minimum twice monthly during active enrollment and follow-up.

8.7.3 Sponsor Medical Monitor

A Medical Monitor, representing the IND Sponsor, has been appointed for oversight of safety in this clinical study. The Sponsor Medical Monitor will be responsible for performing safety assessments as outlined in a Safety Review and Communications Plan (SRCP) (see Section 8.7.6).

8.7.4 Pausing Rules

The following automatic pausing rules to suspend study drug administration are in place for this study:

- 2 (or more) subjects experience the same Grade 4 AE and/or SAE that is unexpected and possibly, probably, or definitely related to the study drugs OR
- 4 (or more) subjects experience the same Grade 3 or higher AE that is unexpected and possibly, probably, or definitely related to the study drugs

- If any of pausing rules are triggered, the following will occur:
  - The Sponsor's Medical Monitor will undertake a review of cumulative AE data within 72 hours of being made aware of the events specified above, AND
  - The DSMB will be notified of the above Medical Monitor review, and of the Medical Monitor's findings.
- If the Medical Monitor is unable to complete the review of the safety data within 72 hours of awareness by the Clinical Safety Office, study enrollment will be halted until the review can take place. The DSMB will again be notified of any such review, and of the findings of the Sponsor's Medical Monitor. In the event of a study pause, after the above appropriate review, the sponsor in consultation with the Sponsor's Medical Monitor may allow enrollment or ask the DSMB for a review of the safety data.
- The IND Sponsor, in collaboration with the PI, may also pause for an individual subject or entire group if a safety concern is identified during routine aggregate data analysis.

8.7.5 Reporting a Pause

If a pausing requirement is met, a description of the adverse event(s) or safety issue must be reported by the Site Investigator by fax or email within one business day to the Sponsor CSO, PI, the IRB, and the DSMB.

The IND Sponsor will notify all sites that the study has been paused.

8.7.5.1 Review of Pauses and Resuming Rules

The site IRB/IECs will be notified by the site PI of any study pause, and the DSMB will be notified by the study chair of any study pause. The IND Sponsor, in consultation with the Sponsor's Medical Monitor and the DSMB, will conduct the review and make the decision to

resume or close the study for events that meet the criteria for stopping the study. As part of the review, the reviewers will also advise on whether the study needs to be paused again for any subsequent event of the same type. The IND Sponsor will notify the Site Investigators of this decision. The Site Investigators will notify their local IRB(s) of the decision to resume administration of the study agent prior to resumption.

The reviewing IRB/IEC has the right to pause enrollment at the sites it is responsible for at any time.

8.7.6 Safety Review and Communications Plan (SRCP)

A Safety Review and Communication Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document which delineates the safety oversight responsibilities of the Protocol chairs, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

8.7.7 Data and Safety Monitoring Board

The DSMB will review and approve a detailed DSMB monitoring plan before the study commences. The Board will review the study per the monitoring plan, but as long as subjects are enrolled into the study, the Board will review the study at least annually, and may convene additional reviews as necessary. The Board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All SAEs, all unanticipated problems, and all IND Safety Reports will be reported by the PI to the DSMB at the same time they are submitted to the IRB or IND Sponsor. The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the Board at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

The DSMB will review study-related data at the following intervals:

- Before enrollment
- Periodic safety review during the study, at least twice a year

These reviews may contain data that have not been monitored and therefore not verified against the source documents. Details of the review will be provided in the DSMB monitoring plan. A copy of the randomization codes will be provided to the DSMB executive secretary in a sealed envelope in case the DSMB requires this information to make its recommendation.

8.7.7.1 Interim Analysis

The study will undertake a formal interim efficacy examination. An unblinded statistician that is not associated with a site, not part of the protocol team and not part of the operational support for this study will perform this analysis.

After half of the total sample size has completed 28 days of follow-up, primary analysis using the Fay-Shaw statistic (13) will be conducted. The study will be stopped for benefit if the one-sided p-value for benefit is less than .001. The study will be stopped for futility if the one-sided p-value for harm is less than .025.

9 STATISTICAL CONSIDERATIONS

This section briefly describes the statistical analyses to be used for the study. A Statistical Analysis Plan will provide further details.

9.1 General Considerations

This is a double-blind, randomized, placebo, controlled trial in patients with hospitalized influenza-like-illness to evaluate the efficacy of NTZ compared to placebo on the time to hospital discharge. The primary analysis will use the Shaw-Fay formulation of the 2 sample Mann-Whitney-Wilcoxon rank-sum statistic. The primary endpoint will be time to hospital discharge, censored at 28 days. Patients who withdraw consent will be counted as being censored at the time of withdrawal. Patients who die by Day 28 will receive the worst ranks, (i.e., worse than any patients who remain hospitalized). Furthermore, patients who die earlier will receive worse ranks than those who die later. Patients who withdraw consent prior to day 28 are thus censored for death and discharge at the time of withdrawal. Such censoring is addressed by the method of Shaw and Fay (2013). A permutation method will be used to determine statistical significance. The primary analysis will follow the intent-to-treat principle where all randomized patients will be used in the analysis. The primary analysis will use a two-sided alpha level of .05.

9.2 Sample Size and Power Calculations

Sample size calculations are based on the primary endpoint only (i.e. Duration of hospitalization). Secondary endpoints may or may not be adequately powered with the chosen sample size.

When there is no censoring or deaths, power for the two-sample Mann-Whitney-Wilcoxon rank-sum test can be approximated assuming that days of hospitalization follows a log normal distribution. The parameters used in the calculation were from the observed distribution of days to hospital discharge for hospitalized subjects who enrolled in the ILI002 observational study. We obtained data on patients who survived with hospitalization less than 28 days. The data were truncated at 28 days to avoid the influence of aberrant values on the estimates. This eliminated about 4% of the data. The mean and variance of the days in the hospital were 7.7 and 33.2. We assumed that the effect of treatment would be to reduce the number of days in the hospital by Delta but that there would be no effect of treatment on the variance of the number of days in the hospital. Thus for the treatment group the mean and variance are given by 7.7-Delta and 33.2, where Delta is varied. We next assume that the distribution of days to hospital discharge followed a log-normal distribution in each group, which is equivalent to assuming that the log(Days) follows a normal distribution, where Days is the number of days until hospital discharge. A log-normal distribution is specified by its moments E{log(Days)} = m and var(log(Days)=v. These moments are related to the mean, E(D), and variance, V(D), of the number of days (D) in the hospital by the formulas.

$$m = \log\{E(D)\} - v/2$$

$$v = \log\{1 + V(D)/[E(D) \times E(D)]\}.$$

For simplicity, we determine the sample size for different assumptions of treatment effects of nitazoxanide, using a two sample t-test with unequal variances using Satterthwaite's method (Table 8). This method requires the mean and variance of log (Days) for the 2 groups, which are obtained by solving the above equations separately for the placebo and treatment groups. Calculations were performed using Nquery 7.0.

Table 9: The total sample size required to achieve different powers under different assumptions about the benefit of nitazoxanide (Delta) using a two-sided .05 level test

Moments for the log days in hospital						
	Placebo Group		Treatment Group			
Delta[a]	**m□□**	**Sqrt(v□)**	**m□**	**Sqrt(v□)**	**Power**	**N[b]**
1.50 Days	1.83	.66	1.52	.78	80%	174
1.50 Days			1.52	.78	90%	232
1.25 Days	1.83	.66	1.58	.76	80%	**258**
1.25 Days			1.58	.76	90%	344
1.00 Day	1.83	.66	1.64	.74	80%	430
1.00 Day			1.64	.74	90%	576

[a] Delta is the assumed reduction in the mean number of days in the hospital under treatment compared to placebo.
[b] N is the total sample size.

We see that with about 258 patients total the study is 80% powered to detect a 1.25 day improvement in the time to hospital discharge. This difference is similar to our assumptions. To allow for up to 10% dropouts we will recruit 290 patients. If the difference was 1.5 days, the study would have 90% power to detect the difference in the treatment arms.

While a 1.25 day improvement is clinically meaningful and easily interpretable for a population with a mean hospital stay of 7.7 days, it is also a reasonable estimate of the effect of NTZ we might see. The NTZ IB describes a Phase 2b-3 study in adults and adolescents with acute uncomplicated ILI. In all subjects, the mean time to resolution of symptoms was 95 hours in the NTZ group compared to 108 hours in the placebo group, thus there is about a 12% reduction in the time to resolution (12% = (108 hr-95 hr)/108 hr x 100%). Our hoped for treatment effect of a 1.25 day reduction in hospital duration is a 16% reduction in the time to hospital discharge (16% = (1 day/7.7 day x 100%).

Kaplan Meier curves of time to hospitalization discharge over 28 days will be used to compare the 2 groups. In these curves, deaths will be counted as being in the hospital at day 28. The between group difference in median time to discharge will be used to estimate the treatment effect and will be reported along with a 95% two-sided confidence interval.

Important additional analyses will be to repeat the primary analysis for those who are influenza positive. Other subgroups include age (<12 years of age, ≥12 years of age) and pathogen type.

9.3 Subject Populations

Intention to Treat (ITT) Population: The ITT population will consist of all participants who are randomized and received at least 1 dose of the study drug. For interim reviews, this will be limited to data entered into the electronic database up to the cut-off date.

9.4 Statistical Analysis

The Statistical Analysis Plan will provide the procedure for accounting for missing, unused, and spurious data.

10 DATA MANAGEMENT AND MONITORING

10.1 Study Monitoring

The trial will be conducted in compliance with this protocol, International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP), the Food and Drug Administration's (FDA) Code of Federal Regulations (CFR) and any applicable regulatory requirement(s). This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID will visit the clinical research site to monitor all aspects of the study in accordance with the appropriate regulations. The objectives of a monitoring visit will be:

1. To verify the prompt reporting of all data points, including reporting SAEs, and also to check the availability of signed informed consent.
2. To compare individual subjects records and the source documents (supporting data, laboratory specimen records and medical records to include physician progress notes, nurse' notes, subjects' hospital charts).
3. To ensure compliance with the protocol, and accuracy and completeness of records.

The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Mexican and US) are being followed. During the monitoring visits, the Investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit. The Sponsor will retain original copies of the Form FDA 1572 and copies of other study documents as deemed necessary.

The Investigator (and/or designee) will make study documents (e.g., consent forms and eCRFs) and pertinent hospital or clinical records readily available for inspection by the local IRB/IEC, the local and national regulatory authorities, the US FDA, the site monitors, and the NIAID staff for confirmation of the study data.

10.2 Source Documents

The primary source document for this study will be the subject's medical record. If the Investigators maintain separate research records, both the medical record and the research records will be considered the source documents for the purposes of auditing the study. The

investigator will retain the source documents. The Investigator will permit monitoring and auditing of this data, and will allow NIAID, IRB/IEC, and regulatory authorities access to the original source documents, regardless of media.

The Investigator is responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected on the eCRF. All eCRFs should be reviewed by the Investigator and signed as required with written or electronic signature, as appropriate. Data for eCRFs will be collected directly from subjects, clinical assessments, and tests during study visits or will be abstracted from subjects' medical records. The subject's medical record must record his/her participation in the clinical trial, including obtaining informed consent, concomitant medications (with doses and frequency), medical interventions or treatments that were administered, and adverse reactions experienced during the trial.

10.3 Data Management Plan

Study data will be collected at the study site(s) and maintained on eCRFs. These forms are to be completed on an ongoing basis during the study. Corrections on any study related source record must be made by striking through the incorrect entry with a single line (taking care not to obliterate or render the original entry illegible) and entering the correct information adjacent to the incorrect entry. Corrections to paper sources must be initialed and dated by the person making the correction.

10.4 Data Capture Methods

Clinical data will be entered into an Internet Data Entry System (IDES) (US 21 CFR 11-compliant). The data system includes password protection and internal quality checks, to identify data that appear inconsistent, incomplete, or inaccurate.

10.5 Study Record Retention

The Investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. The US FDA requires study records to be retained for up to 2 years after marketing approval or disapproval (21 CFR 312.62), or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational agent for a specific indication. These records are also to be maintained in compliance with IRB/EC, state, and federal medical records retention requirements, whichever is longest.

All stored records are to be kept confidential to the extent provided by federal, state, and local law. It is the Investigator's responsibility to retain copies of source documents until receipt of written notification to the contrary from OCRPRO/NIAID. No study document should be destroyed without prior written agreement between OCRPRO /NIAID and the Principal Investigator. Should the Investigator wish to assign the study records to another party and/or move them to another location, the Investigator must provide written notification of such intent to OCRPRO /NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location.

NIAID must be notified in writing and written NIAID permission must be received by the site prior to destruction or relocation of research records.

11 HUMAN SUBJECTS

11.1 IRB/IEC Approval

This protocol, informed consent document, diary card, relevant supporting information, and all types of patient recruitment or advertisement information must be submitted to the IRB/IEC for review and must be approved before the study is initiated.

Any amendments must also be approved by the IRB/IEC prior to implementing changes in the study.

The Investigator is responsible for keeping the IRB/IEC appraised of the progress of the study as deemed appropriate, but in any case at least once a year. The Investigator must also keep the IRB/IEC informed of any significant AEs.

11.2 Compliance with Good Clinical Practices (GCP)

This study will be conducted in compliance with the conditions stipulated by NIAID and local IRB/IEC, informed consent regulations, US FDA and local regulatory agencies, and supported by ICH/GCP guidelines. In addition, all local regulatory requirements will be adhered to, in particular those which afford greater protection to the safety of the trial subjects.

11.3 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers about the essential information about the study, which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions of essential information about the research will include the study's purpose, duration, experimental procedures, alternatives, risks, and benefits, and subjects will have the opportunity to ask questions and have them answered.

The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The researcher will document the signing of the consent form in the subject’s medical record. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Children will receive an assent according to their institutional and or IRB/EC requirements.

11.4 Anonymity and Confidentiality

The information obtained during the conduct of this clinical study is confidential. The results of the research study may be published, but patient names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the investigators at each site will keep records in locked cabinets and the results of tests will be coded to prevent association with the subject’s names.

11.5 Compensation

Each site is responsible for generating a compensation scheme as applicable (accounting for travel cost and time lost from work for outpatient visits), and that fits the local legal and regulatory requirements as well as the cultural norm. Any compensation must be reviewed by the local IRB/IEC.

12 STORED SPECIMENS, AND DATA

12.1 Samples/Specimens

The samples collected during this study are for clinical laboratory and virologic (respifinder) testing only. No samples are stored as part of this protocol.

12.2 Storage of Data

Data will be stored using codes assigned by the sponsor. Data will be kept in password-protected computers, which are located in locked rooms.

12.3 Storage of Genetic Sample

No samples are being stored for genetic testing on the subjects.

12.4 Reporting Loss or Destruction of Samples/Specimens/Data

Any loss or unanticipated destruction of data (for example, misplacing a printout of data with identifiers) will be reported to the corresponding IRB/IECs.

Any inadvertent release or unanticipated destruction of centrally maintained data will be reported to all IRB/IECs.

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