A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED TRIAL TO EVALUATE THE EFFICACY AND SAFETY OF NITAZOXANIDE IN THE TREATMENT OF COLDS DUE TO *ENTEROVIRUS/RHINOVIRUS* INFECTION

PROTOCOL NO. RM08-3005
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INVESTIGATOR'S AGREEMENT

CONFIDENTIALITY

The information in this protocol is provided to you, as an Investigator or consultant, for review by you, your staff, and an applicable institutional review committee. By accepting this document, you agree that information contained herein will be considered confidential and will not be disclosed to others, without written authority from The Romark Institute for Medical Research, except to the extent necessary to obtain: (a) Institutional Review Board approval and (b) informed consent from those persons to whom the investigational medicinal product will be administered.

APPROVAL OF FINAL PROTOCOL

My signature below constitutes agreement with this protocol. I am providing the necessary assurances that this study will be conducted by me and my staff according to all stipulations of the protocol, including all statements regarding confidentiality, and in complete accordance with all applicable regulations including current Good Clinical Practice guidelines and the ethical guidelines set by the World Medical Assembly (Declaration of Helsinki, last amendment in Fortaleza, Brazil October 2013). Furthermore, my signature below indicates that source documents will be available for review by the Sponsor or their designated representative.

Principal Investigator Signature:

_____ _____

Principal Investigator Date

Print name: _____

With the signature below, the Sponsor approves of this protocol.

Sponsor Signature:

[redacted] 29 NOV 2018

The Romark Institute for Medical Research Date

Print name: [redacted]

SYNOPSIS

Title:	A Phase 3, Randomized, Double-Blind, Placebo Controlled Trial to Evaluate the Efficacy and Safety of Nitazoxanide in the Treatment of Colds Due to *Enterovirus/Rhinovirus* Infection
Study Number:	RM08-3005
IND Number:	140,445
Indication:	Treatment of colds due to *Enterovirus/Rhinovirus* infection
Design:	Multicenter, randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety of nitazoxanide (NTZ) in the treatment of colds due to *Enterovirus/Rhinovirus* infection
Number of Subjects:	At least 600 (up to maximum of 700) with colds due to laboratory confirmed *Enterovirus/Rhinovirus* infection; estimated to require up to 1,750 subjects in total
Population:	Males and females ≥ 12 years of age with colds due to *Enterovirus/ Rhinovirus* infection
Randomization:	1:1
Study Dose and Administration:	Group 1 (NTZ): Two NTZ 300 mg tablets orally twice daily (b.i.d.) for 5 days. Group 2 (Placebo): Two placebo tablets orally b.i.d. for 5 days.
Objective:	Evaluate the effect of NTZ administered 600 mg orally b.i.d. for 5 days in reducing the duration of symptoms compared to that of a placebo.
Primary Efficacy Parameter:	Time from first dose to Symptom Response over 21 days of follow up based upon the FLU-PRO© instrument
Key Secondary Efficacy Parameter:	Time from first dose to Ability to Perform All Normal Activities
Other Secondary Efficacy Parameters:	Proportions of subjects experiencing one or more complications of colds due to *Enterovirus/Rhinovirus* infection including pneumonia, otitis media, bronchitis, sinusitis, exacerbations of asthma or COPD, worsening of pre-existing health conditions, secondary infections requiring systemic antibiotic use, hospitalization due to cold or complications of the cold, and death due to complications of the cold

Safety Parameters:	Adverse events
Biological Samples:	Blood samples will be collected for all subjects at baseline and Day 7. Urine samples will be collected at baseline and Day 7. Nasopharyngeal swabs will be collected at baseline, Days 2, 3, 7 and 22 for all subjects. A blood sample will be collected on Day 3 for pharmacokinetics.
Study Centers:	Multicenter
Trial Duration:	August 2018 – April 2019

1. INTRODUCTION

1.1. Enteroviruses/Rhinoviruses

There are more than 500 million non-influenza viral respiratory infections or "common colds" each year in the United States, with adults experiencing as many as 2-6 illnesses per year and children experiencing 6-8 (Fendrick et al. 2003, Turner 1997, Worrall 2011).

Colds have been reported to account for as much as 40% of time lost from work in the employed population and 30% of school absences (Kirkpatrick 1996). In a study conducted from November of 2000 to February of 2001, the total economic impact of non-influenza viral respiratory tract infections was nearly $40 billion (Fendrick et al. 2003). Adjusted for inflation (February 2001 to January 2018), the economic impact today would exceed $56 billion (USDOL Bureau of Labor Statistics 2018).

Fifty (50) to 67% of non-influenza viral respiratory tract infections are associated with viruses from the genus *Enterovirus* (EV), which belong to the *Picornaviridae* family (Atkinson et al. 2016). Within the EV genus, there are presently fifteen species including *Rhinovirus A*, *Rhinovirus B*, *Rhinovirus C* and *Enterovirus A* through *L* (The Pirbright Institute 2018). There are hundreds of EV serotypes, preventing the development of an effective vaccine or production of sufficient individual immunity to avoid multiple infections annually (Jacobs et al. 2013). A large majority of respiratory tract infections caused by viruses from the Enterovirus genus are due to Rhinovirus species, and RT-PCR assays do not distinguish between the different species of enteroviruses. Therefore, in this document we will refer to these viruses as Enterovirus/Rhinovirus (EV/RV).

EV/RV infection has been described as a "cytokine disease" because infection is characterized by massive upregulation of IFN, IL-1, IL-6, IL-8, TNF-α, granulocyte macrophage colony-stimulating factor and RANTES (Atkinson et al. 2016). Common symptoms include rhinorrhea, nasal congestion, sore throat, cough, headache, fever, and malaise. A natural history study of EV/RV infections demonstrated that patients were able to self-diagnose a cold in less than a day, with most patients self-diagnosing less than 8 hours from symptom onset (Arruda et al. 1997).

Colds due to EV/RV infection are generally considered self-limiting and do not cause respiratory distress. With advances in viral detection methods, however, colds due to EV/RV infection have been linked to exacerbations of COPD, asthma development, severe bronchiolitis in pediatric patients, and fatal pneumonia in elderly and immunocompromised adults. These severe outcomes are in addition to the milder commonly accepted complications of the common cold including secondary otitis media, sinusitis, pneumonia, and, in pediatric patients, croup and bronchiolitis (Jacobs et al. 2013).

Because there are no FDA-approved antiviral drugs for treatment of colds due to EVs, the standard of care is treatment of symptoms with over-the-counter (OTC) symptom relief medication including antihistamines, decongestants, analgesics, antitussives, NSAIDs, and combinations thereof. Most of these treatments have not shown clear benefit (Allan and Arroll 2014). Antiviral drugs to mitigate the impact of EV/RV infection are therefore needed.

1.1.1. Antivirals Studied for Treatment of Colds Due to EV/RV Infection

In the past 25 years, only two antiviral drugs for the treatment of colds due to EV/RV infection have been studied in Phase 2 or 3 clinical trials, pleconaril (ViroPharma) and vapendavir (Aviragen).

Pleconaril

Pleconaril is a broad-spectrum anti-picornaviral agent that integrates into hydrophobic pockets within VP1 of the capsid of picornaviruses. Two Phase 2 studies of pleconaril conducted in 1998 and 1999 enrolled otherwise healthy subjects ≥14 years of age with moderate or severe acute respiratory illness within 36 hours of symptom onset. Subjects were required to grade symptoms including rhinorrhea, nasal congestion, cough, sore throat, malaise, and myalgia as absent, mild, moderate, or severe daily for 21 days. The first of the studies (n=1024, 41% picornavirus-positive) evaluated time to complete resolution of symptoms, defined as all symptoms graded absent for two days with no subsequent relapse. The second study (n=875, 43% picornavirus-positive) evaluated time to alleviation of symptoms, defined as rhinorrhea graded absent and all other symptoms graded absent or mild for at least 48 hours. Neither study alone achieved statistically significant results in spite of trends toward efficacy, but pleconaril-treated subjects had a 1.5 day (8.5 vs. 10 days) median benefit in time to alleviation of symptoms in a pooled analysis of the two studies (P=0.029) (Hayden et al. 2002).

Based on the Phase 2 studies, two parallel Phase 3 studies of pleconaril were designed to evaluate efficacy using time to alleviation of symptoms as a primary endpoint. Symptom alleviation was defined as rhinorrhea graded absent and the other five symptoms graded absent or mild for 48 hours without the use of symptom relief medication. Enrollment was restricted to subjects presenting to the clinic within 24 hours of symptom onset. Other important subject selection criteria included otherwise healthy subjects at least 18 years of age with moderate to severe rhinorrhea and at least one other moderate or severe respiratory symptom (nasal congestion, cough, or sore throat). Subjects were excluded if they had fever, recent history of allergic rhinitis, if they had received asthma treatment within the previous two months, or if they had chronic cough, any known immunodeficiency, or any underlying medical condition that would confound study results. Enrollment was stratified based on smoking status and pre-treatment use of symptom relief medication, and subjects were allowed to take acetaminophen or dextromethorphan as needed for symptoms. The studies were conducted from August through November 2000 and enrolled a total of 2,096 subjects including 1,363 (65%) with laboratory-confirmed picornavirus infection. Time to alleviation of symptoms was significantly shorter for the pleconaril group compared to placebo in both studies, with median treatment benefits of 0.6 days (6.6 compared to 7.2 days, P=0.037) and 1.5 days (6.2 compared to 7.7 days, P=0.001) separately and 1.0 days (6.3 compared to 7.3 days, P<0.001) in a pooled analysis. Pleconaril-treated subjects also had improvements in time to resolution of individual symptoms and time to self-assessed "no cold" status (Hayden et al. 2003). Subjects who used symptom relief medication prior to enrollment had longer times to alleviation of symptoms. It is unclear whether the symptom relief medication use was the result of more severe symptoms or if the prolonged symptoms were the result

of suppression of cytokines and resulting prolonged viral replication (USDHHS FDA Division of Antiviral Drug Products 2003).

Vapendavir

Vapendavir is an antiviral capsid binder that has been studied in two Phase 2 studies for the treatment of symptomatic human rhinovirus infection in asthmatic adults. Both studies enrolled subjects with a diagnosis of asthma and presumptive rhinovirus qualified by (1) runny nose, sore throat, and scratchy throat; and (2) a minimum total WURSS-21 score of 20; or (3) significant fever. The first of the studies was conducted August 2010 – March 2012 and enrolled 300 subjects including 92 rhinovirus-infected. The primary endpoint was ANCOVA daily change in WURSS-21 severity score over days 2-4. Treatment with vapendavir resulted in a statistically significant reduction of the WURSS-21 severity score in the primary analysis (P=0.020) (Clinicaltrials.gov 2018a).

The second Phase 2 study of vapendavir further restricted eligible subjects to those with moderate to severe asthma who were within 48 hours of symptom onset. The primary endpoint of the study was comparison of two doses of vapendavir to placebo using least squares mean change in ACQ-6 total score from baseline to Day 14 (Clinicaltrials.gov 2018b). The study enrolled 455 subjects including 168 with rhinovirus infection. The study did not demonstrate a statistically significant improvement compared to placebo in the primary analysis, but vapendavir therapy was reported to show "evidence of an antiviral effect" in subjects who received the drug within 24 hours after symptom onset (Genetic Engineering & Biotechnology News 2017).

1.2. Nitazoxanide (NTZ)

NTZ is a thiazolide anti-infective with *in vitro* activity against parasites, anaerobic bacteria, and viruses (Anderson and Curran 2007).

Alinia® (NTZ) for Oral Suspension (patients 1 year of age and older) and Alinia (NTZ) Tablets (patients 12 years and older) are marketed in the United States for the treatment of diarrhea caused by *Giardia lamblia* or *Cryptosporidium parvum*. Alinia for Oral Suspension and Alinia Tablets have not been shown to be superior to placebo for the treatment of diarrhea caused by *Cryptosporidium parvum* in HIV-infected or immunodeficient patients (Alinia prescribing information 2017).

1.2.1. NTZ Inhibits Viral Replication and Cytokine Secretion

Tizoxanide, the active metabolite of NTZ, has been shown to possess antiviral activity in cell culture against a broad range of viruses including influenza A and B viruses, parainfluenza (Sendai) virus, respiratory syncytial virus (RSV) A-2, canine coronavirus S-378, rotavirus, norovirus, hepatitis C virus genotypes 1a and 1b, Japanese encephalitis virus, dengue fever virus-2, yellow fever virus, hepatitis B virus, and human immunodeficiency virus (HIV) (Rossignol 2014). The wide spectrum of antiviral activity suggests a cell-mediated effect rather than a specific viral target.

Laboratory studies to evaluate the potential for resistance of influenza A virus to tizoxanide have been unable to select for resistant virus (Romark Study Report RM01-0417, data on file). These studies suggest a low potential for resistance.

Experiments in HeLa R19 cells have shown that NTZ inhibits replication of Rhinovirus A serotypes 2 and 16 with EC_{50}s of approximately 1 to 5 μM. Studies in PBMCs have also shown that tizoxanide suppresses secretion of pro-inflammatory cytokines that are upregulated by EV/RV infection. Concentrations required to suppress cytokine secretion by 50% for IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and TNF-α ranged from 2.0 to 9.8 μM. (Romark, data on file) Nitazoxanide has also been shown to suppress IL-6 production in TG-injected mice (Hong et al. 2012).

Ongoing studies of the mechanism of action of NTZ have shown that NTZ and tizoxanide modulate mitochondrial function by uncoupling oxidative phosphorylation. Studies have shown that tizoxanide decreases cellular ATP in a dose-dependent manner in MDCK cells and in MDCK cells infected with influenza viruses. Maximum inhibition of ATP in influenza-infected or uninfected MDCK cells reaches up to 45% after 24 hours of exposure to 100 μM tizoxanide. In these experiments, a 10% reduction of ATP achieved by adding less than 10 μM tizoxanide is sufficient to inhibit influenza virus replication by approximately 90%. The decrease in cellular ATP does not affect cell viability and is reversible after eliminating tizoxanide from the culture. (Romark, data on file) Studies of a number of different viruses have shown that viral replication is ATP-dependent (Braakman et al. 1992, Braakman et al. 1991, Doms et al. 1987, Chang et al. 2009, Mirazimi and Svensson 2000). In addition, inhibition of ATP and its downstream effect on AMP-activated protein kinase (AMPK) activation has been shown to suppress secretion of pro-inflammatory cytokines (Lee et al. 2017, Sag et al. 2008, Wang et al. 2003).

The activity of NTZ in inhibiting replication of a broad range of respiratory viruses as well as secretion of pro-inflammatory cytokines upregulated during respiratory virus infection has prompted clinical development of NTZ as a treatment of colds and flu due to influenza A and B viruses and enteroviruses.

1.2.2. Pharmacokinetics of NTZ in Humans

NTZ is not detectable in the plasma following oral administration of the drug. The main metabolites of NTZ in humans are tizoxanide and tizoxanide glucuronide. Tizoxanide is highly bound to plasma proteins (>99%). The absorption of NTZ in immediate release tablets is significantly enhanced (C_{max} and AUC of tizoxanide and tizoxanide glucuronide in plasma are more than doubled) when it is administered with food. In fasted human volunteers receiving a single 500 mg dose of ^{14}C 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. (Investigators Brochure for NTZ 300 mg Tablets, July 2018)

The pharmacokinetics of tizoxanide and tizoxanide glucuronide during repeated oral dosing of nitazoxanide (NTZ) 300 mg extended release tablets administered 600 mg twice daily with food were evaluated in healthy volunteers. Twelve (12) subjects received two NTZ controlled release tablets twice daily with food for 7 days. The pharmacokinetics were studied in plasma up to 12 hours post-dose after the morning dose on Day 1 and Day 7, and before the morning dose on Day 2-6. Based on mixed effect analysis of variance, the steady state tizoxanide and tizoxanide glucuronide plasma

concentrations was reached by Day 2, after one day of treatment with NTZ at 600 mg b.i.d. The main pharmacokinetics parameters of tizoxanide and tizoxanide glucuronide are summarized in the table below:

Table 1: Summary of Tizoxanide and Tizoxanide Glucuronide Pharmacokinetics Parameters

PK Parameter (unit)	Tizoxanide		Tizoxanide Glucuronide	
	Day 1 N=12	Day 7 N=12	Day 1 N=12	Day 7 N=12
C_{max} (µg/mL)	5.23 ± 2.71	8.16 ± 4.16	4.88 ±1.72	8.96 ±3.48
t_{max} (h)	6.00 (4.00-9.00)	5.00 (3.00-8.00)	6.00 (5.00-11.00)	5.50 (0.00-7.02)
t_{lag} (h)	0.00 (0.00-2.00)	0.00 (0.00-0.00)	0.50 (0.00-2.00)	0.00 (0.00-0.00)
AUC_{0-t} (µg.h/mL)	26.9 ± 16.1	52.5 ± 33.7	29.5 ± 12.1	75.0 ± 37.8
AUC_{0-T} (µg.h/mL)	28.6 ± 16.3[a]	48.3 ± 31.9[b]	30.5 ± 12.2[c]	75.2 ± 37.9
C_T (µg/mL)	0.709 ± 0.987	1.72 ± 2.04	1.39 ± 0.846	3.89 ± 2.88
$AUC_{0-\infty}$ (µg.h/mL)	27.8 ± 17.8[b]	52.3 ± 35.6[b]	35.3 ± 16.5[d]	62.1 ± 24.2[e]
$t_{1/2}$ (h)	1.66 ± 0.408[b]	2.19 ± 0.485[b]	2.70 ± 0.848[c]	4.99 ± 5.20
λ_z (1/h)	0.441 ± 0.106[b]	0.331 ± 0.0733[b]	0.282 ± 0.0902[c]	0.201 ± 0.0813
C_{avg} (µg/mL)	NA	4.02 ± 2.66[b]	NA	6.27 ± 3.16
C_{min} (µg/mL)	NA	1.50 ± 1.83	NA	3.63 ± 3.00
PTF (%)	NA	193 ± 39.1[b]	NA	101 ± 40.7
Swing (%)	NA	1039 ± 656	NA	268 ± 194

Values are arithmetic mean ± SD, except median (range) for t_{max} and t_{lag}
N = number of subjects with data; NA = not applicable
[a] N=10; [b] N=11; [c] N=11; [d] N=8; [e] N=7

Table 2: Summary of Tizoxanide and Tizoxanide Glucuronide Trough Plasma Concentrations

PK Parameter (unit)	Trough Concentrations (µg/mL Mean (CV%)						
Dose Group Nitazoxanide	Day						
	1 (a)	2	3	4	5	6	7 (b)
Tizoxanide	0.709 (139)	1.55 (100)	2.36 (101)	3.24 (99.7)	3.80 (146)	3.26 (119)	3.02 (98.6)
Tizoxanide Glucuronide	1.39 (61.0)	3.68 (72.3)	4.38 (72.8)	5.35 (77.2)	5.83 (98.8)	5.94 (91.2)	5.65 (68.7)

1.2.3. Phase 2 Clinical Trials of NTZ in Subjects with Influenza-like Illness: Studies RM02-2022 and RM01-2021

Two randomized, double-blind, placebo controlled clinical trials were conducted in subjects with symptoms of viral respiratory infection (VRI) in Cajamarca, Peru. For these studies, subjects were enrolled based upon symptoms with very limited virology testing being conducted by rapid direct immunofluorescence assay.

Study RM02-2022: One-hundred (100) children aged 12 months through 11 years of age with fever, at least one respiratory symptom (cough, nasal discharge or congestion, sneezing or sore throat) and at least one constitutional symptom (myalgia, malaise, fatigue, headache, chills/sweat) of less than 72 hours duration were randomized to receive NTZ 100 mg/5 mL suspension or a matching placebo suspension b.i.d. for 5 days. The dose of the suspension was 5 mL for children aged 12 through 47 months and 10 mL for children aged 48 months through 11 years. A nasopharyngeal swab was obtained at baseline and subjected to rapid direct immunofluorescence assay for respiratory syncytial virus (RSV), influenza A, influenza B, parainfluenza 1, 2 and 3, and adenovirus. Physical examinations were performed at baseline and on study Day 7. Subjects or their parents or guardians maintained a daily diary to record administration of study medication and adverse events and to grade each of their symptoms on a 4-point scale: 0= absent, 1= mild, 2= moderate, 3= severe. A study nurse visited each subject daily during the treatment period to ensure compliance with the study and to collect plastic zipper storage bags containing tissue used during the preceding day. The primary endpoint of the study was time from first dose to alleviation of symptoms (all symptoms absent or mild) compared using a survival analysis (log rank test). The median times from first dose to alleviation of symptoms were 4 days (IQR 2-7) for the NTZ treatment group and >7 days (IQR 3- >7) for the placebo group ($P<0.001$, log rank test). This analysis was supported by secondary analyses. Subjects randomized to the placebo treatment group were more likely to have at least one illness-related physical exam abnormality at Day 7 follow up (81% vs. 28%, $P<0.0001$), and they were more likely to require antibiotic treatment at the Day 7 follow up (64% vs. 9%, $P<0.0001$) than subjects treated with NTZ. The most common illness-related physical exam findings at the Day 7 follow-up visit were erythematous oropharynx (72% vs. 20%, $P<0.0001$), hypertrophic tonsils (74% vs. 17%, $P<0.0001$), nasal congestion (49% vs. 9%, $P<0.0001$), ronchi (40% vs. 7%, $P=0.0006$) and adenomegaly (26% vs. 7%, $P=0.02$). Treatment with NTZ was associated with a significant decline in weight of daily tissue collections during the treatment period ($P=0.0347$). By rapid direct immunofluorescence assay, respiratory viruses were identified in 17% of the subjects, 12 subjects with adenovirus, 2 with influenza A, 2 with parainfluenza 1, and 1 with RSV. The small number of subjects with identified VRI did not allow for analyses for subsets with specific VRIs. Notably, the rapid assay did not include a test for EV/RV. Given that EV/RVs are the most common cause of VRIs, it is likely that most of the subjects were infected with EV/RV. There were no serious adverse events. All adverse events were mild to moderate in severity. The nature and frequency of adverse events reported by subjects in the active and placebo groups were similar, the only exception being a higher incidence of chromaturia (yellow discoloration of urine), which was reported by 64% of subjects in the NTZ group and 28% of subjects in the placebo group ($P=0.0006$).

Study RM01-2021: In a second clinical trial, 86 adults and adolescents ≥12 years of age with fever, at least one respiratory symptom (cough, nasal discharge or congestion, sneezing or sore throat) and at least one constitutional symptom (myalgia, malaise, fatigue, headache, chills/sweat) of less than 72 hours duration were randomized to receive NTZ 500 mg tablets or a placebo b.i.d. for 5 days. A nasopharyngeal swab was obtained at baseline and subjected to rapid direct immunofluorescence assay for respiratory syncytial virus (RSV), influenza A, influenza B, parainfluenza 1, 2 and 3, and adenovirus. Physical examinations were performed at baseline and on study Day 7. Subjects maintained a daily diary to record administration of study medication and adverse events and to grade each of their symptoms on a 4-point scale: 0= absent, 1= mild, 2= moderate, 3= severe. A study nurse visited each subject daily during the treatment period to ensure compliance with the study and to collect plastic zipper storage bags containing tissue used during the preceding day. The primary endpoint of the study was time from first dose to alleviation of symptoms (all symptoms absent or mild) compared using a survival analysis (log rank test). The median time from first dose to alleviation of VRI symptoms was 4 days (IQR 3-5) for the NTZ treatment group and 7 days (IQR 3->7) for the placebo group (P=0.0365, log rank test). This analysis was supported by secondary efficacy analyses. Subjects randomized to the placebo treatment group were more likely to have at least one illness-related physical exam abnormality at the Day 7 follow up (71% vs. 37%, P=0.0032), and they were more likely to require antibiotic treatment at the Day 7 follow up (36% vs. 18%, P=0.132) than subjects treated with NTZ. The most common illness-related physical exam findings at the Day 7 follow up visit were erythematous oropharynx (69% vs. 26%, P<0.001) and hypertrophic tonsils (50% vs. 21%, P=0.01). The mean weight of tissue collected over the treatment period was also higher for subjects in the placebo group than for subjects treated with NTZ (14.23 grams vs. 10.58 grams, P=0.20), with the difference arising primarily during the first two to three days of the study. By rapid direct immunofluorescence assay, respiratory viruses were identified in 12% of the subjects, 4 subjects with adenovirus, 5 with RSV and 1 with influenza A. Notably, the rapid assay did not include a test for EV/RV. Given that EV/RVs are the most common cause of VRIs, it is likely that most of the subjects were infected with EV/RV. There were no serious adverse events. All adverse events were mild to moderate in severity. The nature and frequency of adverse events reported by subjects in the active and placebo groups were similar, the only exception being a higher incidence of chromaturia (yellow discoloration of urine), which was reported by 60% of subjects in the NTZ group and 19% of subjects in the placebo group (P=0.0001).

1.2.4. Phase 2b/3 Clinical Trial of NTZ in Uncomplicated Influenza: Study RM08-3001

A randomized, double-blind, placebo controlled dose-range finding study was conducted to compare the efficacy of 300 mg NTZ twice daily for 5 days, 600 mg NTZ twice daily for 5 days or a placebo for the treatment of uncomplicated influenza in subjects 12 to 65 years of age. The study was conducted in 74 outpatient primary care centers located throughout the United States during the 2010-2011 influenza season. 624 subjects were enrolled in the study based upon symptoms and presence of influenza in the community within 48 hours of symptom onset. Nasopharyngeal swabs were collected at baseline for identification of 19 different viral respiratory infections by RT-PCR. Subjects maintained diaries to record time of medication intake, oral temperature, the severity of

each of 9 symptoms (runny nose, nasal congestion, sore throat, cough, headache, muscle aches, tiredness/fatigue, feverishn, and sweats/chills) graded as absent, mild, moderate or severe), ability to perform normal activities (scale of 0-10), time lost from work or school, concomitant medications and adverse events. Diary data was recorded twice daily through study Day 7 or, if longer, until the subject no longer had any moderate or severe influenza symptoms. Subjects returned to the clinic for follow-up on Day 7 and Day 28. Blood and urine samples were collected at baseline and Day 7 for laboratory safety tests, and blood samples were collected at baseline and Day 28 for influenza antibody titer. The primary efficacy endpoint was time from first dose to alleviation of symptoms (all 9 symptoms absent or mild and remained so for at least 24 hours) in subjects with laboratory-confirmed influenza. In accordance with the study statistical analysis plan, the primary efficacy analysis was repeated for the subset of subjects with EV/RV infection.

Eighty-eight (88) subjects enrolled were diagnosed with EV/RV as the sole infection at baseline. A virus species was identified by sequencing for 83% of these subjects. Sixty-four percent (64%) were *Rhinovirus A*, 4% *Rhinovirus B*, 29% *Rhinovirus C*, and 3% were *Enterovirus C*. Within each species there was a wide range of serotypes. Analyses of time from first dose to alleviation of all symptoms for these subjects are presented in Table 3 below. Due to the small sample size for a virus with significant variability in presentation, as well as inadequate study design for these viruses, no treatment benefit was expected or observed in this subset. While no apparent treatment benefit was observed for the NTZ 600 mg treated subjects compared to placebo-treated subjects in this subset, it is important to note that the sample size was small (n=29 and n=26 for NTZ 600 mg and placebo groups, respectively) for an illness with significant variability in presentation, the median time to symptom alleviation for the placebo-treated subjects was relatively short (90.8 hours), the patient-reported outcomes instrument has not been validated according to current FDA standards, and the primary endpoint used for this study has been shown to be relatively insensitive in detecting treatment benefit in studies of approved influenza antiviral medications.

Table 3: Study RM08-3001: Median (IQR) time (hrs.) to alleviation of all symptoms for subjects infected with EV/RV as sole identified pathogen

Group	NTZ 600 mg	NTZ 300 mg	Placebo
N (censored)	29 (1)	33 (3)	26 (2)
Median (IQR)	105.1 (60-159)	86.7 (70-119)	90.8 (59-134)
Difference vs. placebo	+14.3 (+1, +25)	-4.1 (+11, -15)	-
P value vs. placebo, Wilcoxon	0.4364	0.8849	-
P value vs. placebo, Log-Rank	0.5196	0.8699	-

1.2.5. Randomized 2x2 Factorial Trial of NTZ and OST in Treating Uncomplicated Influenza: Study RM08-3002

A randomized, double-blind, factorial trial of NTZ and OST was conducted in 130 outpatient primary care clinics in the United States, Canada, Belgium, Australia and New

Zealand between March 2013 and April 2015. 1,941 subjects 13 to 65 years of age were enrolled based upon symptoms and the presence of influenza in the community. Subjects were enrolled within 48 hours of symptom onset and randomized to receive NTZ 600 mg, OST 75 mg, NTZ 600 mg plus OST 75 mg or placebo twice daily for five days. Nasopharyngeal swabs were collected at baseline for identification of 19 different viral respiratory infections by RT-PCR. Subjects maintained diaries to record time of medication intake, oral temperature, the severity of each of 7 symptoms (cough, nasal obstruction, sore throat, fatigue, headache, myalgia or feverishness graded as absent, mild, moderate or severe), ability to perform normal activities (scale of 0-10), time lost from work or school, concomitant medications and adverse events. Diary data was recorded twice daily through study Day 14 or, if longer, until the subject no longer had any moderate or severe influenza symptoms. Subjects returned to the clinic for follow-up on Day 7, Day 14 and Day 28. Blood and urine samples were collected at baseline and Day 7 for laboratory safety tests, and blood samples were collected at baseline and Day 28 for influenza antibody titer. The primary efficacy endpoint was time from first dose to alleviation of symptoms (all 7 symptoms absent or mild and remained so for at least 24 hours). In accordance with the study statistical analysis plan, the primary efficacy analysis was repeated for the subset of subjects with EV/RV infection.

Two hundred twenty-eight (228) subjects enrolled were diagnosed with EV/RV as the sole infection at baseline. A virus species was identified by sequencing for 86% of these subjects. Sixty-four percent (64%) were *Rhinovirus A*, 12% *Rhinovirus B*, 23% *Rhinovirus C*, and 1% were either *Enterovirus A* or *Enterovirus B*. Within each species there was a wide range of serotypes. Analyses of response data for these subjects are presented below as background and serve in part as the basis for hypotheses tested in the present study (RM08-3005).

In subjects with EV/RV identified as the sole cause of respiratory illness at baseline, treatment with NTZ was associated with a reduction of time to alleviation of symptoms compared to treatment with placebo or OST.

Table 4: Study RM08-3002: Median (IQR) time (hrs.) to alleviation of all symptoms for subjects infected with EV/RV as sole identified pathogen

Group	NTZ	OST	NTZ+OST	Placebo
N (censored)	55 (1)	51 (5)	61 (5)	61 (7)
Median (IQR)	115.6 (69-210)	138.8 (83-309)	113.7 (65-179)	146.5 (92-298)
Difference vs. placebo	-30.9 (-23, -88)	-7.7 (-9, +11)	-32.8 (-27 -119)	
P value vs. placebo, Wilcoxon	0.0390	0.7015	0.0205	
P value vs. placebo, Log Rank	0.0128	0.9141	0.0660[1]	

[1]P value vs. placebo is shown for this sensitivity analysis because OST is not effective against EV/RV.

Analysis of time to alleviation of all symptoms comparing all NTZ-treated subjects (NTZ and NTZ+OST groups) to placebo-treated subjects is presented below.

Table 5: Study RM08-3002: Median (IQR) times (hrs.) to alleviation of all symptoms for subjects infected with EV/RV as sole identified pathogen, NTZ vs. placebo

Group	NTZ	Placebo
N (censored)	116 (6)	61 (7)
Median (IQR)	114.1 (65-187)	146.5 (92-298)
Difference vs. placebo	-32.4 (-27, -111)	
P value vs. placebo, Wilcoxon	0.0133	
P value vs. placebo, Log Rank	0.0161	

The magnitude of treatment benefit was larger in subjects treated earlier in the course of illness. The following table presents an analysis of time to alleviation of symptoms for subjects enrolled within 24 hours of symptom onset and for those enrolled >24 hours after symptom onset.

Table 6: Study RM08-3002: Median (IQR) times (hrs.) to alleviation of all symptoms for subjects infected with EV/RV as sole identified pathogen

	Enrolled ≤24 Hours from Symptom Onset		Enrolled >24 Hours after Symptom Onset	
Group	**NTZ**	**Placebo**	**NTZ**	**Placebo**
N (censored)	47 (4)	23 (3)	69 (2)	38 (4)
Median (IQR)	103.5 (63-212)	174.2 (114-309)	124.7 (72-181)	137.5 (79-261)
Difference vs. placebo	-70.7 (-51, -97)		-12.8 (-7, -80)	
P value vs. placebo, Wilcoxon	0.0103		0.3004	
P value vs. placebo, Log Rank	0.0493		0.1235	

Treatment with NTZ also showed benefit in analysis of time to return to ability to perform all normal activities with the median improvement being approximately 4.69 days (112.5 hours). See the table below.

Table 7: Study RM08-3002: Median (IQR) times (hrs.) to return to normal activities for subjects infected with EV/RV as sole identified pathogen, NTZ vs. placebo

Group	NTZ	Placebo
N (censored)	115 (29)	60 (22)
Median (IQR)	189.5 (109-402)	302.0 (135-643)
Difference vs. placebo	-112.5 (-26, -241)	
P value vs. placebo, Wilcoxon	0.0452	
P value vs. placebo, Log Rank	0.0326	

The magnitude of treatment benefit for time to return to normal activities was also larger for subjects enrolled within 24 hours of symptom onset: median benefit = 7.44 days (178.6 hours) for subjects enrolled within 24 hours of symptom onset compared to 2.68 days (64.2 hours) for subjects enrolled >24 hours after symptom onset. See the table below.

Table 8: Study RM08-3002: Median (IQR) times (hrs.) to return to normal activities for subjects infected with EV/RV as sole identified pathogen

	Enrolled ≤24 Hours from Symptom Onset		Enrolled >24 Hours after Symptom Onset	
Group	**NTZ**	**Placebo**	**NTZ**	**Placebo**
N (censored)	46 (10)	22 (8)	69 (19)	38 (14)
Median (IQR)	165.1 (105-309)	343.7 (241->700)	214.3 (112-431)	278.5 (119->700)
Difference vs. placebo	-178.6 (-136, >-391)		-64.2 (-8, <-269)	
P value vs. placebo, Wilcoxon	0.0051		0.7738	
P value vs. placebo, Log Rank	0.0116		0.4816	

Treatment with NTZ 600 mg twice daily for five days as monotherapy or in combination with OST was well tolerated by all subjects. The most commonly reported adverse events (reported by ≥2% of subjects in any treatment group) regardless of causality were:

Table 9: RM08-3002: Most common adverse events (reported by 2% of subjects in any treatment group) regardless of causality

Adverse event (≥2%)	NTZ (%)	OST (%)	NTZ+OST (%)	Placebo (%)
Nausea	3.9	4.4	6.4	5.3
Vomiting	2.5	4.2	6.4	2.7
Diarrhea	6.2	2.5	3.9	4.9
Abdominal pain upper	2.1	0.8	2.3	1.2
Chromaturia	5.2	-	5.5	0.4
Dizziness	2.5	3.1	3.1	1.0

There were no serious adverse events related to any of the treatments.

1.2.6. Overview of Other Experience in Clinical Trials and Post-marketing Surveillance

NTZ has been marketed for diarrheal disease caused by *Giardia* or *Cryptosporidium* in the United States since 2003 and in Latin America since 1996. It is estimated that more

than 250 million patients have been exposed to NTZ worldwide. No drug-related serious adverse events have been reported during post-marketing experience with NTZ.

Phase 2 and 3 clinical studies have been conducted in approximately 5,600 subjects to evaluate the safety and efficacy of NTZ in treating parasitic, bacterial and viral infections. During these studies, no drug-related serious adverse events have been observed. The side effects have been usually of a mild transient nature, and less than 1% of subjects have discontinued therapy because of an adverse event. The most common adverse events reported in clinical trials include abdominal pain, chromaturia, diarrhea, dizziness, headache, nausea and vomiting and did not differ significantly from those of placebo except for chromaturia which was reported by 4 to 5% of subjects and is attributed to urinary excretion of NTZ metabolites. Clinical chemistry and hematology obtained before and after treatment have not revealed any abnormalities attributable to the test drug (Clinicaltrials.gov 2018b).

1.3. Rationale for the Study

There is an important need for a treatment for colds due to EV/RV infection that could reduce the duration of symptoms, the time until patients are able to perform their normal activities, and potentially the risk of complications. This product should have a favorable safety profile and low risk for resistance.

This study is a multicenter randomized, double-blind, placebo-controlled trial designed to evaluate efficacy and safety of NTZ 600 mg administered orally twice daily for five days compared to a placebo in the treatment of colds due to EV/RV infection.

2. STUDY OBJECTIVES

The primary objective of this study is to evaluate the effect of NTZ administered orally 600 mg b.i.d. for 5 days in reducing the duration of symptoms of colds due to EV/RV infection compared to that of a placebo during 21 days of follow-up based upon the FLU-PRO© patient-reported outcome instrument.

A key secondary efficacy objective is to evaluate the effect of NTZ compared to placebo on time to return to ability to perform all normal activities.

Another secondary efficacy objective is to evaluate the effect of NTZ compared to placebo on the proportion of subjects experiencing one or more complications of colds due to EV/RV infection including pneumonia, otitis media, bronchitis, sinusitis, exacerbations of asthma or COPD, worsening of pre-existing health conditions, secondary infections requiring systemic antibiotic use, hospitalization due to cold or complications of the cold, and death due to cold or complications of the cold.

Exploratory efficacy objectives include evaluating the effect of treatment with NTZ on the time to response for each FLU-PRO symptom, time to response for each FLU-PRO domain, time to return to usual health, changes in viral titers from baseline to each of Days 2, 3 and 7, time to symptom response for all subjects treated, and time to symptom response for subjects with individual non-EV/RV virus infections.

Other important objectives include evaluation of the safety of NTZ by analysis of adverse events and evaluation of relationships between pharmacokinetics and clinical or virologic responses.

3. STUDY DESIGN

The study will be a multicenter, randomized, double-blind trial to evaluate the efficacy of NTZ compared to placebo in treating colds due to EV/RV infection.

Subjects will be randomized 1:1 to one of the following groups:

- Group 1 (NTZ): Two NTZ 300 mg tablets b.i.d. for 5 days
- Group 2 (Placebo): Two placebo tablets b.i.d. for 5 days

Enrollment and the primary efficacy analysis will be stratified by (1) time from onset of illness to study enrollment (0-24, >24-≤36, or >36 hours from onset), (2) pre-enrollment use of symptom relief medication, and (3) presence of underlying lung condition including asthma, COPD, or past or present history of smoking.

This study is expected to run from August 2018 through April 2019.

Rationale for important issues in the study design is described below:

- *Choice of NTZ dose and duration of treatment.* The dose of NTZ used for this clinical trial (600 mg b.i.d. for 5 days) was associated with a reduction of the duration of symptoms and time to ability to perform all normal activities in subjects with EV/RV infection in a previous trial of NTZ for the treatment of influenza (Study RM08-3002). In clinical trials involving 2,886 subjects, adverse events reported by subjects receiving 600 mg NTZ b.i.d. for 5 days were similar to those of subjects receiving the placebo, with the exception of a higher rate of diarrhea (8.1% compared to 5.8% for the placebo). Studies of NTZ at higher doses have shown dose-dependent increases in the frequency of reported diarrhea and other gastrointestinal side effects, therefore no higher dose is planned for this study.
- *Choice of control groups.* A placebo control is appropriate for the study due to lack of any approved active control.
- *Choice of patient population.* The population to be studied includes adults and adolescents at least 12 years of age with EV/RV infection. This is a population similar to those selected for studies RM08-3001, RM08-3002, RM08-3003 and RM08-3004 of the NTZ 300 mg extended release tablets in more than 3,000 subjects with influenza-like illness. No safety concerns have been identified from these clinical trials.

  For the present study, some subjects (e.g., subjects >65 years of age, underlying asthma or COPD, diabetes mellitus if not poorly controlled, etc. – see Inclusion/Exclusion criteria in sections 4.1 and 4.2) who are at higher risk of complications or exacerbations of underlying conditions will be allowed to participate if, in the judgment of the investigator, they are able to comply with protocol requirements and are not expected to require hospitalization. There is no approved antiviral for colds due to EV/RV, and subjects will not be prevented from being prescribed rescue medication for

complications. Therefore, participation in the study will not pose unreasonable risk to any eligible subject. Enrollment will be stratified by presence of underlying lung condition including asthma, COPD, and history of smoking.

Data from pharmacokinetics studies in healthy volunteers 12 to 17 years of age or 18 to 65 years of age indicate that the pharmacokinetics of the major NTZ metabolites, tizoxanide and tizoxanide glucuronide, following oral administration of a NTZ 500 mg tablet are similar for these age groups. Safety data is also similar for these age groups. Therefore, the adult dose is deemed appropriate for pediatric patients down to 12 years of age. Informed consent will be required for subject participation in this study (see Protocol section 15). A signed assent form will be required for any minors enrolled (≤ 18 years of age or as local regulations apply) as well as signed parental/legal guardian consent by their parent/legal guardian allowing for the minor's participation.

- *Choice of Inclusion Criteria.* In non-asthmatic individuals, symptoms of EV/RV infection are generally limited to the upper respiratory tract. Rhinorrhea and nasal obstruction are the most prominent symptoms in uncomplicated cases of EV/RV infection. Lower respiratory symptoms including cough, shortness of breath, chest tightness, and wheezing may be the most prominent symptom in patients who have underlying asthma or other chronic lung disease. Rhinorrhea rated as moderate or severe was the main inclusion criterion for two Phase 3 studies of pleconaril in the treatment of picornavirus-associated viral respiratory infection, accompanied by nasal congestion, cough, or sore throat, resulting in a picornavirus-positive rate of 62% and 68% (Allan and Arroll 2014). The same criteria have been used to enroll natural history and epidemiology studies of EV/RV infection with up to an 82% picornavirus-positive rate (Arruda et al. 1997).

  Fever, especially a low-grade fever, is consistent with EV/RV infection in epidemiological studies, with presentation guidelines for physicians, and with presentation of more than 300 EV/RV-infected subjects enrolled in Phase 3 studies of NT-300 for treatment of influenza (Chughtai et al. 2017, Rotbart and Hayden 2000, Aring 2016). Exclusion of subjects with fever is likely to exclude EV/RV-infected subjects with severe cytokine response. Therefore, the present study will require a negative result for influenza by a rapid diagnostic test if the subject has an oral temperature >100°F or when local influenza activity has been confirmed in order to minimize the number of subjects enrolled with influenza infection.

  Human challenge studies indicate that the onset of symptoms of Rhinovirus infection occurs approximately 24 hours post-inoculation (p.i.); symptoms peak approximately 48-72 hours p.i.; and virus titer peaks approximately 96 hours p.i. (Fullen et al. 2016). Enrollment within 40 hours of symptom onset will maximize potential treatment benefit by initiating treatment before symptom severity and virus titer peak. See sections 1.1.1 and 1.2.4 above for presentation of data indicating improved efficacy of investigational antiviral drugs and NTZ in subjects enrolled earlier during the course of EV/RV infection.

- *Choice of Patient-Reported Outcome Instrument.* This clinical trial will use a new, recently released patient-reported outcome questionnaire, InFLUenza Patient-Reported Outcome Questionnaire (FLU-PRO©).

FLU-PRO was developed with the support of the U.S. Department of Health and Human Services through the National Cancer Institute and the National Institutes of Allergy and Infectious Diseases, National Institutes of Health, in response to the need for improved metrics to evaluate treatment effect in clinical trials of drugs for the treatment of influenza and other respiratory tract viral diseases. It was developed and validated in accordance with FDA's guidance, "Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims." It has also been validated for use on an electronic device as part of an electronic diary ("eDiary") that can time stamp diary entries to ensure timely recording, thereby mitigating risks of recall bias. We will use an electronic diary for this clinical trial. FLU-PRO was separately validated for use in a population with non-influenza ILI (Powers et al. 2018).

PRO instruments used in previous studies of EV/RV-associated illness include the modified Jackson index, the Wisconsin Upper Respiratory Symptom Survey (WURSS), and a six-symptom scale including rhinorrhea, nasal congestion, sore throat, malaise, cough, and myalgia. The Jackson scale is the oldest PRO for evaluation of colds and influenza, but it was not developed or validated in accordance with FDA guidance on PROs, therefore content validity and measurement properties may be incomplete (Barrett et al. 2009, Jackson and Dowling 1959). The WURSS was developed and validated for patients with self-diagnosed colds in general accordance with practices recommended in the FDA guidance on PROs (Barrett et al. 2002). However, the WURSS is intended to measure the overall impact of ILI on a patient, as evidenced by presence of both symptom and quality of life assessments within the survey. Because the purpose of the present study is to measure how a patient feels due to EV/RV infection (concept measured), use of the WURSS scale is not appropriate for measurement of efficacy data. The FLU-PRO scale measures the appropriate concept and was validated for use in the intended population (Powers 2018).

In an ongoing study RM08-3004 enrolling subjects with influenza or influenza-like illness, diary compliance, based upon electronic date and time-stamped data, has been 87% with an average time to complete the FLU-PRO of less than four minutes, indicating patient acceptability and lack of patient burden. Therefore, use of FLU-PRO is expected to provide robust and reliable data for analysis of the symptoms caused by EV/RV infection.

- *Choice of Endpoints.* The efficacy endpoints to be analyzed sequentially for this study are (1) Time to Symptom Response based upon the FLU-PRO© instrument, (2) Time to Ability to Perform All Normal Activities, and (3) proportions of subjects experiencing complications of EV/RV infection.

  The primary efficacy endpoint for this study will be Time to Symptom Response based upon the FLU-PRO© instrument. This is directed to the way the patient "feels" as measured by his/her symptoms. A Symptom Response definition will be developed based upon a blinded review of FLU-PRO symptom scores (pooled for all subjects without regard to treatment group assignment so as to maintain blinding) and correlation of those scores to the time at which subjects report that they have returned to their usual state of health (a "yes" or "no" question incorporated into the daily Questionnaire). Two Symptom Response definitions will be determined in this manner: one for subjects with

underlying lung conditions including asthma, COPD or history of smoking; and one for subjects with no underlying lung condition. This methodology will allow for analysis of all subjects together while accounting for differences expected in the usual state of health for subjects with lung conditions (e.g., chronic cough). The Time to Symptom Response used for evaluating the primary endpoint will be calculated based upon these Symptom Response definitions and programmed prior to database unblinding for the primary analysis. This anchor-based approach to defining responders ensures that the Symptom Response definition is valid and meaningful for the subjects participating in each given clinical trial.

The key secondary endpoint to be analyzed second in sequence will be Time to Ability to Perform All Normal Activities. This endpoint will provide important information with respect to the way a patient "functions." Each of the previous clinical trials of NTZ in subjects with influenza have indicated improvement in this endpoint while data collection from those studies has typically not extended out long enough to fully characterize the benefit. As noted in section 1.2.4 above, subjects with EV/RV infection as their sole identified cause of respiratory illness at baseline also showed improvement in this endpoint for subjects treated with nitazoxanide compared to placebo. This endpoint is more meaningful than time to return to work or school because the decision to return to work or school is influenced by factors independent of how the patient feels or his/her ability to perform work or school activities (Mitchell 2011).

Another secondary endpoint will be proportions of subjects experiencing complications of EV/RV infection, including pneumonia, otitis media, bronchitis, sinusitis, exacerbations of asthma or COPD, worsening of pre-existing health conditions, secondary infections requiring systemic antibiotic use, hospitalization due to cold or complications of the cold, and death due to cold or complications of the cold. We do not expect to see enough complications in this population to demonstrate differences between treatment groups, and therefore, this endpoint is lesser in priority compared to Time to Ability to Perform All Normal Activities. All other efficacy endpoints will be considered exploratory.

4. SUBJECT SELECTION

The criteria for inclusion and exclusion are defined below:

4.1. Inclusion Criteria

1. Male and female subjects at least 12 years of age
2. Presence of clinical signs and/or symptoms consistent with an acute illness compatible with EV/RV infection (each of the following is required):
   a. Presence of moderate or severe rhinorrhea defined as "attempting to relieve nasal symptoms by blowing, wiping, or sniffling at least twice per hour for any one hour within 12 hours preceding study entry," AND
   b. Presence of cough, sore throat or nasal obstruction.

3. Negative rapid influenza diagnostic test (required only if the subject has an oral temperature >100°F in the clinic or if the latest CDC weekly influenza report shows influenza prevalence "Regional" or higher for the institution's state). A result from a rapid influenza diagnostic test performed on the same day that informed consent is obtained will be sufficient to meet this criterion if documentation of test results is available as part of medical history.
4. Onset of illness no more than 40 hours before enrollment in the trial. Onset of illness is defined as the first time at which the subject experienced rhinorrhea, cough, sore throat or nasal obstruction.
5. Willing and able to provide written informed consent (including assent by legal guardian if under 18 years of age) and comply with the requirements of the protocol, including completion of the subject diary

4.2. Exclusion Criteria

1. Persons requiring or anticipated to require in-hospital care
2. Cystic fibrosis
3. Cardiac arrhythmia
4. Immunologic disorders or receiving immunosuppressive therapy (e.g., for organ or bone marrow transplants, immunomodulatory therapies for certain autoimmune diseases)
5. Untreated HIV infection or treated HIV infection with a CD4 count below 350 cells/mm3 in the last 6 months
6. Persons with sickle cell anemia or other hemoglobinopathies
7. Poorly controlled insulin-dependent diabetes mellitus (HbA1C >8.0%)
8. Concurrent infection at the screening examination that requires systemic antimicrobial therapy
9. Females of childbearing potential who are either pregnant or sexually active without the use of birth control. Female subjects of child-bearing potential that are sexually active must have a negative baseline pregnancy test and must agree to continue an acceptable method of birth control for the duration of the study and for 1 month post-treatment. A double barrier method, oral birth control pills administered for at least 2 monthly cycles prior to study drug administration, an IUD, or medroxyprogesterone acetate administered intramuscularly for a minimum of one month prior to study drug administration are acceptable methods of birth control for inclusion into the study. Female subjects are considered of childbearing potential unless they are postmenopausal (absence of menstrual bleeding for 1 year - or 6 months if laboratory confirmation of hormonal status), or have had a hysterectomy, bilateral tubular ligation or bilateral oophorectomy.
10. Females who are breastfeeding
11. Receipt of any dose of NTZ within 30 days prior to screening
12. Prior treatment with any investigational drug therapy within 30 days prior to screening

13. Subjects with active respiratory allergies or subjects expected to require anti-allergy medications during the study period for respiratory allergies
14. Known sensitivity to NTZ or any of the excipients comprising the NTZ tablets
15. Subjects unable to take oral medications
16. Subjects who, in the judgment of the Investigator, will be unlikely to comply with the requirements of this protocol including completion of the subject diary

5. STUDY PROCEDURES

5.1. Screening Evaluation (day 1)

Before screening, subjects and their parents/guardian(s) will be informed of the nature of study, and written consent must be obtained prior to participation. After giving informed consent, the subject will be assigned a subject number, and the following procedures will be carried out:

1. Complete medical history.
2. Full physical examination including body weight and vital signs (blood pressure, pulse, respiratory rate and oral temperature using standard electronic oral thermometer provided by Sponsor).
3. Collection of demographic information and smoking history.
4. Urine pregnancy test for all females of childbearing potential.
5. Recording of time of onset of respiratory symptoms.
6. Evaluation according to eligibility (inclusion and exclusion) criteria.

5.2. Baseline (day 1, same day as screening evaluation)

At baseline, the following procedures will be carried out:

1. Collection of two nasopharyngeal swabs (one from each nostril) using nylon flocked dry swabs (Copan Diagnostics) for RT-PCR and culture.
2. Collection of blood sample for laboratory safety tests.
3. Collection of a urine sample for routine urinalysis (glucose, protein and blood).
4. Review and recording of any concomitant medications.
5. Provision of an electronic diary to subject via his/her own smartphone or a provisioned device, and subject completion of baseline electronic diary under supervision and instruction of study site personnel, including the Practice Diary, FLU-PRO Questionnaire, Activity Diary, and Study Drug Intake and Temperature Diary.
6. Randomization and dispensing of study medication (medication assigned in sequential order).

7. Administration of the first dose of study medication with food (< 1 hour after food intake) under observation of Investigator or a member of Investigator's staff, and entry in Study Drug Intake and Temperature Diary.
8. Instruct subject regarding:
   a. *Administration of study medication.* Subjects will be instructed to take the study medication (two tablets) twice daily with food (< 1 hour after food intake, preferably a high-fat meal but at a minimum a cereal bar). The subject should take his/her second dose as close as possible to 12 hours after the first dose. Then he/she will take the study medication every 12 hours for the remaining 4 days.
   b. *Completion of subject diary.* Subjects will be instructed on completion of the electronic diary.
   c. *Use of birth control.* Female subjects of childbearing potential, if sexually active, will be instructed to continue an acceptable method of birth control for the duration of the study and for 1 month post-treatment. Acceptable methods of birth control include a double barrier method, oral birth control pills, an IUD, or medroxyprogesterone acetate administered intramuscularly.
   d. *Follow-up visits:* Subjects will be instructed to return to the clinic for follow-up on Day 7 and Day 22.
   e. *Seeking emergency care or contacting the study physician or nurse*: Subjects must be informed to seek emergency medical care or contact the study physician or nurse if they develop any of the following symptoms listed below during the full 22-day study and follow-up period.

      CALL 911 (United States) OR LOCAL EMERGENCY SERVICES (outside of United States) OR HOSPITAL OR CONTACT STUDY PHYSICIAN, IF:
      - Trouble breathing including shortness of breath
      - Severe headache, stiff neck, confusion or excessive somnolence

      CALL STUDY NURSE OR PHYSICIAN, IF:
      - Extremely high fever >104°F (40°C)
      - Fever (≥100.4°F or ≥38°C) lasts for longer than 3 days
      - Increased difficulty breathing
      - Wheezing develops
      - New pain develops or pain localizes to one area, such as an ear, the throat, the chest, or the sinuses
      - Symptoms become more severe or frequent
      - Fever (≥100.4°F or ≥38°C) returns after being absent for 24 hours
      - Symptoms recur or any difficulty breathing following 5-10 days resolution of illness
      - An allergic-like reaction occurs or is suspected
      - Abnormal behavior
9. Review and record adverse events

5.3. Day 2 – Day 22

A study physician, nurse or other site personnel will review each subject's electronic diary entries daily to ensure compliance with collection of diary data. If a subject has not completed his/her diary or if errors are suspected, the study personnel will contact the subject to implement appropriate corrective actions.

5.4. Day 2-5 Telephone Monitoring

A study physician, nurse or other site personnel will make daily telephone calls to subjects on each of Days 2, 3, 4 and 5 of dosing to screen for potential complications and review adverse events. Subjects will be referred for immediate care as needed based on the screening. All information gained from telephone monitoring will be included in the EDC system for each subject. [Note: In lieu of a telephone call, this information may be obtained during the Day 2 and 3 home or office visits (see below).]

5.5. Day 2 and 3 Evaluations

A study physician, nurse or other study personnel will visit each subject at home (or at the clinic or another location as agreed with the subject) on each of Days 2 and 3 to (i) collect two nasopharyngeal swabs for detecting and quantifying EV/RV, and (ii) screen for potential complications and adverse events. Subjects will be referred for immediate care as needed based on the screening. All information gained from these visits will be included in the case report forms for each subject. In addition, a blood sample for pharmacokinetics will be collected on Day 3. The Day 2 and Day 3 visits will occur at approximately the same time of day that the patient took his/her first dose of study medication (approximately 24 and 48 hours after the first dose). On Day 3, the blood sample for pharmacokinetics will be collected before the first dose of that day.

Study sites or subjects may opt out of these Day 2 and Day 3 visits due to site staffing, subject availability or other practical considerations or preferences; nevertheless at least 75% of subjects enrolled in the trial are expected to complete the Day 2 and Day 3 visits and related procedures (for viral kinetics and pharmacokinetics).

5.6. Day 7 Follow-up (±1 day)

Subjects will return to the clinic on Day 7, and the following procedures will be performed:

1. Brief physical examination (body weight and vital signs with nursing physical assessment) including symptom directed physician physical examination as required by subject symptoms. Vital signs will include blood pressure, pulse, respiratory rate and oral temperature (use standard electronic oral thermometer provided by Sponsor).
2. Collection of two nasopharyngeal swabs using nylon flocked dry swabs (Copan Diagnostics) for RT-PCR and culture.
3. Collection of blood sample for laboratory safety tests.
4. Collection of urine sample for routine urinalysis.
5. Review of compliance with study medication, collection of medication bottle with any unused medications, and completion of the pill count log form.

6. Review and recording of concomitant medications.
7. Review and recording of adverse events/side effects and complications.

5.7. Day 22 Follow-up (+3 days)

Subjects will return to the clinic on Day 22, and the following procedures will be performed:

1. Brief physical examination (body weight and vital signs with nursing physical assessment) including symptom directed physician physical examination as required by subject symptoms. Vital signs will include blood pressure, pulse, respiratory rate and oral temperature (use standard electronic oral thermometer provided by Sponsor).
2. Collection of two nasopharyngeal swabs using nylon flocked dry swabs (Copan Diagnostics) for RT-PCR and culture (to be tested for presence of virus only if the sample collected at the preceding time point had detectable virus).
3. Review and recording of concomitant medications.
4. Review and recording of adverse events/side effects and complications. Note that all adverse events and complications must be followed until their resolution or stabilization even beyond the 22-day study period.

5.8. Unscheduled Visit

Subjects returning to the clinic for an unscheduled visit due to worsening symptoms or adverse events will be evaluated at the discretion of the Investigator as is medically warranted. Tests and/ or procedures performed at this visit may include, but are not limited to, the following:

1. Brief physical examination (body weight and vital signs with nursing physical assessment) including symptom directed physician physical examination as required by subject symptoms. Vital signs will include blood pressure, pulse, respiratory rate and oral temperature (use standard electronic oral thermometer provided by Sponsor).
2. Collection of two nasopharyngeal swabs using nylon flocked dry swabs (Copan Diagnostics) for RT-PCR and culture.
3. Collection of blood sample for laboratory safety tests.
4. Collection of urine sample for routine urinalysis.
5. Review of compliance with study medication.
6. Review and recording of concomitant medications.
7. Review and recording of adverse events/side effects.

5.9. Study Discontinuation

Rules for discontinuation of a subject or for discontinuing the study are provided in section 11.2. All subjects discontinued from the study before Day 7 will be evaluated at study discontinuation using the procedures described above for Day 7. All subjects discontinued from the study after Day 7 will be evaluated at study discontinuation using the procedures described above for Day 22.

5.10. Electronic Subject Diary

Electronic subject diaries will be completed twice daily through Day 21. The electronic diaries will capture the following information:

1. Medication intake (twice daily at time of medication intake).
2. Oral temperature (twice daily at time of medication intake). Use standard electronic oral thermometer provided by Sponsor.
3. Use of other medications taken by the subject not previously captured in the subject's medication history. Document in detail including dose in milligrams or other applicable units, time of ingestion, and the reason for use of the medication (at time of medication intake).
4. FLU-PRO Questionnaire (once daily between 7 pm and 11 pm) to characterize symptoms.
5. Activity assessment using an 11-point visual analog scale (0= unable to perform normal activity, 10= fully able to perform normal activity) (once daily between 7 pm and 11 pm).
6. Adverse experiences (once daily between 7 pm and 11 pm).

5.11. Plan for Laboratory Safety Tests

A central laboratory will be used for laboratory safety testing. Blood tests will include hemoglobin, hematocrit, complete blood count (total and differential), platelet count, random blood sugar, total cholesterol, HDL, LDL, triglycerides, albumin, AST, ALT, GGT, alkaline phosphatase, bilirubin (total/direct), BUN, creatinine, sodium, potassium, chloride. Routine urinalysis will include glucose, protein, and blood.

5.12. Plan for Virology Testing and Monitoring Resistance

Virology testing of nasopharyngeal swab samples will be conducted as described on the following pages.

Table 10: Virology testing of nasopharyngeal swabs

	Day 1	Day 2	Day 3	Day 7	Day 22
RT-PCR by ePlex RPP for Virus Detection	X	X[1]	X[2]	X[3]	X[4]
Sequencing for EV/RV Typing	X[5]				
Quantitative PCR	X[6]	X[6]	X[6]	X[7]	
Culture & Susceptibility Testing for Resistance Monitoring	X[8]			X[8]	X[8]
Nucleotide Sequencing	X[9]			X[9]	X[9]

[1] If Day 1 sample is negative for EV/RV by RT-PCR
[2] If Day 2 sample is negative for EV/RV by RT-PCR
[3] Only if one of Day 1, 2, or 3 samples is positive for EV/RV for RT-PCR
[4] If Day 7 sample is positive for EV/RV by RT-PCR
[5] Only the earliest sample positive for EV/RV by RT-PCR of those collected at Days 1, 2, and 3 will be sequenced for EV/RV type
[6] A sample will only be subjected to quantitative PCR if one of the Day 1, 2, or 3 samples is positive for EV/RV by RT-PCR
[7] The Day 7 sample will be subjected to quantitative PCR only if one of the Day 1, 2, or 3 samples AND the Day 7 sample is positive for EV/RV by RT-PCR
[8] If the Day 1 and Day 7 or Day 1, 7, and 22 samples are positive for EV/RV by RT-PCR each sample will be cultured and tested for susceptibility to tizoxanide. Results observed for the Day 7 and Day 22 samples will be compared to the results observed for the Day 1 sample.
[9] Sample pairs exhibiting resistance to tizoxanide will be subjected to sequencing.

1. Collection of samples:

   Two nasopharyngeal swabs will be collected from each subject at Baseline (Day 1) and Days 2, 3, 7 and 22.

2. Testing of biological samples:

   a. Each Baseline sample will be subjected to RT-PCR using the ePlex ® Respiratory Pathogen Panel (GenMark, Carlsbad, CA) to detect influenza A (non-specific as to subtype); influenza A H1, H1N1 (2009), H3 subtypes; influenza B; respiratory syncytial virus A and B (RSV); parainfluenza 1, 2, 3 and 4; human metapneumovirus (hMPV); adenovirus; human EV/RV; coronavirus NL63, HKU1, 229E and OC43; human bocavirus; *Chlamydophila pneumoniae*; *Legionella pneumophila*; and *Mycoplasma pneumoniae*.
   b. If a subject's Baseline sample is negative for EV/RV by RT-PCR, his/her Day 2 and Day 3 samples will be subjected to RT-PCR using the ePlex® Respiratory Pathogen Panel to detect respiratory pathogens.
   c. If the Baseline sample or the Day 2 or Day 3 sample is positive for EV/RV, the earliest positive sample will be sequenced to determine virus type and each of the Baseline, Day 2 and Day 3 samples will be subjected to quantitative PCR testing to quantify viral shedding.
   d. All Day 7 samples will be subjected to RT-PCR using the ePlex® Respiratory Pathogen Panel. Day 7 samples positive for EV/RV will also be subjected to

quantitative PCR testing to quantify viral shedding if one of the Day 1, 2, or 3 samples was also positive for EV/RV.

e. Day 22 samples will be subjected to RT-PCR using the ePlex ® Respiratory Pathogen Panel only if the sample collected at the preceding time point (Day 7) had detectable EV/RV.

3. Drug susceptibility testing:

   a. If any Day 7 or Day 22 sample is positive for EV/RV by RT-PCR and one of the Day 1, 2, or 3 samples was also positive for EV/RV, the virus will be isolated (if possible) and tested for susceptibility to tizoxanide ($TCID_{50}$).
   b. The Baseline isolate for subjects with virus cultured at Day 7 or 22 will also be tested for susceptibility to tizoxanide, and the results will be compared to the results observed for the Day 7 or 22 isolate.

4. Nucleotide sequencing:

   a. All Day 7 or 22 samples with resistance ($IC_{50} \geq 10x$ IC_{50} at Baseline) to tizoxanide will be subjected to sequencing. The corresponding Baseline sample will also be subjected to nucleotide sequencing. Initial genotypic assessments of resistance to tizoxanide will focus on isolates displaying the largest shifts in susceptibility.
   b. Sequencing data for Day 7 or 22 isolates with resistance to tizoxanide will be compared to sequence data for the Baseline sample to identify mutations that may confer resistance.

5. Storage of samples: All samples collected during the study will be stored for at least 2 years for potential future testing.

6. Clinical Virology Laboratory for diagnostic testing:

7. Clinical virology laboratory for sequencing, quantification of viral shedding, and susceptibility testing:

5.13. Pregnancy

Fertility and reproduction studies in animals have revealed no evidence of impaired fertility or harm to the fetus due to NTZ. However, there are no adequate and well controlled studies of fertility or reproduction in humans. All pregnancies including those of partners of male subjects included in the study will be reported by the investigational staff within 24 hours of their knowledge of the event using a pregnancy reporting form.

6. RANDOMIZATION

An independent third party ( ) will prepare a master randomization list and maintain the masking of the study. Randomization will be performed using permuted block randomization. Subjects who qualify for the study will be assigned to treatment using centralized randomization procedures stratified by time from onset of illness to study enrollment, presence of underlying chronic lung condition (asthma, COPD, history of smoking) and pre-enrollment use of symptom-relief medication. The treatment numbers will appear on the bottles containing the masked study medication. The randomization list will be masked to study participants including Sponsor, Investigators, study monitors, subjects and laboratory personnel. Unmasking for any individual subject will occur only if necessitated by emergency and knowledge of the medication being taken will influence the medical management of the subject. Furthermore, if the event warrants submission of an IND Safety Report, then the subject will be unmasked for completion of the report.

7. DATA MANAGEMENT

7.1. Electronic Data Entry

will provide the electronic data collection (EDC) system,

is a source data collection solution that will allow the site staff to record subject data on electronic protocol-driven source forms by using tablets provided by . Its purpose is to eliminate the need for data transcription into a traditional electronic case report form, and to allow both real-time access to source data by authorized Sponsor personnel and real-time edit checks.

is a mobile application for the capture of patient-reported outcomes (PROs). Study subjects will be allowed to use on their own smartphones or a provisioned device provided by in order to record PROs, concomitant medications and adverse events (in the form of an electronic subject diary) and to receive personalized study-specific reminders and education.

is the web portal. It delivers real-time access to source data, and enables authorized Sponsor personnel to instantly review, clean and analyze study data.

The site staff will use as they meet with study subjects and perform study procedures. Computerized data cleaning checks will be used in addition to manual review, including listings review, to check for discrepancies and to ensure consistency and completeness of the data. If the system is not available for any reason, source data may be captured on paper and transcribed to the system at a later time.

The responsible study monitor(s) will verify data, which can be performed remotely or at the clinical study site. The Investigator will ensure that the data recorded are accurate and complete.

Queries emerging during data cleaning will be generated by data management or clinical research associates in [redacted] The Investigator or his/her designee will answer the queries and update the source data, if needed.

Adverse events will be coded by data management using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA); medications will be coded by data management using the World Health Organization (WHO) DRUG dictionary.

After the Last Subject Last Visit (LSLV) has taken place, the database will be cleaned as necessary. As soon as the database is considered clean, it will be locked. The locked database will be used in the final statistical analysis for study reporting. Measures will be undertaken to protect subject data handled during inspections against disclosure to unauthorized third parties. Subject confidentiality will be maintained at all times.

All listings, summaries and analyses will be produced using SAS Statistical Software (SAS Institute, Inc., Cary, NC).

7.2. Protocol Deviations

Protocol deviations will be documented by the Investigator, reported to the institutional review board (IRB) as appropriate, and also reviewed by the assigned clinical research associate (CRA). Deviations will be reported in the clinical trial management system. Each deviation will be classified as major or minor according to the following definitions:

Major protocol deviation:	A deviation that has an impact on subject safety, may substantially alter risks to subjects, may have an effect on the integrity of the study data, or may affect the subject's willingness to participate in the study
Minor protocol deviation:	All other protocol deviations.

7.3. Data Quality Assurance

The accuracy and reliability of the study data will be assured by the selection of qualified Investigators and appropriate study centers, review of protocol procedures with the Investigator and associated personnel prior to the study, and by periodic monitoring visits by the Sponsor. Written instructions will be provided for collection, preparation, and shipment of samples.

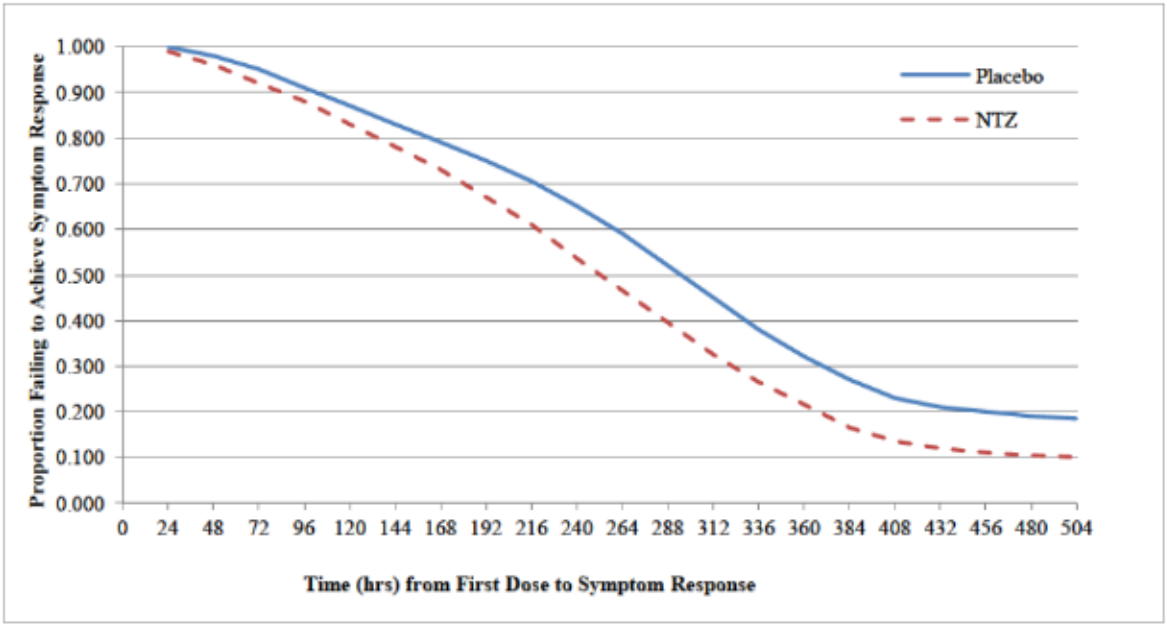
The monitor will review the source data for accuracy and completeness, and any discrepancies will be resolved with the Investigator or designee, as appropriate.

The Sponsor will be entitled to inspect and audit the facilities used in the clinical and laboratory parts of the study, as well as to make anonymized copies of all the data files pertaining to the study. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

8. STATISTICAL CONSIDERATIONS

8.1. Sample Size Calculations

The Symptom Response definitions for this study will be derived from the subjects' FLU-PRO questionnaire responses as described in section 8.3 below. The FLU-PRO-derived endpoint is expected to track very closely to return to usual health status (a daily yes/no question in the FLU-PRO questionnaire), which in turn is expected to track very closely to ability to perform 100% of normal activities. We assume, therefore, that the cumulative distributions of time to Symptom Response for the placebo treatment group will closely reflect historical data from Romark Study RM08-3002 for time from first dose until the EV/RV-infected subjects report that they are able to perform 100% of usual activities (see placebo curve in chart below). Then, to calculate sample size, we assume that treatment with NTZ treatment should result in reduction of at least 48 hours in median time to symptom response. The following curves have been developed for determining sample size.



Based upon these curves, a sample size of 530 subjects with laboratory-confirmed EV/RV infection (i.e. 265 for each of the 2 groups) will provide 90% power to detect a statistically significant difference in the survival distributions between the NTZ and placebo groups (2-sided alpha of 0.05). This calculation was performed for the Gehan rank test using SAS PROC POWER (SAS 9.4) with the curves shown above (proportion not recovering by the end of each day was used as input) and censoring at day 21 (hour 504). Assuming that up to 12% of subjects will have incomplete data, we arrive at a sample size of 600 subjects in total, 300 per treatment group to yield at least approximately 530 with complete data (all 600 subjects will be included in the primary analysis with subjects with incomplete data censored as of their last entry).

Based upon these calculations, the study will enroll at least 600 subjects with laboratory-confirmed EV/RV infection up to a maximum of 700 subjects with laboratory-confirmed EV/RV infection. We estimate that this will require enrollment of up to 1,750 subjects in total assuming an EV/RV-positive rate of at least 40%.

8.2. Efficacy Variables

Primary Efficacy Parameter: Time from first dose until Symptom Response

Secondary Efficacy Parameters:

i. Time from first dose until subjects are able to perform 100% of normal activities (i.e., a score of 10 on the scale of 0-10)

ii. Proportions of subjects experiencing one or more complications of colds due to EV/RV infection including pneumonia, otitis media, bronchitis, sinusitis, exacerbations of asthma or COPD, worsening of pre-existing health conditions, secondary infections requiring systemic antibiotic use, hospitalization due to cold or complications of the cold, and death due to cold or complications of the cold

Exploratory Efficacy Parameters:

i. Time to Individual Symptom Response

ii. Time to FLU-PRO Domain Response

iv. Time to Return to Usual Health

v. Change in virus titer (quantitative PCR) from baseline to Day 2, from baseline to Day 3, and from baseline to Day 7

vi. Proportions of subjects with virus detected in nasopharyngeal swabs collected at each of Days 2, 3 and 7 by culture and by RT-PCR

vii. Time to Symptom Response for all subjects treated (ITT population)

viii. Time to Symptom Response for subjects infected with individual non-EV/RV viruses

8.3. Response Definitions

Symptom Response: To be defined based upon a blinded review of symptom scores (pooled for all EV/RV-infected subjects without regard to treatment group assignment) that correlate to the time at which subjects report that they return to usual health (a daily global assessment question). Methodology for the correlation will be defined in the Statistical Analysis Plan. Symptom scores must be maintained at the defined level for at least 2 daily diary periods without any symptom relief medication during those 2 daily diary periods.

Time of Symptom Response: The start of the first daily diary period in which Symptom Response is achieved and is maintained for at least 2 daily

	diary periods without any symptom relief medication during those 2 daily diary periods.
Time to Symptom Response:	Time (hours) from first dose to the Time of Symptom Response.
Ability to Perform All Normal Activities:	Subject reports a score of 10 on the 0-10 scale for ability to perform normal activities, which is maintained for at least 2 daily diary periods without symptom relief medication during those 2 daily diary periods
Time of Ability to Perform All Normal Activities:	The start of the first daily diary period in which the subject reports a score of 10 on the 0-10 scale for ability to perform normal activities, which is maintained for at least 2 daily diary periods without symptom relief medication during those 2 daily diary periods
Time to Ability to Perform All Normal Activities:	Time (hours) from first dose to the Time of Ability to Perform All Normal Activities
Individual Symptom Response:	For each individual symptom (n=32), a score of ≤ the maximum response value specified in the Symptom Response definition maintained for at least 2 daily diary periods without symptom relief medication during those 2 daily diary periods
Time of Individual Symptom Response:	The start of the first daily diary period in which the Individual Symptom Response is achieved and is maintained for at least 2 daily diary periods without symptom relief medication during those 2 daily diary periods
Time to Individual Symptom Response:	Time (hours) from first dose to Time of Individual Symptom Response
FLU-PRO Domain Response:	For each individual domain (n=6), each item scored ≤ the maximum response value specified in the Symptom Response definition maintained for at least 2 daily diary periods without symptom relief medication during those 2 daily diary periods
Time of FLU-PRO Domain Response:	For each domain, the start of the first daily diary period in which the FLU-PRO Domain Response is achieved and is maintained for at least 2 daily diary periods without

	symptom relief medication during those 2 daily diary periods
Time to FLU-PRO Domain Response:	Time (hours) from first dose to FLU-PRO Domain Response for each domain
Return to Usual Health:	"Yes" response to the daily FLU-PRO global assessment question, "Have you returned to your usual health today?" maintained for at least 2 daily diary periods without symptom relief medication during those 2 daily diary periods
Time of Return to Usual Health:	The start of the first daily diary period in which Usual Health is achieved and is maintained for at least 2 daily diary periods without symptom relief medication during those 2 daily diary periods
Time to Return to Usual Health:	Time (hours) from first dose to Time of Return to Usual Health
Complication of Colds:	Complications of colds due to EV/RV infection include pneumonia, otitis media, bronchitis, sinusitis, exacerbations of asthma or COPD, worsening of pre-existing health conditions, secondary infections requiring systemic antibiotic use, hospitalization due to cold or complications of the cold, and death due to cold or complications of the cold. Exacerbations of asthma, exacerbations of COPD, bacterial pneumonia, acute bacterial otitis media and acute bacterial sinusitis will be reported as complications of colds due to EV/RV infection only if each of the following criteria are satisfied: <u>Exacerbation of asthma:</u> 1. Episode requires treatment with systemic corticosteroids, and 2. Episode is considered by the Investigator to be related to viral respiratory infection <u>Exacerbation of COPD:</u> 1. Episode requires a change in regular medication (e.g., addition of antibiotics and/or oral corticosteroids), and 2. Episode is considered by the Investigator to be related to viral respiratory infection <u>Bacterial pneumonia:</u>

1. At least two of the following symptoms: difficulty breathing, cough, production of purulent sputum, chest pain, and
2. At least two of the following vital sign abnormalities: fever, hypotension, tachycardia, tachypnea, and
3. At least one of the following findings: hypoxemia, clinical evidence of pulmonary consolidation, elevated white blood cell count or leukopenia, and
4. Chest radiograph findings of new infiltrates in a lobar or multilobar distribution, and
5. Microbiologic criteria: appropriate sputum specimen with fewer than 10 squamous epithelial cells and more than 25 polymorphonuclear cells per low power field

Acute bacterial otitis media:

1. Symptoms of ear pain or earache, ear fullness or decreased hearing, and
2. One or more of the following otoscopic findings performed by a clinician experienced in otoscopy:
   - Bulging or fullness of the tympanic membrane (convexity of the plane of the eardrum), with loss of anatomic landmarks on visualization,
   - Opacification of the tympanic membrane regardless of color,
   - Erythema of the tympanic membrane, or
   - Abnormal tympanic membrane mobility on biphasic pneumatic otoscopy; a tympanic membrane in the neutral position or retracted is not sufficient evidence of acute bacterial otitis media because these findings are not specific enough to distinguish the disease from otitis media with effusion

Bacterial sinusitis:

1. At least two of the following symptoms:
   - Maxillary tooth pain (unilateral findings can be more specific)
   - Facial pain (unilateral findings can be more specific)
   - Frontal headache

- Purulent nasal discharge (unilateral findings can be more specific)
- New onset fetor oris (bad breath)
- Morning cough
- Nasal obstruction, and

2. At least one of the following signs:
   - Purulent secretions from sinus ostia on examination
   - Abnormal sinus transillumination
   - Pain on palpation over sinuses
   - Facial swelling, and
3. Radiographic findings consistent with acute sinusitis

8.4. Statistical Methodology

The statistical methodology is described briefly below and will be described in detail in the Statistical Analysis Plan.

8.4.1. Efficacy Analyses

Efficacy analyses will be based on a population consisting of all subjects that received at least one dose of study drug and had laboratory-documented EV/RV infection (infected intent-to-treat or ITTI population). Laboratory-documented EV/RV infection is defined by identification of EV/RV in nasopharyngeal secretions by RT-PCR at baseline, Day 2 or Day 3. For time to event analyses a test of significance (as described in the following) will be performed with descriptive statistics provided including the use of Kaplan-Meier figures.

There will be one primary efficacy analysis:

- Time to Symptom Response for the NTZ treatment group will be compared to that of the placebo treatment group using a stratified Gehan-Wilcoxon test (α =0.05) where stratification will follow that used for randomization. Subjects without a Symptom Response being recorded will be treated as censored as of their last diary without a documented Symptom Response.

If the primary analysis is significant at the 0.05 level, a key secondary efficacy analysis will be formally evaluated at the 0.05 level as follows:

- Time to Ability to Perform All Normal Activities for the two treatment groups will be compared using a stratified Gehan-Wilcoxon test (α =0.05) where stratification will follow that used for randomization. Subjects without an Ability to Perform All Normal Activities response being recorded will be treated as censored as of their last diary without a documented Ability to Perform All Normal Activities response.

If both the primary analysis and the key secondary efficacy analysis are significant at the 0.05 level, another secondary efficacy analysis will be formally evaluated at the 0.05 level as follows:

- Proportions of subjects experiencing one or more complications of colds due to EV/RV infection will be compared between the treatment groups using a Cochran-Mantel-Haenszel test stratified by the randomization strata (α =0.05).

Exploratory analyses will be performed as follows:

- For each of the 32 individual FLU-PRO symptoms, Time to Individual Symptom Response for the NTZ and placebo treatment groups will be compared using the Gehan-Wilcoxon test.
- For each of the 6 FLU-PRO domains, Time to FLU-PRO Domain Response for the NTZ and placebo treatment groups will be compared using the Gehan-Wilcoxon test.
- Time to Return to Usual Health for the NTZ and placebo treatment groups will be compared using the Gehan-Wilcoxon test.
- Changes in viral titers from baseline to Day 2, from baseline to Day 3, and from baseline to Day 7 for the NTZ and placebo treatment groups will be compared using a t-test.
- For subjects with laboratory-confirmed EV/RV, the proportions of subjects with EV/RV detected by culture in nasopharyngeal swabs collected at each of Days 2, 3 and 7 will be compared for the two treatment groups using a Fisher's exact test.
- For the ITT population, Time to Symptom Response for the NTZ and placebo treatment groups will be compared using the Gehan-Wilcoxon test.
- For subsets of subjects infected with individual non-EV/RV viruses (e.g., coronavirus), Time to Symptom Response for the NTZ and placebo treatment groups will be compared using the Gehan-Wilcoxon test.

Sensitivity analyses for the primary endpoint will include:

- Repeat the primary efficacy analysis, assigning a Time to Symptom Response of 21 days for subjects that discontinue prior to Day 21 without achieving Symptom Response.
- Repeat the primary efficacy analysis for a "per protocol" subset.
- Repeat the primary efficacy analysis excluding NTZ-treated subjects who reported experiencing chromaturia.

8.4.2. Population Pharmacokinetics Analysis

On Day 3, the plasma samples will be collected before the morning dose (at the trough). These data will allow for analysis of relationships between trough plasma concentrations and clinical and virologic response.

Day 3 trough plasma concentrations of tizoxanide and tizoxanide glucuronide will be summarized descriptively for each of the treatment groups. Exploratory analyses will be conducted to evaluate the relationships between plasma concentrations and age, race, gender, body weight, body mass index, concomitant medications, changes in viral titer over time, time from first dose to Symptom Response and adverse events.

8.4.3. Safety Analyses

All randomized subjects who receive the study medication will be evaluated for drug safety. Safety analyses will be done descriptively.

9. INVESTIGATIONAL PRODUCTS

9.1. Drug Regimens, Administration and Duration

Group 1 (NTZ): Subjects will receive two NTZ 300 mg tablets b.i.d. with food (< 1 hour after food intake) for 5 days.

Group 2 (Placebo): Subjects will receive two placebo tablets b.i.d. with food (< 1 hour after food intake) for 5 days.

The food prior to drug intake should preferably be a high-fat meal, but at minimum a cereal bar.

9.2. Identity of Investigational Products

NTZ 300 mg and placebo tablets were manufactured for Romark Laboratories, L.C. by [redacted] in the United States. The active formulation for this study is a yellow, film-coated tablet that contains 300 mg NTZ plus standard excipients. The placebo tablet will have the same appearance and inactive ingredients as the active tablet.

9.3. Packaging and Labeling

NTZ or placebo tablets will be packaged for each subject in a white HDPE bottle, each containing 20 tablets. The subjects will take two tablets at each dosing time point. The bottles will be stored at room temperature and will bear a label with the following information:

Table 11: Study Medication Label

20 Tablets	Study N° RM08-3005	Treatment N°: XXXX
Lot:	**Principal Investigator:**	

Take 2 Tablets by Mouth with Food Twice Daily
Caution: New Drug-Limited by Federal Law to Investigational Use
STORE AT ROOM TEMPERATURE • DO NOT USE BEYOND END OF STUDY
KEEP OUT OF REACH OF CHILDREN
Study Sponsor: The Romark Institute for Medical Research
[redacted]

9.4. Drug Accountability

Medication will be dispensed at baseline. Medication compliance will be reviewed with each subject during the Day 2-5 Telephone Monitoring. At the Day 7 visit, subjects will be asked to return the bottle in which the medication was dispensed along with any unused medication.

The Investigator or designee is required to maintain adequate records of the disposition of all study drug, including dates, quantity and use by subject. Unused supplies must be returned to the Sponsor.

9.5. Subject Compliance

Subject compliance with the protocol will be checked by the Investigator and recorded in the EDC system at each visit.

Subjects will be considered non-compliant (major protocol violation) if they have missed more than two doses of the study medication during the first three days of the study. Non-compliance will not be cause for discontinuation of subject participation in the study.

9.6. Disallowed Medication

The following medications will not be allowed during the study: topical or systemic decongestants, nasal corticosteroids, and any prescription or non-prescription medications classified as (i) expectorants and cough preparations, (ii) analgesics and antipyretics or (iii) antihistamines (American Hospital Formulary Service (AHFS) classification). As an exception to this rule, subjects will be allowed to use acetaminophen and/or dextromethorphan as necessary for disabling symptoms .

Medications for pre-existing conditions that are not excluded (see exclusion criteria) should be continued as prescribed. The use of such medication will be recorded in the EDC system.

10. ADVERSE EVENTS

The term "adverse event" is defined for purposes of this study as any unwanted physical, psychological or behavioral change experienced by a subject during the course of the study and after taking the first dose of study medication regardless of its severity or relation to the study. Adverse events may include symptoms, signs, unexpected worsening of pre-existing conditions, clinically significant changes in laboratory values, diseases and syndromes, and significant and unexpected failures of pharmacological action of other medications. Symptoms of colds due to EV/RV infection (cough, sore throat, nasal obstruction, fatigue, headache, myalgia, feverishness) and complications of colds will not be reported as adverse events.

Adverse events will be recorded on the appropriate EDC forms throughout the study, and the severity of each adverse event will be graded on a four-point scale: mild, moderate, severe, or life-threatening (See Appendix II). The duration of the adverse event and relationship to the study drug will also be recorded. All adverse events must be followed until their resolution or stabilization even beyond the planned study period.

10.1. Definitions

The following definitions will apply to the reporting of adverse events:

1. Serious Adverse Event: Any adverse experience occurring at any dose that is fatal or life threatening; requires in-patient hospitalization or prolongation of an existing hospitalization; is a persistent significant disability/incapacity; is a congenital anomaly or birth defect; or is an important medical event that may not result in death, be life-threatening, or require hospitalization but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.
2. Unexpected Adverse Event: Any adverse experience that is not identified in nature, severity, or frequency in the Investigator's Brochure for NTZ.
3. Severity of adverse events will be assessed by the Investigator using the Toxicity Grading Scale Tables provided in Appendix II (derived and adapted from "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", US Dept. of HHS, FDA, CDER, September 2007 and the National Institutes of Health, National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0).
4. Causality (relationship to treatment) will be assessed as follows:
    - *Definitely Related:* The adverse event is clearly related to the investigational agent(s) or research intervention: the adverse event has a temporal relationship to the administration of the investigational agent(s) or research intervention, follows a known pattern of response, and no alternative cause is present.
    - *Probably Related:* The adverse event is likely related to the investigational agent(s) or intervention: the adverse event has a temporal relationship to the administration of the investigational agent(s) or research intervention, follows a known or suspected pattern of response, but an alternative cause may be present.
    - *Possibly Related:* There is a reasonable possibility that the event may have been caused by or is linked in a significant way to the research; the adverse event has a temporal relationship to the administration of the investigational agent(s) or research intervention, follows a suspected pattern of response, but an alternative cause is present.
    - *Unrelated (or Not Related):* The adverse event is clearly NOT related to the investigational agent(s) or intervention: the adverse event has no temporal relationship to the administration of the investigational agent(s) or research intervention, follows no known or suspected pattern of response, and an alternative cause is present.

Under double-blind treatment conditions, it should be assumed that all subjects are taking the test drug.

10.2. Clinical Adverse Events

At the time of each return visit, the subject will be questioned regarding the occurrence and nature of any adverse events. All events must be recorded in the subjects' medical records and in the EDC system. Any subject affected will be examined by the Investigator as deemed necessary to ascertain the course of the event and any residual effects.

All moderate and severe adverse events will be reviewed by the Principal Investigator who will determine using his/her best clinical judgment whether they warrant the subject to be discontinued from the study. The Sponsor will be notified immediately if a subject is discontinued from the study. For all adverse events that require the subject to be discontinued from the study, relevant clinical assessments and laboratory tests will be repeated as clinically indicated until final resolution or stabilization of the event(s).

All subjects will be instructed to contact the Investigator, Investigator's assistants, or clinical personnel should the subject have any serious adverse experiences. Serious adverse events (SAEs), including death regardless of the cause, must be reported to the Sponsor immediately (within 24 hours of the initial report).

A serious event requiring immediate notification by telephone is an event that:

- results in death
- is life threatening
- requires inpatient hospitalization or prolongation of an existing hospitalization
- is a persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- an important medical event that may not result in death, be life-threatening, or require hospitalization but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

An overdose is defined as any intentional or unintentional consumption of the drug by any route that exceeds the highest dose stated in the Investigator's Brochure or in an investigational protocol, whichever dose is larger. Overdoses without an associated adverse event should be recorded, but not reported as an adverse event.

10.3. Reporting Requirement

The Principal Investigator is required to notify The Romark Institute for Medical Research (Sponsor) immediately of any unexpected, fatal, or life-threatening experience and all unusual, alarming, or serious reactions to medication regardless of any opinions as to the cause/effect relationship. All SAEs will also be reported to the IRB. Adverse events should be reported to:

The Romark Institute for Medical Research
Medical Affairs
[redacted]

Tel: [redacted]

10.4. Medication Modification/Withdrawal Due to an Adverse Event

No dose adjustment is permitted during the 5-day treatment period. If a severe adverse reaction develops during therapy, the study medication should be discontinued, and the subject should be withdrawn from the study.

10.5. Medication Errors

A medication error is defined as any preventable event related to dosing instructions, product labeling, or packaging that causes or leads to inappropriate medication use or subject harm while the medication is in the control of the investigative site or subject. Medication errors which result in adverse events should be recorded and reported as adverse events. All other medication errors should be reported to the study Sponsor through the Medical Affairs department within 7 days of identification by the site.

11. DISCONTINUATION

11.1. Study Discontinuation

The study may be discontinued under the following circumstances:

1. The Sponsor reserves the right to discontinue the study at any time.
2. Adverse event listings will be produced for safety monitoring at least once every two weeks during recruitment. The data will be tested to determine if there are greater than 5% of the subjects who have had ≥ Grade 3 adverse events considered by the Investigator to be possibly, probably or definitely related to the study drug (defined by the Toxicity Grading Scale Tables provided in Appendix II).

   If greater than 5% of the subjects have at least one Grade 3 or Grade 4 adverse event considered to be possibly, probably or definitely related to the study drug by the study Medical Monitor, then the study must be stopped and the Institutional Review Boards, and FDA's Division of Antiviral Drug Products will be notified. All safety and activity data will be submitted to the FDA in a timely manner.

11.2. Subject Discontinuation

Treatment will be discontinued for individual subjects for the following reasons:

1. An allergic reaction occurs or is suspected.
2. Medical conditions that may require study discontinuation in the Investigator's judgment.
3. Subject desire to discontinue participation.

In the case of an allergic reaction or other medical condition requiring subject discontinuation, appropriate treatment will be instituted by the Investigator.

12. ELECTRONIC DATA COLLECTION (EDC) SYSTEM

An EDC system will be used for this study ([redacted]). Prior to study initiation, site staff and authorized Romark personnel will be trained to use this system.

All electronic source forms are to be filled out completely by the examining site staff and reviewed and signed off on by the Investigator(s). Electronic diaries will be completed by the study subjects and reviewed by study personnel and authorized Sponsor representatives.

13. RETENTION OF RECORDS

Essential Documents (EDs) are documents that individually and collectively permit evaluation of the conduct of a trial and quality of the data produced. They demonstrate the compliance of the Investigator, Sponsor, and monitor with the GCP standards and with all applicable regulatory requirements.

In compliance with the ICH/GCP guidelines, the Investigator/institution will maintain all eCRFs and all EDC source forms and source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). A list of these documents is found in Appendix III: List of Essential Document for the Investigative Site. The Investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents listed in Appendix III must be retained for the duration required by applicable regulatory authorities or until the Sponsor informs the Investigator/institution these documents are no longer needed.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation relating to this study, the Investigator must permit access to such reports.

The Sponsor is responsible for organizing and maintaining the Trial Master File which is a clear documentation of the course of the study.

14. MONITORING THE STUDY

Monitoring will be conducted by the Sponsor and/or a contract research organization (CRO) according to the Sponsor's standard operating procedures. Site visits will be conducted by the Sponsor at regular intervals to conduct inspections.

Any data transcribed into the EDC system will be 100% source verified.

The Investigator will grant representatives of the Sponsor's clinical operations team and quality team, as well as regulatory agencies and ethical committees access to inspect facilities and records (including subject charts) relevant to this study and agrees to assist the monitors in their activities, if requested.

Given the short duration of this study, the large clinical experience with NTZ, and the population being studied, the data from this study will not be monitored by an independent data monitoring committee.

15. INFORMED CONSENT

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The informed consent form (ICF) must be signed before performance of any study-related activity. The informed consent form will be approved by both the Sponsor and by the reviewing IRB. They will be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Before enrollment in the study, the Investigator or an authorized member of the investigational staff must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care he/she will receive. Finally, they will be told that the Investigator will maintain a subject identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access, and agrees to allow his or her study physician to re-contact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the subject.

If the subject is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and should personally date and sign the ICF after the oral consent of the subject is obtained, if permitted by local law.

Assent from any minor (≤ 18 years of age or as local regulations apply) enrolled into the study will be obtained along with documented consent from their parent/legal guardian to allow the minor to participate in the study. The assent and consent forms must be signed prior to the performance of any study related activity.

Signed ICFs must remain in the subject's file(s) and be available for verification by representatives of Romark, the IRB, and FDA/relevant regulatory agencies at any time.

16. ETHICS

The clinical trial will be performed in accordance with the guidelines set by the World Medical Assembly (Declaration of Helsinki, last amendment in Fortaleza, Brazil, October 2013). Prior written approval of the study protocol and of the informed consent form will be obtained from the appropriate local Medical Ethics Review Board.

16.1. Study-Specific Design Considerations

Potential subjects will be fully informed of the risks and requirements of the study and during the study subjects will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential AEs of the study, and provide their consent voluntarily will be enrolled.

16.2. Investigator Responsibilities

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP), and applicable regulatory and country specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and wellbeing of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical study data are credible.

16.3. Institutional Review Board (IRB)

Before the start of the study, the Investigator (or Sponsor where required) will provide the IRB with current and complete copies of the following documents:

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any other written materials to be provided to the subjects)
- Investigator's Brochure (or equivalent information) and addenda
- Sponsor-approved subject recruiting materials
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by IRB)
- Information regarding funding, name of the Sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects
- Any other documents that the IRB requests to fulfill its obligation

This study will be undertaken only after the IRB has given full approval of the final protocol, amendments (if any), the ICF, applicable recruiting materials, and subject compensation programs, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IRB and the documents being approved.

During the study, the Investigator (or Sponsor where required) will send the following documents and updates to the IRB for their review and approval, where appropriate:

- Protocol amendments
- Revision(s) to ICF and any other written materials to be provided to subjects
- If applicable, new or revised subject recruiting materials approved by the Sponsor
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's Brochure addenda or new edition(s)
- Summaries of the status of the study at intervals stipulated in guidelines of the IRB (at least annually)
- Reports of AEs that are serious, unlisted, and associated with the investigational drug
- New information that may adversely affect the safety of the subjects or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects
- Report of deaths of subjects under the Investigator's care
- Notification if a new Investigator is responsible for the study at the site
- Annual IND Update Report, Short Term Study Specific Safety Summary and Line Listings, where applicable
- Any other requirements of the IRB

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable informed consent form and assent form revisions must be submitted promptly to the IRB for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to the study subjects. If a deviation from, or a change to the protocol was implemented to eliminate an immediate hazard to study subjects, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IRB as soon as possible.

The re-approval of the clinical study by the IRB should be documented in writing.

At the end of the study, the Investigator (or Sponsor where required) will notify the IRB about the study completion.

16.4. Privacy of Personal Data

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the safety, quality, and utility of the investigational study drug(s) used in this study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or

alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of study subjects confidential.

The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the Investigator to allow direct access to his or her original medical records for study-related monitoring, audit, IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

17. DATA CONFIDENTIALITY, DISCLOSURE OF DATA, AND PUBLICATION

Data generated for the study should be stored by the Investigator in a limited-access file area and be accessible only to representatives of the study site, Romark, the IRB, and FDA/relevant regulatory agencies. Medical information resulting from a subject's participation in this study may be given to the subject's personal physician or to the appropriate medical personnel responsible for the subject's welfare.

No information that can be related to a specific individual subject may be released or used in any fashion without the signed written consent of that subject. All reports and communications relating to study subjects will identify subjects only by initials and subject identification number. The Investigator will keep complete subject identification for purposes of long-term follow-up, if needed. This information will be treated with strict adherence to professional standards of confidentiality.

Site personnel will enter data relating to each subject's participation in the study into the EDC system provided by the Sponsor. In the EDC system, identification numbers and initials will be used to identify subjects. Subject names will not be used in the CRFs. Management of data from the EDC system and the production of the clinical study report will be the responsibility of the Sponsor. Access to the database will be restricted to employees who have been trained to use the system. Access to the EDC system and study report will be limited to the IRB, FDA or other regulatory agencies and the Sponsor.

Presentation and/or publication of the results of the study is encouraged provided that The Romark Institute for Medical Research is notified in advance of the author's intent and is given the opportunity to review the manuscript or abstract 45 days prior to its submission for presentation at a scientific meeting or for publication in a scientific journal. The Investigators will have complete autonomy regarding the content and wording of any abstracts, presentations, and scientific publications arising from this study, including the decision of whether or not to publish.

18. DATA AND REPORT REQUIREMENTS

Data required by The Romark Institute for Medical Research prior to approval and initiation of the study are as follows:

1. Curriculum vitae of the Principal Investigator and all Co-Investigators.
2. Copy of the IRB-approved Informed Consent and subject information forms.
3. Copy of the IRB approval for the conduct of the study.

Data and materials required by The Romark Institute for Medical Research before the study can be considered complete and terminated are as follows:

1. Pre- and post-treatment history, physical examination and subject evaluations.
2. Pre- as well as interim and post-treatment laboratory findings and all special test results.
3. EDC forms properly completed and signed by the Principal Investigator.
4. Drug Inventory Logs indicating drug dispensed and return of the unused supplies to the Sponsor or destruction by study site.
5. Signed Informed Consent/Assent from each subject.

19. CONTACT INFORMATION

Table 12: Contact Information

Medical Monitors		
Name:	[redacted]	[redacted]
Title:		
Tel.:		
Fax:		
E-mail:		
Sponsor Medical Affairs		
Name:	[redacted]	
Title:		
Tel.:		
Mobile:		
Fax:		
E-mail:		
Sponsor Project Management		
Name:	[redacted]	[redacted]
Title:		
Tel.:		
Mobile:		
Fax:		
E-mail:		
Central Laboratory ([redacted])		
Name:	[redacted]	
Title:		
Tel.:		
Fax:		
E-mail:		
Investigational Product Supplier		
Name:	[redacted]	
Title:		
Tel.:		
Mobile:		
Fax:		

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21. APPENDICES

Appendix I: Study Schedule
Appendix II: Toxicity Grading for Adverse Events
Appendix III: List of Essential Documents for the Investigative Site
Appendix IV: Protocol Revision History
Appendix V: Declaration of Helsinki

21.1. Appendix I: Study Schedule

Table 13: Schedule of Assessments

	Screening (Day 1)	Baseline (Day 1)	Days 2-22	Days 2-5	Days 2, 3	Day 7±1	Day 22+3	Unscheduled Visit
Signed informed consent	X							
Complete medical history	X							
Physical examination/weight/vital signs	X					X[1]	X[1]	X
Demographics/smoking history	X							
Urine pregnancy test	X							
Record oral temperature	X					X	X	X
Record symptoms and time of onset	X							
Evaluate according to inclusion/exclusion criteria	X							
Collect nasopharyngeal swabs		X			X[2]	X[3]	X[4]	X
Blood sample for pharmacokinetics					X[5]			
Blood sample for laboratory safety tests[6]		X				X		X
Urine sample for routine urinalysis[6]		X				X		X
Record concomitant medications		X				X	X	X
Complete baseline symptoms in diary and dispense diary		X						
Randomization/dispense study medication		X						
First dose in office and enter in diary		X						
Instructions re: dosing, concomitant medications, subject diary, birth control, follow-up visits and seeking emergency care		X		X		X		X
Review/record adverse events		X		X	X	X	X	X
Review electronic subject diary entries			X[7]					X[7]
Screen for EV-related complications				X	X			
Review compliance with study medication, collect container with unused medication, complete pill count log form					X	X		X[8]

[1] Day 7 and 22 physical exam is a brief physical exam (vital signs and nursing physical assessment) including symptom directed physician physical examination as required by subject symptoms. Vital signs include blood pressure, pulse, respiratory rate and oral temperature.

[2] Nasopharyngeal swabs on days 2 and 3 will be collected at the subject's home (or clinic or another location as agreed with the subject).

[3] Nasopharyngeal swabs collected on day 7 will only be tested for the presence of virus if a sample from day 1, 2 or 3 was positive for enterovirus/rhinovirus.

[4] Nasopharyngeal swabs collected on day 22 will only be tested for the presence of virus if the sample from day 7 was positive for enterovirus/rhinovirus.

[5] Blood sample collected pre-dose on day 3.

[6] Laboratory safety tests include hemoglobin, hematocrit, complete blood count (total and differential), platelet count, random blood sugar, total cholesterol, HDL, LDL, triglycerides, albumin, AST, ALT, GGT, alkaline phosphatase, bilirubin (total/direct), BUN, creatinine, sodium, potassium, chloride and routine urinalysis (glucose, proteins and blood).

[7] All subjects will maintain an electronic diary until day 22 (+3). Site staff will contact subjects as needed during study to ensure timely completion of electronic diary.

[8] Collection of unused IMP and completion of pill count log form will be performed at unscheduled visit if applicable.

21.2. Appendix II: Toxicity Grading for Adverse Events

[Derived and adapted from "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", US Dept. of HHS, FDA, CDER, September 2007]

Table 14: Table for Clinical Abnormalities: Vital Signs

Vital Signs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C)**	38.0 – 38.4	38.5 – 38.9	39.0 – 40	> 40
(°F)**	100.4 – 101.1	101.2 – 102.0	102.1 – 104	> 104
Tachycardia – beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia – beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) – mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Subject should be at rest for all vital sign measurements.

** Oral temperature; no recent hot or cold beverages or smoking.

*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Table 15: Table for Clinical Abnormalities: Systemic (General)

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/ vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools or <400 gms/24 hours	4-5 stools or 400-800 gms/24 hours	6 or more watery stools or >800 gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Table 16: Table for Clinical Abnormalities: Systemic Illness

Systemic Illness	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

The laboratory values provided in the tables below serve as guidelines and are dependent upon institutional normal parameters.

Table 17: Table for Laboratory Abnormalities: Serum

Serum*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Chloride – mEq/L***	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis

Serum*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN****	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests – ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Liver Function Tests – GGT*** increase by factor	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---

Serum*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Triglycerides – mg/dL***	150 mg/dL – 300 mg/dL	>300 mg/dL – 500 mg/dL	>500 mg/dL - 1000 mg/dL	>1000 mg/dL
HDL – mg/dL***	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
LDL – mg/dL***	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** Derived from the National Institutes of Health, National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. ADL- Activities of Daily Living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. selfcare ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

*** "ULN" is the upper limit of the normal range.

Table 18: Table for Laboratory Abnormalities: Hematology

Hematology*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) – gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) – gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hematocrit - %**	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000

Hematology*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN***	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** Derived from the National Institutes of Health, National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. ADL- Activities of Daily Living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. selfcare ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

*** "ULN" is the upper limit of the normal range.

Table 19: Table for Laboratory Abnormalities: Urine

Urine*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 – 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

21.3. Appendix III: List of Essential Documents for the Investigative Site

Table 20: List of Essential Documents for the Investigative Site

Study No: RM08-3005	
Title of Document	
1.	Investigator's Brochure and Updates
2.	Signed protocol (all versions) and amendments, if any, and sample EDC forms
3.	Information given to trial subject: • Informed consent form (all versions), any other written information, advertisement for subject recruitment (if used)
4.	Financial agreement between the Investigator/institution and the Sponsor for the trial
5.	Signed agreement between involved parties • Financial Disclosure of Investigator/institution and Sponsor • Confidential Disclosure Agreement of Investigator/institution and Sponsor
6.	Dated, documented approval/favorable opinion of IRB of the following: • Protocol and any amendments • EDC system (if applicable) • Informed consent form and any revisions • Any other written information to be provided to the subjects • Advertisement for subject recruitment (if used) • Subject compensation (if any) • Any other documents given approval • Continuing review of the trial
7.	Institutional Review Board composition
8.	Regulatory notice of Principal Investigator and sub-Investigators, FDA Form 1572
9.	Curriculum vitae and/or other relevant documents evidencing qualifications of Investigator and sub-Investigators
10.	Normal values/ranges and updates for medical/laboratory/technical procedures and/or tests included in the protocol
11.	Medical/laboratory technical procedures/tests and updates: • certification or • accreditation or • established quality control and/or external quality assessment or • other validation (where required)
12.	Shipping records for investigational product(s) and trial-related materials
13.	Site initiation monitoring report
14.	Relevant communications other than site visits: • Letters/ emails • Meeting notes • Notes of telephone calls
15.	Signed informed consent forms
16.	Source documents
17.	Signed, dated, and completed EDC forms to include documentation of EDC form corrections
18.	Notification by originating Investigator to Sponsor of serious adverse events and related reports
19.	Notification by Investigator, where applicable, to IRB of unexpected serious adverse drug reactions and of other safety information
20.	Notification by Sponsor to Investigator of safety information

Study No: RM08-3005	
Title of Document	
21.	Interim or annual reports to IRB
22.	Subject Screening log
23.	Subject identification code list
24.	Subject enrollment log
25.	Investigational product accountability records (receipt, storage, dispensing, shipment)
26.	Signature sheet
27.	Record of retained body fluids/tissue samples (if any)
28.	Final report by Investigator/institution to IRB

21.4. Appendix IV: Protocol Revision History

Table 21: Protocol Revision History

Summary of Changes	
Amendment 1	
Purpose:	Administrative update to the protocol as follows: • Correct the IND number • Update EC50 range • Clarified conditions for nucleotide sequencing • Update the name of the clinical virology laboratory for diagnostic testing due to organization name change.
Effective Date:	August 7, 2018
Change 1:	Page 7, *"IND Number"* Changed IND number from "*107,316*" to "*140,445*"
Change 2:	Page 12, Section 12.1, "*NTZ Inhibits Viral Replication and Cytokine Secretion*" Changed EC_{50} range from "*approximately 5μM*" to "*approximately 1 to 5 μM*"
Change 3:	Page 29, Section 5.12, "*Plan for Virology Testing and Monitoring Resistance*" Removed "*reduced susceptibility or*" in footnote 9 to Table 7
Change 4:	Page 30, Section 5.12, "*Plan for Virology Testing and Monitoring Resistance*" Changed name of clinical virology laboratory for diagnostic testing from "*North Shore Long Island Jewish Health System Laboratories*" to "*Northwell Health Laboratories*"
Action:	A revised protocol version 1.1 dated August 7, 2018 was generated

Table 21: Protocol Revision History (Continued)

Summary of Changes	
Amendment 2	
Purpose:	Update the protocol in accordance with FDA comment.
Effective Date:	August 31, 2018
Change 1:	Page 7, "*Number of Subjects*" Corrected estimated number of subjects from "*900-1,200*" to "*800-1,200*"
Change 2:	Page 8, "*Biological Samples*" Corrected number of subjects from "*900-1,200*" to "*800-1,200*"
Change 3:	Page 12, Section 1.2.2, "*Pharmacokinetics of NTZ in humans*" Updated Investigator's Brochure version from "*November 2017*" to "*July 2018*"
Change 4:	Page 14, Section 1.2.3, "*Phase 2 Clinical Trials*", Study RM02-2022 Clarified: "*Notably, the rapid assay did not include a test for EV/RV. Given that EV/RVs are the most common cause of VRIs, it is likely that most of the subjects were infected with EV/RV.*"
Change 5:	Page 15, Section 1.2.3, "*Phase 2 Clinical Trials*", Study RM01-2021 Clarified: "*Notably, the rapid assay did not include a test for EV/RV. Given that EV/RVs are the most common cause of VRIs, it is likely that most of the subjects were infected with EV/RV.*"
Change 6:	Page 15, Section 1.2.4, "*Phase 2b/3 Clinical Trial*" Added summary of Phase 2b/3 clinical trial of NTZ in uncomplicated influenza
Change 7:	Page 20, Section 2.0, "*Study Objectives*" Added exploratory endpoints, "*time to symptom response for all subjects treated, and time to symptom response for subjects with individual non-EV/RV virus infections.*"
Change 8:	Pages 20 and 21, Section 3.0, "*Study Design*" Removed discussion of not limiting symptom relief medication in *Choice of Control Groups* and *Choice of Patient Population*.

Summary of Changes	
Change 9:	Page 21, Section 3.0, "*Study Design*" Updated *Choice of Inclusion Criteria* to clarify that a negative result for influenza by a rapid diagnostic test will be required if the subject has an oral temperature >100°F.
Change 10:	Page 24, Section 4.1, "*Inclusion Criteria*" Updated Inclusion 3 from "*Negative rapid influenza diagnostic test (not required if latest CDC weekly influenza report shows influenza prevalence less than "Regional" for the institution's state).*" To "*Negative rapid influenza diagnostic test (required only if the subject has an oral temperature >100°F in the clinic or if the latest CDC weekly influenza report shows influenza prevalence "Regional" or higher for the institution's state).*"
Change 11:	Page 34, Section 8.1, "*Sample Size Calculations*" Corrected number of subjects per group from "*285*" to "*265*"
Change 12:	Page 35, Section 8.2, "*Efficacy Variables*" Added exploratory analyses: "*vii. Time to Symptom Response for all subjects treated (ITT population) viii. Time to Symptom Response for subjects infected with individual non-EV/RV viruses*"
Change 13:	Page 40, Section 8.4.1, "*Efficacy Analyses*" Added exploratory analyses: • "*For the ITT population, Time to Symptom Response for the NTZ and placebo treatment groups will be compared using the Gehan-Wilcoxon test.* • *For subsets of subjects infected with individual non-EV/RV viruses (e.g., coronavirus), Time to Symptom Response for the NTZ and placebo treatment groups will be compared using the Gehan-Wilcoxon test.*" Added sensitivity analysis: "*Repeat the primary efficacy analysis excluding NTZ-treated subjects who reported experiencing chromaturia.*"
Change 14:	Page 42, Section 9.6, "*Disallowed Medication*" Added section to outline disallowed medication.
Action:	A revised protocol version 1.2 dated August 31, 2018 was generated.

Table 21: Protocol Revision History (Continued)

Summary of Changes	
Amendment 3	
Purpose:	Update the protocol to increase the estimated number of subjects to be enrolled in order to achieve the protocol-specified 600-700 EV/RV-positive subjects (primary efficacy population) and other clerical edits
Effective Date:	November 29, 2018
Change 1:	Page 7, "*Number of Subjects*" Corrected Number of Subjects from "*800-1,200*" to "*up to 1,750*"
Change 2:	Page 18, Section 1.2.5, "*Randomized 2x2 Factorial Trial of NTZ and OST in Treating Uncomplicated Influenza: Study RM08-3002*" Added Tables 7 and 8 that were previously inadvertently omitted
Change 3:	Page 35, Section 8.1, "*Sample Size Calculations*" Corrected number of number of subjects from "*We estimate that this will require enrollment of 800-1,200 subjects in total assuming an EV/RV-positive rate between 50% and 75%.*" to "*We estimate that this will require enrollment of up to 1,750 subjects in total assuming an EV/RV-positive rate of at least 40%.*"
Change 4:	Page 61, Appendix II, "*Toxicity Grading for Adverse Events*" Added toxicity grading ranges for sodium, potassium, chloride, glucose, blood urea nitrogen (BUN), creatinine, calcium, magnesium, phosphorous, CPK, albumin, and total protein in accordance with FDA's "*Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*".
Action:	A revised protocol version 1.3 dated November 29, 2018 was generated.

21.5. Appendix V: Declaration of Helsinki

Special Communication

World Medical Association Declaration of Helsinki
Ethical Principles for Medical Research Involving Human Subjects

World Medical Association

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington, DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

    Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

    Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

    When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

    All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

    The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

    In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

    The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it

may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

    After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

    All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

    Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

    Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

    and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

    Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

ARTICLE INFORMATION

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