STATISTICAL ANALYSIS PLAN

Based on:
Protocol No. RM08-3004
Final Version 1.5

A Phase III, Randomized, Double-Blind, Placebo Controlled Trial to Evaluate the Efficacy and Safety of Nitazoxanide in the Treatment of Uncomplicated Influenza

Study Sponsor:
The Romark Institute for Medical Research
[redacted]

Version: FINAL 1.2

July 20, 2019

1. REVISION HISTORY

Version	Date	Revision Author	Comments
1.0	04JAN2018		Original SAP
1.1	11DEC2018	[redacted]	Update stratification factors and response definitions for "Time of Return to Ability to Perform Normal Activities" and "Time of Return to Usual Health" in accordance with protocol v1.5
1.2	TBD	[redacted]	Update to: 1. Clarify handling of missing concomitant medications end dates and times of dosing (Section 8.4); 2. Add protocol deviation category "GCP" (Sections 9.1.2, 9.2); 3. Remove subject initials and urine pregnancy test result from Demographic Data listing (Section 9.1.2); 4. Clarify definition of "time from symptom onset" to mean the time in hours between the time of first dose of study medication and the patient-reported time of symptom onset (Sections 9.1.4, 10.2, 10.2.2, 10.4.1, 10.4.3, 10.5.1 - 10.5.4); 5. Clarify source of time of first dose data (Sections 10.2, 10.4.1, 10.4.3, 10.5.1 - 10.5.4); 6. Update cumulative distribution parameters for selection of maximum symptom response criteria from 75%-90% to 90%-95% (Section 10.2.1); 7. Update procedure for selection of maximum symptom response criteria where the number of daily diary records to be evaluated exceeds the capacity of the IT system (Section 10.2.1); 8. Clarify maximum symptom response criteria including improvement of example case (Section 10.2.1); 9. Clarify impact of symptom relief medication use on Time to FLU-PRO Domain Response and Resolution of Acute Febrile Illness analyses (Sections 10.5.2, 10.5.3); 10. Specified that the primary and secondary efficacy analyses will be analyzed in a hierarchical fashion (Section 10.4); and 11. Clarified derivation of analysis variables for analysis of Proportions of Subjects with Detectable Virus at Days 2, 3 and 7 (Section 10.5.6).

2. SIGNATURE PAGE

This plan has been reviewed and approved by the following:

Author: [redacted]
The Romark Institute for Medical Research
21 Jul 2019
Date

Statistics Approval: [redacted]
07/22/2019
Date

Sponsor Approval: [redacted]
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Date

3. TECHNICAL SUMMARY REPORT

Name of Sponsor/Company: The Romark Institute for Medical Research	**Individual Study Table Referring to Part of the Dossier:** **Volume:** **Page:**	***For National Authority Use Only***
Name of Finished Product: Nitazoxanide 300 mg Tablets		
Name of Active Ingredient: Nitazoxanide		

Title Of Study:	A Phase III Randomized, Double-Blind. Placebo Controlled Trial to Evaluate the Efficacy and Safety of Nitazoxanide in the Treatment of Uncomplicated Influenza	
Investigators:	Multicenter	
Studied period:	December 2017 – April 2019	**Phase of development:** III

Objectives:

The primary objective of this study is to evaluate the effectiveness of nitazoxanide (NTZ) administered orally 600 mg b.i.d. with food for 5 days in reducing the duration of symptoms of influenza 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.

Other secondary efficacy objectives include evaluating the effect of NTZ compared to placebo on:

a) the proportion of subjects experiencing one or more complications of influenza including pneumonia, otitis media, bronchitis, sinusitis, worsening of pre-existing health conditions, systemic antibiotic use for infections secondary to influenza infection, hospitalization due to complications of influenza, and death; and

b) the duration of symptoms of influenza during 21 days of follow up based upon the four FLU-PRO domains (Body/Systemic, Throat, Chest/Respiratory and Nose) that correspond to the seven symptoms included in the traditional patient-reported outcomes instrument (feverishness, myalgia, headache, fatigue, sore throat, nasal obstruction and cough).

Other important objectives include evaluation of the safety of NTZ by analysis of adverse events and effect on influenza antibody titers.

Number of Subjects:

A sample size of at least 600 (up to a maximum of 700) subjects with laboratory-confirmed influenza is planned for the study. This is estimated to require approximately 800 to 1200 subjects in total.

Study Design:

Multicenter randomized double-blind placebo controlled trial for evaluation of efficacy and safety of NTZ in the treatment of uncomplicated influenza

Population:

Males and females ≥12 years of age with uncomplicated influenza

Study Dose and Administration:	
Group 1 (NTZ):	Two NTZ 300 mg tablets orally twice daily (b.i.d.) with food for 5 days.
Group 2 (Placebo):	Two placebo tablets orally b.i.d. with food for 5 days.

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5. LIST OF ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
b.i.d.	Twice daily
BUN	Blood urea nitrogen
CSR	Clinical study report
EDC	Electronic data capture
FDA	U.S. Food and Drug Administration
GGT	Gamma-glutamyl transferase
HDL	High density lipoprotein
hMPV	Human metapneumovirus
LDL	Low density lipoprotein
LLN	Lower limit of normal
MedDRA	Medical Dictionary for Regulatory Activities
NTZ	Nitazoxanide
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcriptase polymerase chain reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	System organ class
T	Tizoxanide
$TCID_{50}$	Tissue culture 50% infective dose
TEAE	Treatment-emergent adverse event
TG	Tizoxanide glucuronide
ULN	Upper limit of normal

6. INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the planned analyses and data displays to be included in the Clinical Study Report (CSR) for Protocol RM08-3004.

All decisions regarding final analysis as defined in this SAP will be made prior to unblinding of the study data. Further information can be found in the protocol.

The SAP is based on:

- Protocol RM08-3004, Final Version 1.5, December 12, 2018
- International Conference on Harmonisation Guidelines E4 and E9; and
- Discussions with the U.S. Food and Drug Administration (FDA).

The purpose of this document is to describe analysis populations and to detail methodology for deriving variables, handling missing data, and analyzing efficacy and safety data.

The SAP may be revised or amended to reflect the requirements of protocol amendments or regulatory requests, but must be finalized, approved and placed on file before the database is locked. Deviations from the SAP will be noted in the CSR.

7. STUDY DESCRIPTION

7.1 Study Objectives

The primary objective of this study is to evaluate the effect of nitazoxanide (NTZ) administered orally 600 mg b.i.d. with food for 5 days in reducing the duration of symptoms of influenza compared to that of a placebo.

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.

Other secondary efficacy objectives include evaluating the effect of NTZ compared to placebo on:

a) the proportion of subjects experiencing one or more complications of influenza including pneumonia, otitis media, bronchitis, sinusitis, worsening of pre-existing health conditions, systemic antibiotic use for infections secondary to influenza infection, hospitalization due to complications of influenza, and death; and

b) the duration of symptoms of influenza during 21 days of follow up based upon the four FLU-PRO domains (Body/Systemic, Throat, Chest/Respiratory and Nose) that correspond to the seven symptoms included in the traditional patient-reported outcomes instrument (feverishness, myalgia, headache, fatigue, sore throat, nasal obstruction and cough).

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 resolution of febrile illness, time to return to usual health, and changes in viral titers from baseline to each of days 2, 3 and 7.

Other important objectives include evaluation of the safety of NTZ by analysis of adverse events and evaluation of the effect of nitazoxanide on influenza antibody response and evaluation of relationships between pharmacokinetics and clinical or virologic responses.

7.2 Study Design

The study will be a multicenter, randomized, double-blind trial to evaluate the efficacy of NTZ compared to placebo in treating uncomplicated influenza. Subjects will be selected according to the inclusion and exclusion criteria listed in the protocol. The study is designed to enroll at least 600 subjects (up to 700 maximum) with laboratory-confirmed influenza. Total enrollment required to achieve this number is expected to be approximately 800 to 1200 subjects. Prior to screening for inclusion in the study, informed consent will be obtained.

Immediately after completion of informed consent, screening, and enrollment in the study, two baseline nasopharyngeal swabs will be collected for viral culture and RT-PCR. A blood sample will be collected for laboratory safety tests and anti-influenza antibodies, and a urine sample will be collected for laboratory safety tests. Subjects will receive instruction on completing their electronic diaries, recording concomitant medications and attending follow-up visits.

Then 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

The medication will be taken in the morning and evening with food. The first dose will be taken with food in the physician's office under the observation of the Principal Investigator or a member of the Investigator's staff. The second dose will be taken by the patient as close as possible to 12 hours after the first dose.

A nurse or other study personnel will visit each subject (or the subject will return to the clinic) on study days 2 and 3 to collect two nasopharyngeal swabs, to review patient diaries, and to review symptoms and screen for influenza-related complications including sinusitis, otitis, bronchitis, pneumonia and central nervous system disease. Subjects will be referred for immediate care as needed based on the screening. A blood sample for pharmacokinetics will also be collected on study day 3. These day 2 and 3 visits will occur at approximately the same time of day that the subject took his/her first dose of study medication (roughly 24 and 48 hours post-first dose). On day 3, the blood sample for pharmacokinetics will be collected before the dose. The nasopharyngeal swabs will be used to evaluate quantitative changes in viral shedding. The blood samples will be used to evaluate relationships between pharmacokinetics and virologic or clinical response. Study sites or subjects may opt out of the day 2 and day 3 visits due to site staffing, patient 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).

A study physician, nurse or other site personnel will make daily telephone calls to subjects for the first five days of dosing to review symptoms and screen for influenza-related complications including sinusitis, otitis, bronchitis, pneumonia and central nervous system disease. Subjects will be referred for immediate care as needed based on the screening. [Note: In lieu of a telephone call, this information may be obtained during the day 2 or 3 office or home visits (see the preceding paragraph).] The study sites and subjects will be blinded from virology laboratory data until after study day 22 or, if earlier, until the subject is discontinued from the study. During the study period,

each subject will be presumed to have influenza infection, and any worsening of symptoms or complications will be presumed to be influenza-related.

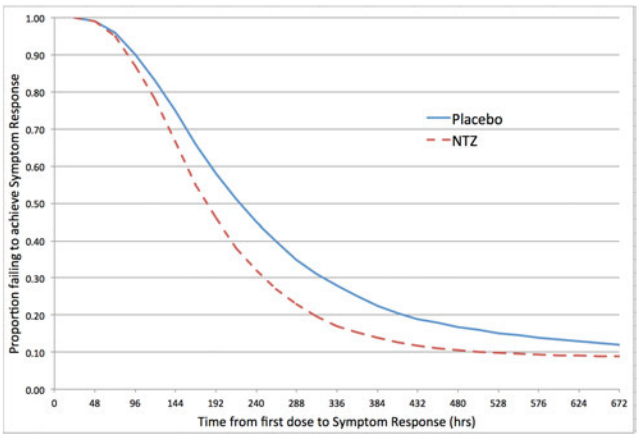
Subjects will return to the clinic on days 7 and 22 for post-treatment follow-up, including two nasopharyngeal swabs, drug accountability, reporting of adverse events and complications of influenza. Blood and urine samples for laboratory safety tests will be collected at day 7, and a blood sample for anti-influenza antibodies will be collected on day 22. A nurse or other study personnel will review the electronic patient diary entries daily (synchronized to a web-based data system) to ensure contemporaneous and complete recording of diary data. Subjects will maintain the electronic patient diaries until study day 22. Any visits that do not occur on the prescribed date, or within 1 day of that date in the case of the day 7 visit or within 3 days after in the case of the day 22 visit will be recorded as protocol deviations.

A central laboratory will be used for laboratory safety tests, anti-influenza antibodies and virology testing. Laboratory safety 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 and routine urinalysis (glucose, protein and blood). Virology testing will include RT-PCR assay to identify influenza A (non-specific as to subtype); influenza A H1, H3 and H1N1 (2009) subtypes; influenza B; respiratory syncytial virus A and B (RSV); parainfluenza 1, 2, 3 and 4; human metapneumovirus (hMPV); adenovirus; human rhinovirus/enterovirus; coronavirus NL63, HKU1, 229E and OC43; human bocavirus; *Chlamydophila pneumoniae*; *Legionella pneumophila*; and *Mycoplasma pneumoniae* (baseline and day 7 nasopharyngeal swabs), culture (baseline and day 7 nasopharyngeal swabs), and quantitation of influenza viruses by $TCID_{50}$ (baseline and days 2, 3 and 7 nasopharyngeal swabs). Follow-up samples (days 2, 3 and 7) collected from subjects who do not test positive for influenza A or influenza B at baseline will not be analyzed for quantitative influenza viral titer.

This study is expected to run from December 2017 through April 2019.

7.3 Sample Size Justification

The Symptom Response definition for this study will be derived from the subjects' FLU-PRO questionnaire responses as described in section 10.2.1 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). Patient-reported outcomes for time to return to usual health status have been shown to track very closely with time to ability to perform 100% of usual activities.[1,2,3] We assume, therefore, that the cumulative distributions of time to symptom response for the placebo treatment group will closely reflect historical data (Romark studies RM08-3002 and RM08-3003 and oseltamivir studies WV15670 and WV15671) for time from first dose until the subjects report that they are able to perform 100% of usual activities. Based upon data from Romark studies RM08-3001, RM08-3002 and RM08-3003, we assume that treatment with NTZ should result in reduction of at least 40 hours in median time to symptom response with differences being less at the first quartile and greater at the third quartile. Based upon this historical data, the following curves have been developed for determining sample size.



Based upon these curves, a sample size of 552 subjects with laboratory-confirmed influenza (i.e. 276 for each of the 2 groups) will provide 90% power to detect a statistically significant difference in the survival distributions between 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 8% of subjects will have incomplete data, we arrive at a sample size of 600 subjects in total, 300 per treatment group.

Based upon these calculations, the study will enroll at least 600 subjects with laboratory-confirmed influenza up to a maximum of 700 subjects with laboratory-confirmed influenza. We estimate that this will require enrollment of 800 to 1,200 subjects in total assuming a flu-positive rate between 50% and 75%.

7.4 Data Collection

Data will be collected using an electronic data capture (EDC) system and electronic subject diaries provided by Clinical Ink, Inc.

Medical history and adverse events will be coded using the latest version of MedDRA. Prior and concomitant medications will be coded using the latest version of WHODrug.

8. DATA DEFINITIONS AND CONVENTIONS

8.1 Analysis Populations

Three populations will be designated for the purpose of these analyses:

Table 1. Definition of Analysis Populations

Analysis Population	Definition
All Treated	All subjects who receive at least one dose of study medication
Confirmed Influenza	The subset of the All Treated population who are positive for influenza by RT-PCR or culture at any of the Baseline, Day 2 or Day 3 visits
Per Protocol	The subset of the Confirmed Influenza population with no major protocol deviations that may have an effect on the integrity of the data or the evaluation of effectiveness

8.2 Data Presentation Conventions

The following conventions are applied to all data presentations and summaries.

Table 2. Data Presentation Conventions

Data	Presentation Convention
Descriptive statistics for continuous variables	Include the number of subjects with available data, mean, standard deviation, median, minimum and maximum values
Descriptive statistics for categorical variables	Include a count of subjects with each response and percentage of subjects with each response out of the total number of subjects per population or treatment group, as applicable
Means and medians for continuous variables	Format to one more decimal place than the measured value
Standard deviations for continuous variables	Format to two more decimal places than the measured value
Minimums and maximums for continuous variables	Format to the same number of decimal places as the measured value
Number and percent for categorical variables	XX (XX.X%)
Date variables	DDMMMYYYY
Time variables	HH:MM (24-hour clock)
P-values	Present five decimal places If less than 0.00001, present as <0.00001 If rounded result is 1.00000, present as >0.99999
All decimal-containing data, where possible	Presentation should be decimal-aligned
Data presented by visit	Summarize by nominal study visit
Randomization groups	Present as "NTZ" and "Placebo" in that order

The expected layout of each table, listing and figure will be approved separately from the SAP. Minor changes to titles or numbering will not necessitate a revision of the SAP nor will they be considered deviations from the planned analyses.

8.3 Handling of Unscheduled Assessments

Results of unscheduled assessments will be included in listings, but will not be used in descriptive statistics.

8.4 Handling of Missing Data

Missing data will be handled as follows:

Table 3. Handling of Missing Data

Data	Handling Convention
Adverse event onset date	If onset date is completely missing, impute with the date of first dose. If year is missing, impute with the year of enrollment. If only year or if year and day are present: • If year = year of first dose, then set month and day to the date of the first dose. • If year < year of first dose, then set month and day to December 31. • If year > year of first dose, then set month and day to January 1. If month and year are present, but day is missing: • If year = year of first dose and ○ If month = month of first dose, then set day to day of first dose. ○ If month < month of first dose, then set day to the last day of the month. ○ If month > month of first dose, then set day to the first day of the month. • If year < year of first dose, then set day to the last day of the month. • If year > year of first dose, then set day to the first day of the month. • For all other cases, set onset date to the date of first dose.
Adverse event end date	If the end date is partially or completely missing, set to the last date the subject was known to be in the study.
Concomitant medications start date	If start date is completely missing, it will not be imputed. If only year or if year and day are present, set the month and day to January 1. If year and month are present and day is missing, set day to the first day of the month.
Concomitant medications end date	If end date is missing, frequency is "1X" and the medication is not listed as "ONGOING", then end date should be set to equal the start date. If the criteria above are not met, the following imputation rules apply: • If end date is completely missing, it will not be imputed. • If only year or if year and day are present, set the month and day to December 31. • If year and month are present and day is missing, set day to the last day of the month.
Concomitant medications time of dosing	If time of dosing is missing for symptom relief medication, it will be imputed as the time of the FLU-PRO Questionnaire completion for that date. If there is no FLU-PRO Questionnaire completed on that date, the time will be imputed as 12:00 p.m.

9. GENERAL CHARACTERISTICS

9.1 General Characteristics Tables and Figures

All tables will present data for the overall study and by treatment group.

9.1.1 Disposition of Subjects

The disposition tables will summarize the count and percentage of subjects who completed or discontinued from the study and the mean (SD), median (IQR), and minimum and maximum number of days in the study.

Analysis Population(s):	All Treated Confirmed Influenza
Derived Variables:	Number of days in study = date of study completion/early termination – date of randomization + 1

9.1.2 Protocol Deviations

The deviations tables will summarize the count and percentage of subjects with protocol deviations by deviation classification (major, minor, or GCP) and the common deviation term.

Analysis Population(s):	All Treated Confirmed Influenza
Derived Variables:	None

9.1.3 Demographics

The demographics tables will summarize sex, age at screening (years), race, height (cm), weight (kg), BMI (kg/m^2), smoking status and vaccination status as collected at screening/baseline using descriptive statistics.

Analysis Population(s):	All Treated Confirmed Influenza
Derived Variables:	• Age in years at screening = date of screening – date of birth • BMI = weight in kg / (height in cm / 100)^2

9.1.4 Disease Characteristics

The disease characteristics tables will summarize oral temperature in-office (°F), oral temperature (°F), time from onset of symptoms to the time of first dose (hours), virus infection(s), respiratory symptoms, and constitutional symptoms as collected at screening/baseline using descriptive statistics.

Analysis Population(s):	All Treated Confirmed Influenza
Derived Variables:	Oral temperature = maximum of oral temperature recorded in the clinic at screening/baseline and self-report by the subject

9.1.5 Prior and Concomitant Medication

The prior and concomitant medication tables will summarize the number and percentage of subjects receiving each medication by Level 4 Anatomical Therapeutic Chemical (ATC) classification and standardized medical name.

Analysis Population(s):	All Treated Confirmed Influenza
Derived Variables:	• Prior medication classification = medications with stop dates prior to screening date • Concomitant medication classification = medications with administration occurring on or after the date of the first administration of study drug until the end of the study

9.1.6 Medical History and Concomitant Diseases

The medical history and concomitant diseases tables will summarize the number and percentage of subjects reporting each medical history events by system organ class.

Analysis Population(s):	All Treated Confirmed Influenza
Derived Variables:	• Medical history classification = events with stop dates prior to screening date • Concomitant disease classification = events with stop dates on or after the date of the first administration of study drug until the end of the study

9.1.7 Treatment Exposure

The treatment exposure tables will summarize the total number of days on therapy and number of tablets taken using descriptive statistics.

Analysis Population(s):	All Treated Confirmed Influenza
Derived Variables:	Number of tablets taken = number of doses reported to have been taken on the diary * 2

9.2 General Characteristics Listings

General characteristics listings will include data specified in Table 4 for all subjects presented by subject number and treatment group.

Table 4. Data Presented in General Listings

Listing	Data Presented
Discontinued Subjects	Date of discontinuation Reason for discontinuation
Subjects with Protocol Deviations	Date of deviation Common deviation term Treatment stopped (Y/N) Subject discontinued (Y/N)

Listing	Data Presented
	Classification (Major/Minor/GCP)
Subjects Excluded from Efficacy Analysis[1]	Reason for exclusion
Demographic Data	Date/time of informed consent Date of birth Age Sex Race Height (cm) Weight (kg) BMI (kg/m^2) Smoking status Number of years smoked Number of cigarettes smoked per day Vaccination status (Y/N)
Baseline Disease Characteristics	Oral temperature in office (°F) Highest oral temperature (°F) Time from onset of symptoms to first study drug intake (hours) Virus infection(s) Respiratory symptoms Constitutional symptoms
Prior Medication Concomitant Medication	Reported medication term Standard medical name Level 4 ATC term Indication Start date Stop date Dose Dose Unit Frequency Route
Medical History Concomitant Diseases	Reported disease, condition or surgery term Standard medical name System organ class Year of diagnosis Active at screening (Y/N) Medication therapy (Y/N)
Treatment Exposure Data	Total number of days on therapy Number of tablets taken Number of tablets dispensed Number of tablets returned Number of tablets missed

[1] Subjects who are not included in the Confirmed Influenza population due to negative RT-PCR and culture assays will not be considered excluded from efficacy analysis

10. EFFICACY

10.1 General

Unless otherwise specified, all statistical tests will use two-sided tests at the α=0.05 level.

The following definitions will apply to multiple efficacy analyses:

Daily Diary Period	Each daily diary period will begin 24 hours prior to the time of diary assessment and end at the time of diary assessment.
Symptom Relief Medication	Any medication taken for a symptom of influenza as indicated by the subject during the study period.

10.2 Primary Efficacy Analysis: Time to Symptom Response

The primary efficacy analysis will be the comparison of the time from first dose of study medication to symptom response between treatment groups using Kaplan-Meier survival curves and the Gehan-Wilcoxon test stratified by (1) time from onset of illness to time of first dose of study medication (0-24, >24-≤36, or >36 hours from onset), and (2) vaccination status (Y/N).

Definitions:

Symptom Response	The set of criteria to be defined based upon a blinded review of symptom scores (pooled for all influenza-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 to be used for the derivation of the Symptom Response definition is described in section 10.2.1 below. Symptom scores must be maintained at the defined level for at least 2 daily diary periods without any symptom relief medication during or between 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 two daily diary periods without any symptom relief medication during or between those two daily diary periods.
Time to Symptom Response	Time (hours) from the first dose of study medication to the Time of Symptom Response.

Variables Used for the Analysis
The primary efficacy analysis will require the following variables:

Variable	Source	Format/Notes
Date and time of first medication intake	CRF	
Date and time of completion for each symptom assessment record	Subject diary	
Symptom score assessment for each symptom for each diary record	Subject diary	The ordinal symptom severity, intensity or frequency score assessment for each of the 32 symptoms will be coded to numerical values 0-4.

Variable	Source	Format/Notes
		It will not be possible for any individual symptom assessment to be missing within a diary record, therefore no individual item imputation will be required. In the event that the diary was not completed at a specified time point (e.g., Day 2), the missing data will not be imputed.
Date and time of symptom relief medication intake	Sponsor-provided listing	The list of symptom relief medications will be selected from the listing of all concomitant medications prior to unblinding.

10.2.1 Derivation of the Symptom Response Definition

The Symptom Response definition will be derived based upon analysis of blinded daily FLU-PRO symptom scores and responses to the daily FLU-PRO question, "Have you returned to your usual health today?" for subjects with laboratory-confirmed influenza.

The Symptom Response definition will consist of a maximum response criterion (0, ≤1, ≤2, ≤3 or ≤4) for each of the 32 FLU-PRO items.

The objective of this procedure is to identify the Symptom Response definition that minimizes the diary-level misclassification rate between Symptom Response and Usual Health status as reported by the influenza-positive subjects enrolled in this study.

Procedure:

1. Evaluate whether each daily diary record has achieved return to usual health (requiring 2 consecutive daily diary assessments with "yes" response to the "usual health" question in FLU-PRO without use of symptom relief medication during or between those daily diary periods). This step will create a "Returned" variable with possible values of "Y" for each daily diary record that constitutes return to usual health and "N" for each daily diary record that does not.

2. Determine the set of possible Symptom Response definitions to be evaluated by selecting potential maximum response criteria that are likely to correlate well with return to usual health.

    a. Examine the cumulative distribution of ordinal symptom assessment responses for the two consecutive diary records at which subjects return to Usual Health.

    b. Maximum response criteria will be selected for consideration as part of the Symptom Response definition if they are included in the ≥90% - ≤95% cumulative distribution of symptom assessments at the time of return to usual health. If a maximum response criterion includes the 95th percentile and exceeds the 95% cumulative distribution, the criterion will still be selected for consideration.

For example, consider the following cumulative distribution of symptom ratings for the two consecutive diary records at which a fictional population of subjects return to usual health:

		Records Reporting 0	Records Reporting ≤1	Records Reporting ≤2	Records Reporting ≤3	Records Reporting ≤4
Coughing	**N**	300	455	478	485	500
	%	60.0%	91.0%	95.6%	97%	100%
Felt cold	**N**	480	499	500	500	500
	%	96.0%	99.8%	100%	100%	100%
(table would include all 32 FLU-PRO symptoms for maximum response criteria selection purposes)						

For "coughing", the maximum symptom response criteria of ≤1 and ≤2 would be selected because the 90th percentile of responses is included in ≤1 and the 95th percentile is included in ≤2. For "felt cold", the maximum symptom response criterion of 0 would be selected because both the 90th and 95th percentiles are included in 0.

Possible Symptom Response definitions will be determined from the set of maximum response criteria selected.

3. For each possible Symptom Response definition, evaluate whether each daily diary record has achieved Symptom Response (two consecutive diary entries with all symptoms assessed ≤ the maximum response values with no symptom relief medication intake during or between those two daily diary periods). This step will create a "Responded" variable with possible values of "Y" for each daily diary record that has achieved Symptom Response and "N" for each daily diary record that has not achieved Symptom Response. This step will be performed on a data storage/VM infrastructure that can support evaluation of 4.2 billion daily diary records with different possible Symptom Response definitions. If the number of diary record evaluations required (number of possible Symptom Response definitions multiplied by number of daily diary records to be evaluated) exceeds 4.2 billion, go back to step 2b above, and increase the ≥90% cumulative distribution threshold by 0.1% increments until the number of possible maximum response criteria is reduced to a number that will result in evaluation of ≤4.2 billion daily diary records.

4. For each possible Symptom Response definition, determine the definition's misclassification rate by comparing the "Responded" and "Returned" variables for each daily diary record. The percentage of daily diary records with mismatched "Responded" and "Returned" values (e.g., "Y" and "N") will constitute that Symptom Response definition's misclassification rate.

5. The Symptom Response definition returning the lowest misclassification rate will be selected as the Symptom Response definition for the study and will be used to calculate the primary efficacy parameter (time to Symptom Response) for each subject.

6. If two or more Symptom Response definitions produce the lowest misclassification rate (a "tie"), the Symptom Response definition with the lower sum of scores will be selected. In the event that the sum of scores are also equal, the Symptom Response definition with the smallest absolute difference between the proportion of false positives ("Responded" but not "Returned") and false negatives ("Returned" but not "Responded") will be selected. If there is still a tie, the definition with the lowest sum of maximum response values for the Body/Systemic domain will be selected, and if that is still a tie, the definition with the lowest sum of maximum response values for the Eye domain will be selected, and so on for the Gastrointestinal, Throat, Nose and Chest/Respiratory domains. If there is still a tie, list the items under the Body/Systemic, Eye,

Gastrointestinal, Throat, Nose and Chest/Respiratory domains in alphabetical order and compare the maximum response values until a definition with the lowest item score can be selected.

The Symptom Response Definition selected in this step will be applied for any subsequent analysis that calls for the primary efficacy analysis to be repeated unless otherwise specified.

10.2.2 Analysis of the Primary Efficacy Parameter

The primary efficacy parameter, time to Symptom Response, will be derived as follows:

Event: Time of Symptom Response – time of the first dose
Censored: Time of last symptom assessment – time of the first dose

If Symptom Response is achieved at the time of the first symptom assessment diary record, the subject will be excluded from the analysis.

If Symptom Response is not achieved prior to a subject's last available diary assessment, but the criteria are met at the last available diary assessment with no evidence of symptom relief medication intake during that diary period, time of Symptom Response will be considered to have occurred at the start of the last daily diary period.

Time to Symptom Response for the NTZ treatment group will be compared to that of the placebo treatment group using a Gehan-Wilcoxon test stratified for (1) time from onset of illness to time of first dose of study medication (0-24, >24-≤36, or >36 hours from onset), and (2) vaccination status (Y/N). The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	Event: Time of symptom response – time of the first dose Censored: Time of last symptom assessment – time of the first dose

10.3 Sensitivity Analyses of the Primary Efficacy Analysis

10.3.1 Sensitivity Analysis Assuming Time of Symptom Response of 21 Days for Discontinued Subjects

Repeat the primary efficacy analysis assuming a time of Symptom Response of 21 days (504 hours from first dose of study medication) for subjects who discontinued the study prior to achieving Symptom Response.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	See Section 10.2: Primary Efficacy Analysis.

10.3.2 Sensitivity Analysis for a "Per Protocol" Subset

Repeat the primary efficacy analysis for the Per Protocol population.

Analysis Population(s):	Per Protocol
Derived Variables:	See Section 10.2: Primary Efficacy Analysis.

10.4 Secondary Efficacy Analyses

If the primary analysis is significant at the 0.05 level, the secondary efficacy analysis of Time to Ability to Perform All Normal Activities will be formally evaluated at the 0.05 level. If the primary analysis and the secondary efficacy analysis of Time to Return to Normal Activities are both significant at the 0.05 level, the secondary efficacy analysis of Proportions of Subjects Experiencing Complications of Influenza will be formally evaluated at the 0.05 level. If any of these analyses is not significant at the 0.05 level, the subsequent analyses or analysis will be performed as exploratory.

10.4.1 Time to Ability to Perform All Normal Activities

Definitions:

Ability to Perform All Normal Activities	Subject reports a score of 10 on the 0-10 scale for ability to perform normal activities and maintains the score for at least two consecutive daily diary periods without symptom relief medication during or between those two daily diary periods
Time of Ability to Perform All Normal Activities	The time of the first diary entry in which the subject achieves Ability to Perform All Normal Activities
Time to Ability to Perform All Normal Activities	Time (hours) from first dose to the Time of Ability to Perform All Normal Activities

Variables Used for the Analysis
The analysis will require the following variables:

Variable	Source	Format/Notes
Date and time of first medication intake	CRF	
Date and time of completion for each ability assessment record	Subject diary	
Ability to perform normal activities score	Subject diary	The ability to perform normal activities score will be recorded by the subject on a 0-10 scale. In the event that the score was not recorded at a specified time point (e.g., Day 2), the missing data will not be imputed.
Date and time of symptom relief medication intake	Sponsor-provided listing	The list of symptom relief medications will be selected from the listing of all concomitant medications prior to unblinding.

Analysis
The efficacy parameter, Time to Ability to Perform All Normal Activities, will be derived as follows:

Event: Time of Ability to Perform All Normal Activities – time of the first dose
Censored: Time of last ability assessment – time of the first dose

If Ability to Perform All Normal Activities is achieved at the time of the first ability assessment diary record, the subject will be excluded from the analysis.

If the subject does not achieve Ability to Perform All Normal Activities prior to the subject's last available diary assessment, but records a "10" for ability to perform all normal activities at the last available diary assessment with no evidence of symptom relief medication intake during that diary period, time of Ability to Perform All Normal Activities will be considered to have occurred at the time of the last available diary assessment.

Time to Ability to Perform All Normal Activities for the NTZ treatment group will be compared to that of the placebo treatment group using a Gehan-Wilcoxon test stratified for (1) time from onset of illness to time of first dose of study medication (0-24, >24-≤36, or >36 hours from onset),, and (2) vaccination status (Y/N). The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	Event: Time of Ability to Perform All Normal Activities – time of the first dose Censored: Time of last ability assessment – time of the first dose

10.4.2 Proportion of Subjects Experiencing Complications of Influenza

Variables Used for the Analysis
The analysis will require the following variables:

Variable	Source	Format/Notes
Complications of influenza	CRF	Investigators will designate an adverse event as a complication of influenza if the event meets protocol-specified criteria

Analysis
Proportions of subjects experiencing one or more complications of influenza will be compared between the treatment groups using a Fisher's exact test.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	None

10.4.3 Time to Modified Symptom Response Excluding FLU-PRO Gastrointestinal and Eye Domains

This analysis will repeat the methodology for deriving and analyzing the Time to Symptom Response, but will exclude from consideration those FLU-PRO items in the Eye and Gastrointestinal domains. Excluded items will be: teary or watery eyes, sore or painful eyes, eyes sensitive to light, felt nauseous (feeling like you wanted to throw-up), stomach ache, vomit (frequency), and diarrhea (frequency).

Definitions:

Modified Symptom Response — The set of criteria to be defined based upon a blinded review of symptom scores from only the Nose, Throat, Chest/Respiratory, and Body/Systemic domains (pooled for all influenza-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 to be used for the derivation of the Modified Symptom Response definition is described in detail below. Symptom scores must be maintained at the defined level for at least 2 daily diary periods without any symptom relief medication during or between those 2 daily diary periods.

Time of Modified Symptom Response — The start of the first daily diary period in which Modified Symptom Response is achieved and is maintained for at least two daily diary periods without any symptom relief medication during or between those two daily diary periods.

Time to Modified Symptom Response — Time (hours) from the first dose of study medication to the Time of Modified Symptom Response.

Variables Used for the Analysis
The analysis will require the following variables:

Variable	Source	Format/Notes
Date and time of first medication intake	CRF	
Date and time of completion for each symptom assessment record	Subject diary	
Symptom score assessment for each symptom for each diary record except those belonging to the Eye and Gastrointestinal domains	Subject diary	The ordinal symptom severity, intensity or frequency score assessment for each of the 25 symptoms will be coded to numerical values 0-4. The numerical values represent ordinal responses and therefore cannot be summed, averaged, or treated as continuous data. It will not be possible for any individual symptom assessment to be missing within a diary record, therefore no individual item imputation will be required. In the event that the diary was not completed at a specified time point (e.g., Day 2), the missing data will not be imputed.
Date and time of symptom relief medication intake	Sponsor-provided listing	The list of symptom relief medications will be selected from the listing of all concomitant medications prior to unblinding.

Derivation of the Modified Symptom Response Definition

Derivation of the Modified Symptom Response definition will be accomplished using the procedure in section 10.2.1 applied to only the 25 FLU-PRO items in the Nose, Throat, Chest/Respiratory, and Body/Systemic Domains.

Analysis
The efficacy parameter, time to Modified Symptom Response, will be derived as follows:

Event: Time of Modified Symptom Response – time of the first dose
Censored: Time of last symptom assessment – time of the first dose

If Modified Symptom Response is achieved at the time of the first symptom assessment diary record, the subject will be excluded from the analysis.

If the Modified Symptom Response is not achieved prior to a subject's last diary available assessment, but the criteria are met at the last available diary assessment with no evidence of symptom relief medication intake during that diary period, Time of Modified Symptom Response will be considered to have occurred at the start of the last daily diary period.

Time to Modified Symptom Response for the NTZ treatment group will be compared to that of the placebo treatment group using a Gehan-Wilcoxon test stratified for (1) time from onset of illness to time of first dose of study medication (0-24, >24-≤36, or >36 hours from onset), and (2) vaccination status (Y/N). The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	Event: Time of Modified Symptom Response – time of the first dose Censored: Time of last symptom assessment – time of the first dose

10.5 Exploratory Efficacy Analyses

10.5.1 Time to Individual Symptom Response

Definitions:

Individual Symptom Response — For each individual symptom (n=32), a score of ≤ the maximum response value specified by the Symptom Response definition maintained for at least two consecutive daily diary periods without symptom relief medication during or between those daily diary periods.

Time of Individual Symptom Response — The start of the first daily diary period in which Individual Symptom Response is achieved and is maintained for at least two daily diary periods without any symptom relief medication during or between those two daily diary periods.

Time to Individual Symptom

Response — Time (hours) from the first dose of study medication to the Time of Individual Symptom Response.

Variables Used for the Analysis
The analysis will require the following variables:

Variable	Source	Format/Notes
Date and time of first medication intake	CRF	
Date and time of completion for each symptom assessment record	Subject diary	
Symptom score assessment for each symptom for each diary record	Subject diary	The ordinal symptom score assessment for each of the 32 symptoms will be coded to numerical values 0-4. In the event that the diary was not completed at a specified time point (e.g., Day 2), the missing data will not be imputed.
Date and time of symptom relief medication intake	Sponsor-provided listing	The list of symptom relief medications will be selected from the listing of all concomitant medications prior to unblinding. Subjects will report the reason for taking each symptom relief medication, and this data will be used to assign concomitant medications to relevant flu symptoms reported in the FLU-PRO questionnaire.

Analysis
The efficacy parameter, time to Individual Symptom Response, will be derived as follows:

Event: Time of Individual Symptom Response – time of the first dose
Censored: Time of last symptom assessment – time of the first dose

If Individual Symptom Response is achieved at the time of the first symptom assessment diary record, the subject will be excluded from the analysis.

If the Individual Symptom Response is not achieved prior to a subject's last available diary assessment, but the criteria are met at the last available diary assessment with no evidence of symptom relief medication intake during that diary period, Time of Individual Symptom Response will be considered to have occurred at the start of the last daily diary period.

Time to Individual Symptom Response for the NTZ treatment group will be compared to that of the placebo treatment group using a Gehan-Wilcoxon test stratified for (1) time from onset of illness to time of first dose of study medication (0-24, >24-≤36, or >36 hours from onset),, and (2) vaccination status (Y/N). The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s):	Confirmed Influenza

Derived Variables:	Event:	Time of Individual Symptom Response – time of the first dose
	Censored:	Time of last symptom assessment – time of the first dose

10.5.2 Time to FLU-PRO Domain Response

Definitions:

FLU-PRO Domain Response — For each FLU-PRO domain (n=6), each item/symptom scored ≤ the maximum response value specified by the Symptom Response definition and maintained for at least two consecutive daily diary periods without symptom relief medication during or between those daily diary periods

Time of FLU-PRO Domain Response — The start of the first daily diary period in which FLU-PRO Domain Response is achieved and is maintained for at least two daily diary periods without any symptom relief medication during or between those two daily diary periods.

Time to FLU-PRO Domain Response — Time (hours) from the first dose of study medication to the Time of FLU-PRO Domain Response.

Variables Used for the Analysis
The analysis will require the following variables:

Variable	**Source**	**Format/Notes**
Date and time of first medication intake	CRF	
Date and time of completion for each symptom assessment record	Subject diary	
Symptom score assessment for each symptom for each diary record	Subject diary	The ordinal symptom severity, intensity or frequency score assessment for each of the 32 symptoms will be coded to numerical values 0-4. In the event that the diary was not completed at a specified time point (e.g., Day 2), the missing data will not be imputed.
Date and time of symptom relief medication intake	Sponsor-provided listing	The list of symptom relief medications will be selected from the listing of all concomitant medications prior to unblinding. Subjects will report the reason for taking each symptom relief medication, and this data will be used to assign concomitant medications to relevant domains captured in the FLU-PRO questionnaire.

Analysis
The efficacy parameter, Time to FLU-PRO Domain Response, will be derived as follows:

Event: Time of FLU-PRO Domain Response – time of the first dose
Censored: Time of last symptom assessment – time of the first dose

If FLU-PRO Domain Response is achieved at the time of the first symptom assessment diary record, the subject will be excluded from the analysis.

If the FLU-PRO Domain Response is not achieved prior to a subject's last available diary assessment, but the criteria are met at the last available diary assessment with no evidence of symptom relief medication intake during that diary period, Time of FLU-PRO Domain Response will be considered to have occurred at the start of the last daily diary period.

Time to FLU-PRO Domain Response for the NTZ treatment group will be compared to that of the placebo treatment group using a Gehan-Wilcoxon test stratified for (1) time from onset of illness to time of first dose of study medication (0-24, >24-≤36, or >36 hours from onset), and (2) vaccination status (Y/N). The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	Event: Time of FLU-PRO Domain Response – time of the first dose Censored: Time of last symptom assessment – time of the first dose

10.5.3 Time to Resolution of Acute Febrile Illness

Definitions:

Resolution of Acute Febrile Illness	Symptom scores for each of "body aches or pains", "chills or shivering", "felt cold", "felt hot" and "sweating" are 0 or 1 and remain so for two consecutive daily diary periods with no record of oral temperature ≥100.4°F (<38°C) or symptom relief medication use for these symptoms during or between those two daily diary periods.
Time of Resolution of Acute Febrile Illness	The start of the first daily diary period in which Resolution of Acute Febrile Illness is achieved and is maintained for at least two daily diary periods without any symptom relief medication during or between those two daily diary periods.
Time to Resolution of Acute Febrile Illness	Time (hours) from the first dose of study medication to the Time of Resolution of Acute Febrile Illness.

Variables Used for the Analysis
The analysis will require the following variables:

Variable	Source	Format/Notes
Date and time of first medication intake	CRF	
Date and time of completion for each symptom assessment and temperature assessment record	Subject diary	
Oral temperature assessment for each temperature assessment record	Subject diary	Oral temperature will be recorded twice daily during the treatment period. This data is recorded contemporaneously with measurement in contrast to symptom diary data, which is intended to characterize maximum symptom intensity or frequency over a 24-hour recall period.
Symptom score assessment for each symptom for each diary record	Subject diary	The ordinal symptom severity, intensity or frequency score assessment for each of the five relevant symptoms will be coded to numerical values 0-4. In the event that the diary was not completed at a specified time point (e.g., Day 2), the missing data will not be imputed.
Date and time of symptom relief medication intake	Sponsor-provided listing	The list of symptom relief medications will be selected from the listing of all concomitant medications diaries prior to unblinding. Subjects will report the reason for taking each symptom relief medication, and this data will be used to assign concomitant medications to relevant symptoms captured in the FLU-PRO questionnaire.

Analysis

The efficacy parameter, Time to Resolution of Acute Febrile Illness, will be derived as follows:

Event: Time of Resolution of Acute Febrile Illness – time of the first dose
Censored: Time of last symptom assessment – time of the first dose

If there is a temperature assessment between diary assessments that is not <100.4°F (<38°C), the earliest a subject may achieve Resolution of Acute Febrile Illness is the start of the next daily diary period.

If the Resolution of Acute Febrile Illness is not achieved prior to a subject's last available diary assessment, but the criteria are met at the last available diary assessment with no evidence of symptom relief medication intake during that diary period, Time of Resolution of Acute Febrile Illness will be considered to have occurred at the start of the last daily diary period.

Time to Resolution of Acute Febrile Illness for the NTZ treatment group will be compared to that of the placebo treatment group using a Gehan-Wilcoxon test stratified for (1) time from onset of

illness to time of first dose of study medication (0-24, >24-≤36, or >36 hours from onset), and (2) vaccination status (Y/N). The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s):	Confirmed Influenza	
Derived Variables:	Event:	Time of Resolution of Acute Febrile Illness – time of the first dose
	Censored:	Time of last symptom assessment – time of the first dose

10.5.4 Time to Return to Usual Health

Definitions:

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 two consecutive daily diary periods without symptom relief medication during or between those two daily diary periods

Time of Return to Usual Health The time of the first diary entry in which the subject achieves Return to Usual Health

Time to Return to Usual Health Time (hours) from first dose to the Time of Return to Usual Health

Variables Used for the Analysis
The analysis will require the following variables:

Variable	**Source**	**Format/Notes**
Date and time of first medication intake	CRF	
Date and time of completion for each symptom assessment record	Subject diary	
Return to usual health global assessment question response	Subject diary	The return to usual health question will have possible response values of "Yes" or "No". In the event that the response was not recorded at a specified time point (e.g., Day 2), the missing data will not be imputed.
Date and time of symptom relief medication intake	Sponsor-provided listing	The list of symptom relief medications will be selected from the listing of all concomitant medications prior to unblinding.

Analysis
The efficacy parameter, Time to Return to Usual Health, will be derived as follows:

Event: Time of Return to Usual Health – time of the first dose
Censored: Time of last ability assessment – time of the first dose

If Return to Usual Health is achieved at the time of the first symptom assessment diary record, the subject will be excluded from the analysis.

If the subject does not achieve Return to Usual Health prior to the subject's last available diary assessment, but records a "Yes" for the return to usual health global assessment question at the last available diary assessment with no evidence of symptom relief medication intake during that diary period, time of Return to Usual Health will be considered to have occurred at the time of the last available diary assessment.

Time to Return to Usual Health for the NTZ treatment group will be compared to that of the placebo treatment group using a Gehan-Wilcoxon test stratified for (1) onset of illness to time of first dose of study medication (0-24, >24-≤36, or >36 hours from onset), and (2) whether the vaccination status (Y/N). The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	Event: Time of Ability to Perform All Normal Activities – time of the first dose Censored: Time of last ability assessment – time of the first dose

10.5.5 Change in Influenza Virus Titer ($TCID_{50}$) from Baseline to Day 2 and from Baseline to Day 3

Variables Used for the Analysis
The analysis will require the following variables:

Variable	**Source**	**Format/Notes**
$TCID_{50}$ virus titer	Laboratory data	$TCID_{50}$ values will be reported as "10^-3.2", for example. For analysis purposes, the values should be negative log-transformed. For the example above, the analysis value would be "3.2".

Analysis
Mean changes in influenza viral titers from baseline to day 2 and from baseline to day 3 for the NTZ and the placebo treatment groups will be compared using a t-test.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	Mean change in influenza viral titer from baseline to day 2: mean of (day 2 $TCID_{50}$ value – baseline $TCID_{50}$ value) for each subject within the treatment groups Mean change in influenza viral titer from baseline to day 3: mean of (day 3 $TCID_{50}$ value – baseline $TCID_{50}$ value) for each subject within the treatment groups

10.5.6 Proportions of Subjects with Detectable Virus at Days 2, 3 and 7

Variables Used for the Analysis
The analysis will require the following variables:

Variable	Source	Format/Notes
$TCID_{50}$ virus titer	Laboratory data	$TCID_{50}$ values will be reported as "10^-3.2", for example. For analysis purposes, any numerical result will be treated as "positive". A result of "No Virus Isolated" will be treated as "negative".

Analysis
The proportion of subjects with a positive result by $TCID_{50}$ at each of days 2, 3, and 7 will be compared across treatment groups using a Fisher's exact test.

Analysis Population(s):	Confirmed Influenza
Derived Variables:	Proportion of subjects with detectable virus at day 2: Number of subjects with "positive" result by $TCID_{50}$ at day 2 divided by the total number of confirmed influenza subjects with a day 2 $TCID_{50}$ result for each treatment group Proportion of subjects with detectable virus at day 3: Number of subjects with "positive" result by $TCID_{50}$ at day 3 divided by the total number of confirmed influenza subjects with a day 3 $TCID_{50}$ result for each treatment group Proportion of subjects with detectable virus at day 7: Number of subjects with "positive" result by $TCID_{50}$ at day 7 divided by the total number of confirmed influenza subjects for each treatment group (subjects with no $TCID_{50}$ result at day 7 will be assumed to have a result of "negative" because day 7 samples were not tested by $TCID_{50}$ if traditional viral culture was negative for the sample)

10.6 Other Exploratory Analyses

10.6.1 Analysis of Influenza Antibody Titers

Variables Used for the Analysis
The analysis will require the following variables:

Variable	Source	Format/Notes
Influenza antibody titer values	Laboratory data	Influenza antibody titer values will be reported as "1/20", for example. The denominator of the fraction will be used for analysis purposes.

Analysis
Changes in influenza antibody titers will be analyzed by comparison of means as well as proportions of subjects seroprotected (day 22 antibody titer ≥40) and seroconverted (day 22 antibody titer >4x baseline titer) at day 22. Means will be compared across treatment groups using a t-test and proportions will be compared across treatment groups using a Fisher's exact test.

Analysis Population(s):	Confirmed Influenza

Derived Variables:	Mean change in influenza antibody titers from baseline to day 22: Mean of (day 22 influenza antibody titer – baseline influenza antibody titer) for each subject across treatment groups Proportion of subjects seroprotected at day 22: Number of subjects with day 22 antibody titer ≥40 divided by the total number of influenza-positive subjects with both baseline and day 22 data for each treatment group Proportion of subjects seroconverted at day 22: Number of subjects with day 22 antibody titer 4x the baseline titer divided by the total number of influenza-positive subjects with both baseline and day 22 data for each treatment group

10.6.2 Population Pharmacokinetics Analysis

Variables Used for the Analysis
The analysis will require the following variables:

Variable	Source	Format/Notes
Day 3 tizoxanide (T) and tizoxanide glucuronide (TG) values	Laboratory data	
Demographic data	CRF	
Concomitant medications use	CRF	
Adverse events	CRF	

Analysis
Plasma concentrations of T and TG will be summarized descriptively for both treatment groups. Exploratory analyses will be conducted to evaluate relationships between plasma concentrations and age, race, gender, body weight, body mass index, concomitant medications (yes/no), changes in viral titer over time, Time to Symptom Response, and adverse events (yes/no). For continuous factors, the relationship with plasma concentrations will be assessed by Spearman's correlation analysis and Pearson's correlation analysis. For categorical factors, plasma concentrations will be compared among groups using the Kruskal-Wallis test.

Analysis Population(s):	Confirmed Influenza, NTZ-treated only
Derived Variables:	• Concomitant medications use: if any concomitant medication is used, then "yes", otherwise "no" • Adverse events: if any adverse event is reported, then "yes", otherwise "no" • Changes in viral titer over time: see section 10.5.5 • Time to Symptom Response: see section 10.2

10.7 Efficacy Listings

Efficacy listings will include data specified in Table 5 for all subjects presented by subject number and treatment group.

Table 5. Data Presented in Efficacy Listings

Listing	Data Presented
Time to Symptom Response	Date/time of first dose Date/time of Symptom Response Event time (hours) Censored time (hours)
Time to Ability to Perform All Normal Activities	Date/time of first dose Date/time of Ability to Perform All Normal Activities Event time (hours) Censored time (hours)
Complications of Influenza	MedDRA SOC MedDRA preferred term Reported term Onset date End date Severity Outcome Change in study drug dosage Treatment required
Time to Modified Symptom Response	Date/time of first dose Date/time of Modified Symptom Response Event time (hours) Censored time (hours)
Time to Individual Symptom Response	Date/time of first dose Date/time of Individual Symptom Response Event time (hours) Censored time (hours)
Time to FLU-PRO Domain Response	Date/time of first dose Date/time of FLU-PRO Domain Response Event time (hours) Censored time (hours)
Time to Resolution of Acute Febrile Illness	Date/time of first dose Date/time of Resolution of Acute Febrile Illness Event time (hours) Censored time (hours)
Time to Return to Usual Health	Date/time of first dose Date/time of Return to Usual Health Event time (hours) Censored time (hours)
Virology Data	Study day RT-PCR result Traditional Culture result $TCID_{50}$ result
Influenza Antibody Titer Data	Study day Influenza type Antibody titer

11. SAFETY

11.1 Safety Tables and Figures

Unless otherwise specified, all safety tables will be based on the All Treated population and present data by treatment group.

Adverse events summary tables will use the following algorithms for counting subject events:

- Preferred term: Each subject will be counted once within each unique preferred term at the maximum grade.
- System organ class: Each subject is counted only once at the maximum grade at each system organ class level.
- Any event: Each subject with an event is counted only once at the maximum grade.

11.1.1 Summary of All Treatment Emergent Adverse Events (TEAEs)

The summary of all TEAEs will summarize the following:

- The total number of TEAEs;
- The number and percentage of subjects with at least one TEAE;
- The number and percentage of subjects with at least one SAE;
- The number and percentage of subjects with a TEAE by the maximum severity grade; and
- The number and percentage of subjects with a TEAE by the closest relationship to the study drug.

11.1.2 Summaries of TEAEs by SOC and Preferred Term

The number and percent of subjects with TEAEs will be summarized by SOC and preferred term with the following variations in presentation:

- Number and percent of subjects with TEAEs will be summarized by SOC and preferred term;
- Number and percent of subjects with TEAEs will be summarized by SOC, preferred term and severity;
- Number and percent of subjects with grade ≥3 TEAEs by SOC and preferred term;
- Number and percent of subjects with TEAEs by SOC, preferred term and relationship to study medication;
- Number and percent of subjects with unexpected TEAEs by SOC and preferred term;
- Number and percent of subjects with SAEs by SOC and preferred term; and
- Number and percent of TEAEs leading to study withdrawal by SOC and preferred term.

11.1.3 Laboratory Results and Changes from Baseline

Numerical hematology and serum biochemistry values will be converted to SI units and classified as Normal, Grade 1, Grade 2, Grade 3 or Grade 4 according to reference ranges indicated in the protocol toxicity grading scale tables (see Appendix II). Urinalysis results will be classified as Normal or Abnormal based on reference ranges provided by the central laboratory.

Hematology, serum biochemistry, and urinalysis laboratory results and change from Baseline will be summarized using descriptive statistics for each visit.

Shift tables will display shifts from Baseline in laboratory results classification (Grade 1/2/3/4 or Normal/Abnormal) by study day.

11.1.4 Summaries of Vital Signs Results and Changes from Baseline

Vital signs data will be classified as Normal, Grade 1, Grade 2, Grade 3 or Grade 4 based on the reference ranges indicated in the protocol toxicity grading scale tables (see Appendix II).

Vital signs results and change from Baseline will be summarized using descriptive statistics for each visit.

Shift tables will display shifts from Baseline in vital signs classification (Grade 1/2/3/4) by study day.

11.1.5 Summaries of Physical Examination Results and Changes from Baseline

The number and percent of subjects with abnormal physical examination findings will be summarized by body system for each visit.

A shift table will display changes in physical examination classification (Normal/Abnormal) at each visit by body system.

11.2 Safety Listings

Safety listings will include data specified in Table 6 for all subjects presented by subject number and treatment group.

Table 6. Data Presented in Safety Listings

Listing	Data Presented
Adverse Events	MedDRA SOC MedDRA preferred term Reported term Onset date End date Severity Serious (Y/N) Relation to study drug Outcome Change in study drug dosage Treatment required
Hematology Laboratory Results Serum Biochemistry Laboratory Results Urinalysis Laboratory Results	Visit Date of visit Laboratory test Result Result units Lower limit of normal Upper limit of normal Classification (L/N/H)
Plasma Tizoxanide and Tizoxanide Glucuronide Results	Visit Date of visit Tizoxanide concentration (µg/mL) Tizoxanide glucuronide concentration (µg/mL)

Listing	Data Presented
Vital Signs Data	Visit Date of visit Weight (kg) Systolic blood pressure (mmHg) Diastolic blood pressure (mmHg) Pulse (bpm) Respiratory rate (bpm) Oral temperature (°F)
Physical Examination Data	Visit Date of visit Body system Result (Normal/Abnormal/Not Done) Abnormality

12. REFERENCES

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Appendix I: 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	X[3]	X
Blood sample for pharmacokinetics					X[4]			
Blood sample for anti-influenza antibodies		X					X	
Blood sample for laboratory safety tests[5]		X				X		X
Urine sample for routine urinalysis[5]		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, patient 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 patient diary entries			X[6]					X[6]
Screen for influenza-related complications				X	X			
Review compliance with study medication, collect container with unused medication, complete pill count log form					X	X		X[7]

[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 patient 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 patient's home (or clinic or another location as agreed with the patient).

[3] Nasopharyngeal swabs collected on day 22 will only be tested for the presence of virus if the sample from day 7 had detectable virus.

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

[5] 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).

[6] 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.

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

Appendix II: Data Displays

Display Number	Title	Type	Analysis Population
General Displays			
14.1.1.1	Disposition of Subjects	Table	All Treated
14.1.1.2	Disposition of Subjects	Table	Confirmed Influenza
14.1.2.1	Summary of Protocol Deviations	Table	All Treated
14.1.2.2	Summary of Protocol Deviations	Table	Confirmed Influenza
14.1.3.1	Summary of Baseline Demographic Characteristics	Table	All Treated
14.1.3.2	Summary of Baseline Demographic Characteristics	Table	Confirmed Influenza
14.1.4.1	Summary of Baseline Disease Characteristics	Table	All Treated
14.1.4.2	Summary of Baseline Disease Characteristics	Table	Confirmed Influenza
14.1.5.1	Summary of Prior Medication	Table	All Treated
14.1.5.2	Summary of Prior Medication	Table	Confirmed Influenza
14.1.6.1	Summary of Concomitant Medication	Table	All Treated
14.1.6.2	Summary of Concomitant Medication	Table	Confirmed Influenza
14.1.7.1	Summary of Medical History	Table	All Treated
14.1.7.2	Summary of Medical History	Table	Confirmed Influenza
14.1.8.1	Summary of Concomitant Diseases	Table	All Treated
14.1.8.2	Summary of Concomitant Diseases	Table	Confirmed Influenza
14.1.9.1	Summary of Treatment Exposure	Table	All Treated
14.1.9.2	Summary of Treatment Exposure	Table	Confirmed Influenza
Efficacy Displays			
14.2.1.1	Evaluation of Symptom Response Definition	Table	Confirmed Influenza
14.2.1.2	Analysis of Time to Symptom Response	Table	Confirmed Influenza
14.2.1.3	Graphical Representation of Time to Symptom Response	Figure	Confirmed Influenza
14.2.2.1.1	Sensitivity Analysis of Time to Symptom Response Assigning a Time to Symptom Response of 21 Days to Discontinued Subjects	Table	Confirmed Influenza
14.2.2.1.2	Graphical Representation of Sensitivity Analysis of Time to Symptom Response Assigning a Time to Symptom Response of 21 Days to Discontinued Subjects	Figure	Confirmed Influenza
14.2.2.2.1	Sensitivity Analysis of Time to Symptom Response for the Per Protocol Population	Table	Per Protocol
14.2.2.2.2	Graphical Representation of Sensitivity Analysis of Time to Symptom Response for the Per Protocol Population	Figure	Per Protocol
14.2.3.1	Analysis of Time to Ability to Perform All Normal Activities	Table	Confirmed Influenza

Display Number	Title	Type	Analysis Population
14.2.3.2	Graphical Representation of Time to Ability to Perform All Normal Activities	Figure	Confirmed Influenza
14.2.4	Analysis of Proportions Experiencing Complications of Influenza	Table	Confirmed Influenza
14.2.5.1	Evaluation of Modified Symptom Response Definition	Table	Confirmed Influenza
14.2.5.2	Analysis of Time to Modified Symptom Response	Table	Confirmed Influenza
14.2.5.3	Graphical Representation of Time to Modified Symptom Response	Figure	Confirmed Influenza
14.2.6.1.x	Analysis of Time to Individual Symptom Response	Table	Confirmed Influenza
14.2.6.2.x	Graphical Representation of Time to Individual Symptom Response	Figure	Confirmed Influenza
14.2.7.1.x	Analysis of Time to FLU-PRO Domain Response	Table	Confirmed Influenza
14.2.7.2.x	Graphical Representation of Time to FLU-PRO Domain Response	Figure	Confirmed Influenza
14.2.8.1	Analysis of Time to Resolution of Acute Febrile Illness	Table	Confirmed Influenza
14.2.8.2	Graphical Representation of Time to Resolution of Acute Febrile Illness	Figure	Confirmed Influenza
14.2.9.1	Analysis of Time to Return to Usual Health	Table	Confirmed Influenza
14.2.9.2	Graphical Representation of Time to Return to Usual Health	Figure	Confirmed Influenza
14.2.10	Analysis of Changes in Influenza Virus Titer ($TCID_{50}$)	Table	Confirmed Influenza
14.2.11	Proportions of Subjects with Detectable Influenza Virus on Days 2, 3, and 7	Table	Confirmed Influenza
14.2.12.1	Analysis of Mean Changes in Influenza Antibody Titers from Baseline	Table	Confirmed Influenza
14.2.12.2	Analysis of Proportions of Subjects with Seroprotected and Seroconverted Antibody Response	Table	Confirmed Influenza
14.2.13	Analysis of Plasma Tizoxanide and Tizoxanide Glucuronide Concentrations	Table	Confirmed Influenza
Safety Displays			
14.3.1.1	Summary of All Treatment Emergent Adverse Events	Table	All Treated
14.3.1.2	Number and Percent of Subjects with Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Table	All Treated
14.3.1.3	Number and Percent of Subjects with Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Severity	Table	All Treated
14.3.1.4	Number and Percent of Subjects with Grade ≥3 Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Table	All Treated
14.3.1.5	Number and Percent of Subjects with Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Relationship to Study Medication	Table	All Treated
14.3.1.6	Number and Percent of Subjects with Treatment Emergent Unexpected Adverse Events by System Organ Class and Preferred Term	Table	All Treated
14.3.1.7	Number and Percent of Subjects with Treatment Emergent Serious Adverse Events by System Organ Class and Preferred Term	Table	All Treated

Display Number	Title	Type	Analysis Population
14.3.1.8	Number and Percent of Subjects with Treatment Emergent Adverse Events Leading to Study Withdrawal by System Organ Class and Preferred Term	Table	All Treated
14.3.2.1	Summary of Hematology Laboratory Results and Changes from Baseline	Table	All Treated
14.3.2.2	Change in Hematology Laboratory Results Over Time: Shift Table	Table	All Treated
14.3.3.1	Summary of Serum Biochemistry Laboratory Results and Changes from Baseline	Table	All Treated
14.3.3.2	Change in Serum Biochemistry Laboratory Results Over Time: Shift Table	Table	All Treated
14.3.4.1	Number and Percent of Subjects with Each Urinalysis Result	Table	All Treated
14.3.4.2	Change in Urinalysis Results Over Time: Shift Table	Table	All Treated
14.3.5.1	Summary of Vital Signs Results and Change from Baseline	Table	All Treated
14.3.5.2	Change in Vital Signs Results Over Time: Shift Table	Table	All Treated
14.3.6.1	Summary of Physical Examination Results by Category	Table	All Treated
14.3.6.2	Number and Percent of Subjects with Physical Examination Changes from Normal at Baseline to Abnormal by Category	Table	All Treated
Listings			
16.1.1	Listing of Discontinued Subjects	Listing	All Treated
16.2.2	Listing of Subjects with Protocol Deviations	Listing	All Treated
16.2.3	Listing of Subjects Excluded from Efficacy Analysis	Listing	Confirmed Influenza
16.2.4.1	Listing of Demographic Data	Listing	All Treated
16.2.4.2	Listing of Baseline Disease Characteristics	Listing	All Treated
16.2.4.3	Listing of Prior Medication	Listing	All Treated
16.2.4.4	Listing of Concomitant Medication	Listing	All Treated
16.2.4.5	Listing of Medical History	Listing	All Treated
16.2.4.6	Listing of Concomitant Diseases	Listing	All Treated
16.2.5	Listing of Treatment Exposure Data	Listing	All Treated
16.2.6.1	Listing of Time to Symptom Response	Listing	Confirmed Influenza
16.2.6.2	Listing of Time to Ability to Perform All Normal Activities	Listing	Confirmed Influenza
16.2.6.3	Listing of Complications of Influenza	Listing	Confirmed Influenza
16.2.6.4	Listing of Time to Modified Symptom Response	Listing	Confirmed Influenza
16.2.6.5	Listing of Time to Individual Symptom Response	Listing	Confirmed Influenza
16.2.6.6	Listing of Time to FLU-PRO Domain Response	Listing	Confirmed Influenza
16.2.6.7	Listing of Time to Resolution of Acute Febrile Illness	Listing	Confirmed Influenza
16.2.6.8	Listing of Time to Return to Usual Health	Listing	Confirmed Influenza
16.2.6.9	Listing of Virology Data	Listing	Confirmed Influenza

Display Number	Title	Type	Analysis Population
16.2.6.10	Listing of Influenza Antibody Titer Data	Listing	Confirmed Influenza
16.2.7	Listing of Adverse Events	Listing	All Treated
16.2.8.1	Listing of Hematology Laboratory Results	Listing	All Treated
16.2.8.2	Listing of Serum Biochemistry Laboratory Results	Listing	All Treated
16.2.8.3	Listing of Urinalysis Laboratory Results	Listing	All Treated
16.2.8.4	Listing of Plasma Tizoxanide and Tizoxanide Glucuronide Results	Listing	All Treated
16.2.9.1	Listing of Vital Signs Data	Listing	All Treated
16.2.9.2	Listing of Physical Examination Data	Listing	All Treated