STATISTICAL ANALYSIS PLAN

Based on:
Protocol No. RM08-3005
Final Version 1.3
NCT03605862

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 Sponsor:
The Romark Institute for Medical Research
[redacted]

Version: FINAL 1.4

July 1, 2019

This study is being conducted in compliance with good clinical practice, including the archiving of essential documents.

1. REVISION HISTORY

Table 1: Summary of Revision History

Ver	Date	Revision Author	Comments
1.0	06Sep2018		Original SAP
1.1	11Dec2018	[redacted]	Update to clarify (1) response definitions for "Time of Return to Ability to Perform Normal Activities" and "Time of Return to Usual Health", (2) source of complications data, (3) imputation rules for quantitative PCR results.
1.2	14Jun2019	[redacted]	Update to: 1. Add exploratory analysis of time to symptom response for subjects who received the first dose of study medication 0-24, >24-≤36, or >36 hours from symptom onset (Sections 3, 8.1, 11.5.8, 11.5.9, 11.5.10, Appendix 2); 2. Add exploratory analysis of time to symptom response by symptom relief medication use (placebo-treated subjects only), time to ability to perform normal activities by symptom relief medication use (placebo-treated subjects only), and proportions experiencing complications of colds by symptom relief medication use (Sections 3, 8.1, 11.6.2, 11.6.3, 11.6.4, Appendix 2); 3. Add the "No pathogen detected" subgroup as a possible analysis population for time to symptom response for subjects without EV/RV infection (Sections 3, 8.1, 11.5.7); 4. Clarify number of subjects required (Section 3); 5. Clarify calculation 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 3, 8.2, 11.2, 11.4.1, 11.5.2, 11.5.3); 6. Remove oral temperature and qualifying symptoms from the Baseline Disease Characteristics table and listing due to lack of relevance and explicit documentation in the database, respectively (Sections 10.1.4, 10.2); 7. Update cumulative distribution parameters for selection of maximum symptom response criteria from 75%-90% to 90%-95% (Section 11.2.1); 8. Clarify maximum symptom response criteria procedure including improvement of example case (Section 11.2.1); 9. Clarify impact of symptom relief medication use on Time to FLU-PRO Domain Response analysis (Section 11.5.2); 10. Clarify that ePlex RPP RT-PCR and quantitative PCR tests should be considered for analysis of proportions of subjects with detectable virus (Section 11.5.5);

Ver	Date	Revision Author	Comments
1.2 (*cont'd*)			11. Clarify that virus subgroup analyses will be performed for subgroups with at least 100 subjects enrolled (previously stated "with at least 50 subjects in each treatment group" – since the derivation of subgroup-specific symptom response definition is to be done on a blinded basis, it cannot be confirmed whether a subgroup meets the previous criteria prior to unblinding) (Section 11.5.7); 12. Clarified data handling convention when two vital signs values are available for one analysis time point (Section 12.1.4); and 13. Correct spelling and punctuation errors.
1.3	24Jun2019	[redacted]	1. Clarified in Section 11.4 that the primary and secondary efficacy analyses will be analyzed in a hierarchical fashion. 2. Added exploratory analysis of Time to Ability to Perform All Normal Activities for the ITT population (Section 11.5.7) and 3. Added exploratory analysis of Proportions Experiencing Complications for the ITT population (Section 11.5.8)
1.4	01Jul2019	[redacted]	Updated 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 11.2.1)

2. SIGNATURE PAGE

This plan has been reviewed and approved by the following:

Author: [redacted] 01 JUL 2019 Date

Statistics Approval: [redacted] 07/01/2019 Date

Sponsor Approval: [redacted] 01 JUL 2019 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:**	***(For National Authority Use Only)*:**
Name of Finished Product: Nitazoxanide 300 mg Tablets	**Page:**	
Name of Active Ingredient: Nitazoxanide		
Title Of Study: 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		
Investigators: Multicenter		
Studied period: August 2018 – April 2019		**Phase of development:** 3

Objectives:

The primary objective of the study is to evaluate the effect of nitazoxanide (NTZ) administered orally 600 mg b.i.d. for 5 days in reducing the duration of symptoms of colds due to *Enterovirus/Rhinovirus* (EV/RV) infection compared to 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, time to ability to perform all normal activities, and proportions experiencing complications for all subjects treated (ITT population); time to symptom response for subjects infected with individual non-EV/RV viruses; and time to symptom response for subjects who received the first dose of study medication 0-24, >24-≤36, or >36 hours from symptom onset. A final exploratory objective is to evaluate the impact of symptom relief medication use on time to symptom response, time to return to ability to perform all normal activities, and proportions experiencing complications of colds for placebo-treated subjects.

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.

Number of Subjects: 530 subjects with laboratory-confirmed EV/RV infection and complete data for analysis purposes, estimated to require at least 600 (up to maximum of 700) with colds due to laboratory-confirmed EV/RV infection

Study Design:

Multicenter, randomized, double-blind, placebo controlled trial to evaluate efficacy and safety of NTZ in the treatment of colds due to EV/RV infection

Population: Males and females ≥12 years of age with EV/RV infection

Study dose and mode of 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

Statistical methods:

Let $S_0(t)$ and $S_1(t)$ be the underlying survival functions regarding the time to Symptom Response for placebo and NTZ, respectively. The null and alternative hypotheses for the primary objective are defined as follows:

$$H_0: S_1(t) = S_0(t) \qquad H_A: S_1(t) \neq S_0(t)$$

The time to Symptom Response for subjects with laboratory-confirmed EV/RV infection (ITTI population) will be compared using a Gehan-Wilcoxon test at the α=0.05 level (two-tailed) 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), (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.

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6. LIST OF ABBREVIATIONS

Abbreviation	Definition
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
b.i.d.	bis in die (twice daily)
BUN	Blood urea nitrogen
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
CSR	Clinical study report
EDC	Electronic data capture
EV/RV	*Enterovirus/Rhinovirus*
FDA	U.S. Food and Drug Administration
FLU-PRO	inFLUenza Patient-Reported Outcomes
GGT	Gamma-glutamyltransferase
HDL	High-density lipoprotein
hMPV	Human metapneumovirus
ITT	Intent to treat
ITTI	Intent to treat infected
LDL	Low-density lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
NTZ	Nitazoxanide
RSV	Respiratory syncytial virus
RT-PCR	Real time polymerase chain reaction
SAP	Statistical analysis plan
SOC	System Organ Class
T	Tizoxanide
TEAE	Treatment-emergent adverse event
TG	Tizoxanide Glucuronide
WHODrug	World Health Organization Drug dictionary

7. 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 RM08-3005.

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

The SAP is based on:

- Protocol RM08-3005, Final Version 1.3, November 29, 2018
- International Conference on Harmonisation Guidelines E4 and E9; and
- Discussions with the U.S. Food and Drug Administration (FDA).

This document will describe analysis populations and 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.

8. STUDY DESCRIPTION

8.1. 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 (ITT population); time to symptom response for subjects infected with individual non-EV/RV viruses; and time to symptom response for subjects who received the first dose of study medication 0-24, >24-≤36, or >36 hours from symptom onset. A final exploratory objective is to evaluate the impact of symptom relief medication use on time to symptom response, time to return to ability to perform all normal activities, and proportions experiencing complications of colds for placebo-treated subjects.

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.

8.2. 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 selected according to the inclusion and exclusion criteria listed in the protocol.

Immediately after completion of informed consent, screening, and enrollment in the study, two Baseline nasopharyngeal swabs will be collected for RT-PCR. Blood and urine samples will be collected for laboratory safety tests. Subjects will receive instruction on completing their electronic diaries, recording concomitant medications and attending follow-up visits.

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

Randomization will be 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), (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.

Study 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 complications of colds. A blood sample for pharmacokinetics will also be collected prior to the first dose of study medication 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). The nasopharyngeal swabs will be used to evaluate quantitative changes in viral shedding. The blood sample 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 complications of colds. [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).]

Subjects will return to the clinic on Days 7 and 22 for post-treatment follow-up, including two nasopharyngeal swabs, drug accountability, and reporting of adverse events and complications of cold. Blood and urine samples for laboratory safety tests will be collected

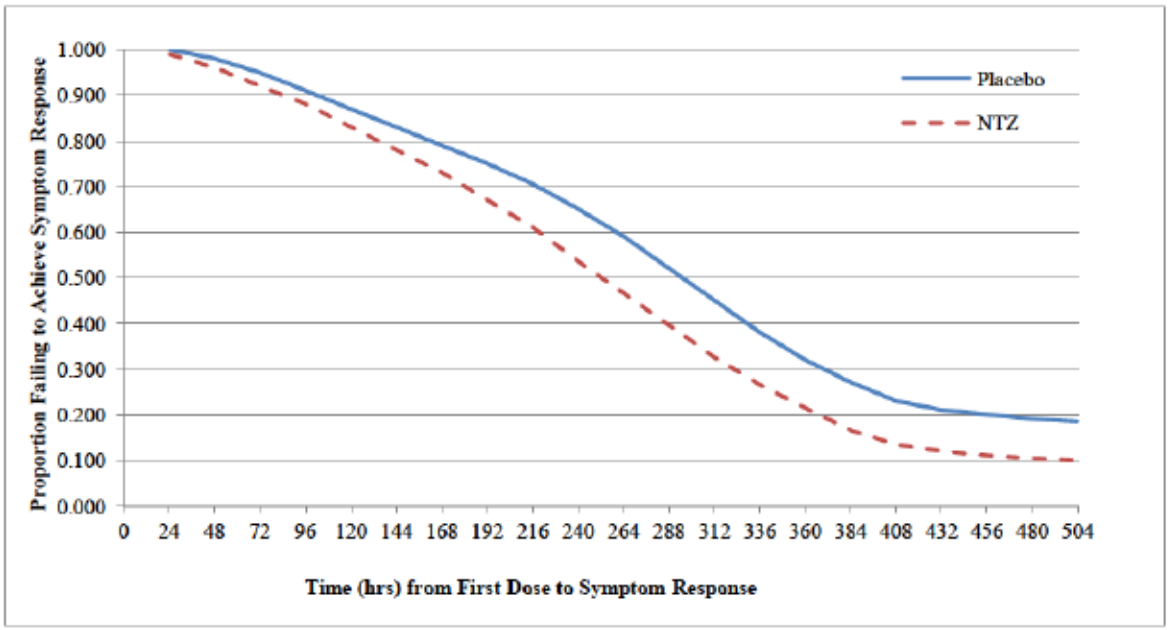
at Day 7. Subjects will maintain the electronic patient diaries through study Day 21. Any visits that do not occur on the prescribed date, or within 1 day of that date in the case of the Day 7 visit or within 3 days after in the case of the Day 22 visit will be recorded as protocol deviations.

A central laboratory will be used for laboratory safety tests 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, H1N1 (2009), and 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*. (Baseline and Day 7 nasopharyngeal swabs), and quantitation of EV/RV by quantitative PCR (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 EV/RV at Baseline, Day 2 or Day 3 will not be analyzed for quantitative virus titer.

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

8.3. Sample Size Justification

The Symptom Response definitions for this study will be derived from the subjects' FLU-PRO questionnaire responses as described in Section 11.2.1. 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 α=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 EV/RV-infected subjects who receive at least one dose of study medication 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.

8.4. Data Collection

Data will be collected using an electronic data capture (EDC) system and electronic subject diaries. 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.

9. DATA DEFINITIONS AND CONVENTIONS

9.1. Analysis Populations

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

Table 2: Definition of Analysis Populations

Table Analysis Population	Definition
Intent to Treat (ITT)	All subjects who receive at least one dose of study medication
Intent to Treat Infected (ITTI)	The subset of the ITT population who are positive for EV/RV by RT-PCR at any of the Baseline, Day 2 or Day 3 visits
Per Protocol	The subset of the ITTI population with no major protocol deviations that may have an effect on the integrity of the data or the evaluation of effectiveness

9.2. Data Presentation Conventions

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

Table 3: Data Presentation Conventions

Data	Presentation Convention
Descriptive statistics for continuous variables	Include the number of subjects with available data, mean, standard deviation, median, first quartile, third quartile, 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

9.3. Handling of Unscheduled Assessments

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

9.4. Handling of Missing Data

Missing data will be handled as follows:

Table 4: 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 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.

10. GENERAL CHARACTERISTICS

10.1. General Characteristics Tables and Figures

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

10.1.1. 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): ITT, ITTI

Derived Variables: Number of days in study = date of study completion/early termination – date of randomization + 1

10.1.2. Protocol Deviations

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

Analysis Population(s): ITT, ITTI

Derived Variables: None

10.1.3. Demographics

The demographics tables will summarize sex, age at screening (years), race, height (cm), weight (kg), BMI (kg/m2), and smoking status as collected at screening/Baseline using descriptive statistics.

Analysis Population(s): ITT, ITTI

Derived Variables: Age in years at screening = date of screening – date of birth

BMI = weight in kg / (height in cm / 100)^2

10.1.4. Disease Characteristics

The disease characteristics tables will summarize time since onset of symptoms and baseline virus infection(s) using descriptive statistics.

Analysis Population(s): ITT, ITTI

Derived Variables: None

10.1.5. 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): ITT, ITTI

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

10.1.6. Medical History and Concomitant Diseases

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

Analysis Population(s): ITT, ITTI

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

10.1.7. 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): ITT, ITTI

Derived Variables: Number of tablets taken = number of doses reported to have been taken in the case report form (CRF) * 2

10.2. General Characteristics Listings

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

Table 5: Data Presented in General Listings

Listing	Data Presented
Discontinued Subjects	Date of discontinuation Reason for discontinuation

Listing	Data Presented
Subjects with Protocol Deviations	Date of deviation Common deviation term Classification (Major/Minor)
Subjects Excluded from Efficacy Analysis[1]	Reason for exclusion
Demographic Data	Subject initials Date/time of informed consent Date of birth Age Sex Race Height (cm) Weight (kg) BMI (kg/m2) Smoking status Number of years smoked Number of cigarettes smoked per day
Baseline Disease Characteristics	Time from onset of illness to first study drug intake (hours) Virus infection(s)
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 ITTI population due to negative RT-PCR assay will not be considered excluded from efficacy analysis

11. EFFICACY

11.1. 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 colds as indicated by the subject during the study period.

11.2. 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 a 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), (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. Separate Symptom Response definitions will be determined for ITTI subjects with no underlying lung condition and for ITTI subjects with underlying lung conditions. Time to Symptom Response will be calculated in the same way for the two groups and all ITTI subjects will be analyzed together.

Definitions:

Symptom Response	The set of criteria to be defined based upon a blinded review of symptom scores (pooled for all ITTI 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 11.2.1 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:

Table 6: Variables Used for the Primary Efficacy Analysis

Variable	Source	Format/Notes
Date and time of first medication intake	CRF	

Variable	Source	Format/Notes
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. 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.

11.2.1. Derivation of the Symptom Response Definition

Two Symptom Response definitions 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 (1) the ITTI population with underlying lung conditions and (2) for the ITTI population without underlying lung conditions.

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 EV/RV-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 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 first 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.

11.2.2. 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 by (1) time from onset of illness to time of first dose of study medication (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. The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s): ITTI

Derived Variables: Event: Time of Symptom Response – time of first dose

Censored: Time of last symptom assessment – time of first dose

11.3. Sensitivity Analyses of the Primary Efficacy Analysis

11.3.1. 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): ITTI

Derived Variables: See Section 11.2: Primary Efficacy Analysis

11.3.2. Sensitivity Analysis for a "Per Protocol" Subset

Repeat the primary efficacy analysis for the Per Protocol population.

Analysis Population(s): ITTI

Derived Variables: See Section 11.2: Primary Efficacy Analysis

11.3.3. Sensitivity Analysis Excluding Subjects Reporting Chromaturia

Repeat the primary efficacy analysis for the ITTI population excluding NTZ-treated subjects who reported chromaturia as an adverse event during the study.

Analysis Population(s): ITTI excluding NTZ-treated subjects who reported chromaturia

Derived Variables: See Section 11.2: Primary Efficacy Analysis

11.4. 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 Colds 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.

11.4.1. 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 daily 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:

Table 7: Variables Used for Analysis of Time to Ability to Perform All Normal Activities

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

by (1) time from onset of illness to time of first dose of study medication (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. The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s): ITTI

Derived Variables: Event: Time of Ability to Perform All Normal Activities – time of first dose

Censored: Time of last ability assessment – time of first dose

11.4.2. Proportion of Subjects Experiencing Complications of Colds

Variables Used for the Analysis

The analysis will require the following variables:

Table 8: Variables Used for Analysis of Complications of Colds

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

Analysis

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

Analysis Population(s): ITTI

Derived Variables: None

11.5. Exploratory Efficacy Analyses

11.5.1. 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:

Table 9: Variables Used for Analysis of Time to Individual Symptom Response

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. 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. Subjects will report the reason for taking each symptom relief medication, and this data will be used to assign concomitant medications to relevant cold 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 by (1) time from onset of illness to time of first dose of study medication (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. The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s): ITTI

Derived Variables: Event: Time of Individual Symptom Response – time of first dose

Censored: Time of last symptom assessment – time of first dose

11.5.2. 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:

Table 10: Variables Used for Analysis of Time to FLU-PRO Domain Response

Variable	Source	Format/Notes
Date and time of first medication intake	CRF	
Date and time of completion for each symptom assessment record	Subject diary	

Variable	Source	Format/Notes
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 by (1) time from onset of illness to time of first dose of study medication (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. The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s): ITTI

Derived Variables: Event: Time of FLU-PRO Domain Response – time of first dose

Censored: Time of last symptom assessment – time of first dose

11.5.3. 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:

Table 11: Variables Used for Analysis of Time to Return to Usual Health

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 diary 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 by (1) time from

onset of illness to time of first dose of study medication (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. The comparison will be graphically depicted using Kaplan-Meier curves.

Analysis Population(s): ITTI

Derived Variables: Event: Time of Return to Usual Health – time of first dose

Censored: Time of last usual health global assessment – time of first dose

11.5.4. Change in Virus Titer from Baseline to Day 2, 3, and 7

Variables Used for the Analysis

The analysis will require the following variables:

Table 12: Variables Used for Analysis of Change in Virus Titer

Variable	Source	Format/Notes
Quantitative PCR virus titer	Laboratory Data	Quantitative PCR values should be log-transformed for analysis purposes.

Analysis

Mean changes in viral titers from Baseline to Day 2, from Baseline to Day 3, and from Baseline to Day 7 for the NTZ and the placebo treatment groups will be compared using a t-test.

Subjects with undetectable virus by quantitative PCR at Baseline will be excluded from this analysis. Any undetectable result on Day 2, Day 3 or Day 7 will be imputed with the lower limit of detection for the quantitative PCR assay. Any positive result that is below the limit of quantitation will be imputed with the limit of quantitation for the quantitative PCR assay.

Analysis Population(s): ITTI

Derived Variables: Event: Time of Return to Usual Health – time of first dose

11.5.5. Proportions of Subjects with Detectable Virus at Day 2, Day 3, or Day 7

Variables Used for the Analysis

The analysis will require the following variables:

Table 13: Variables Used for Analysis of Proportions of Subjects with Detectable Virus at Day 2, Day 3, or Day 7

Variable	Source	Format/Notes
ePlex RPP RT-PCR or quantitative PCR result for *Enterovirus/Rhinovirus*	Laboratory Data	

Analysis

The proportion of subjects with a positive result for EV/RV by ePlex RPP RT-PCR or quantitative PCR at each of Days 2, 3, and 7 will be compared across treatment groups using a Fisher's exact test.

Analysis Population(s): ITTI

Derived Variables: Proportion of subjects with detectable virus at Day 2, 3, or 7 = number of subjects with "positive" result for EV/RV by ePlex RPP RT-PCR or quantitative PCR on Day 2, 3, or 7 divided by the total number of EV/RV-infected (ITTI) subjects with at least one test result for the time point for each treatment group.

NOTE: Day 2 and 3 visits were optional according to the protocol so it is expected that the number of subjects with test results at these time points will be less than the number of ITTI subjects.

11.5.6. Time to Symptom Response for All Subjects Treated (ITT Population)

Time to Symptom Response will be summarized for all subjects who received at least one dose of study medication regardless of viral infection (ITT population). A population-specific Symptom Response definition will be derived in accordance with Section 11.2.1 for (1) the ITT population with underlying lung conditions and (2) for the ITT population without underlying lung conditions. Time to Symptom Response will be analyzed in accordance with Section 11.2.2.

11.5.7. Time to Ability to Perform All Normal Activities for All Subjects Treated (ITT Population)

Time to Ability to Perform All Normal Activities as described in Section 11.4.1 will be repeated for all subjects who received at least one dose of study medication regardless of viral infection (ITT population).

11.5.8. Analysis of Proportions Experiencing Complications, All Subjects Treated (ITT Population)

Analysis of Proportions Experiencing Complications as described in Section 11.4.2 will be repeated for all subjects who received at least one dose of study medication regardless of viral infection (ITT population).

11.5.9. Time to Symptom Response for Subjects Infected with Individual non-EV/RV Viruses or No Pathogen Detected

Time to Symptom Response will be summarized for subgroups of subjects infected with the viruses shown below or with no pathogen detected provided that the subgroup includes at least 100 subjects.

Possible Subgroups:
Influenza-infected
Parainfluenza-infected
hMPV-infected
Adenovirus-infected

Coronavirus-infected
Bocavirus-infected
No pathogen detected

A subgroup-specific Symptom Response definition will be derived in accordance with Section 11.2.1 for (1) the specific virus-infected population with underlying lung conditions and (2) for the specific virus-infected population without underlying lung conditions. Time to Symptom Response will be analyzed in accordance with Section 11.2.2.

11.5.10. Analysis of Time to Symptom Response for Subjects Dosed Within 24 Hours of Symptom Onset

Repeat the primary efficacy analysis for the ITTI population including only subjects who took the first dose of study medication within 24 hours (≤ 24 hours) from symptom onset. Time from onset of illness will not be used as a factor in this analysis since all subjects will be from the same onset category.

Analysis Population(s): ITTI subjects with time of first dose of study medication ≤ 24 hours from symptom onset

Derived Variables: See Section 11.2: Primary Efficacy Analysis

11.5.11. Analysis of Time to Symptom Response for Subjects Dosed >24-≤36 Hours after Symptom Onset

Repeat the primary efficacy analysis for the ITTI population including only subjects who took the first dose of study medication more than 24 hours and less than or equal to 36 hours (> 24-≤36 hours) from symptom onset. Time from onset of illness will not be used as a factor in this analysis since all subjects will be from the same onset category.

Analysis Population(s): ITTI subjects with time of first dose of study medication > 24 and ≤ 36 hours from symptom onset

Derived Variables: See Section 11.2: Primary Efficacy Analysis

11.5.12. Analysis of Time to Symptom Response for Subjects Dosed >36 Hours after Symptom Onset

Repeat the primary efficacy analysis for the ITTI population including only subjects who took the first dose of study medication more than 36 hours (>36 hours) from symptom onset. Time from onset of illness will not be used as a factor in this analysis since all subjects will be from the same onset category.

Analysis Population(s): ITTI subjects with time of first dose of study medication > 36 hours from symptom onset

Derived Variables: See Section 11.2: Primary Efficacy Analysis

11.6. Other Exploratory Analyses

11.6.1. Population Pharmacokinetics Analysis

Variables Used for the Analysis

The analysis will require the following variables:

Table 14: Variables Used for Analysis of Plasma Concentrations

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): ITTI, NTZ-treated only

Derived Variables: Concomitant medication 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 11.5.5

Time to Symptom Response: see Section 11.2

11.6.2. Analysis of Time to Symptom Response by Symptom Relief Medication Use for Placebo-Treated Subjects

Repeat the primary efficacy analysis for the ITTI and ITT populations comparing placebo-treated subjects who did not use symptom relief medication to placebo-treated subjects who did use symptom relief medication. Sum of baseline FLU-PRO symptom assessments (<42 or ≥42) will be included as a factor in the analysis. Pre-enrollment symptom relief medication use will not be used as a factor in the analysis since the groups being compared are classified by symptom relief medication use.

Analysis Population(s): ITTI placebo-treated subjects, ITT placebo-treated subjects

Derived Variables: See Section 11.2: Primary Efficacy Analysis

11.6.3. Analysis of Time to Ability to Perform All Normal Activities by Symptom Relief Medication Use for Placebo-Treated Subjects

Repeat the analysis of time to ability to perform all normal activities for the ITTI and ITT populations comparing placebo-treated subjects who did not use symptom relief medication to placebo-treated subjects who did use symptom relief medication. Sum of baseline FLU-PRO symptom assessments (<42 or ≥42) will be included as a factor in the analysis. Pre-enrollment symptom relief medication use will not be used as a factor in the analysis since the groups being compared are classified by symptom relief medication use.

Analysis Population(s): ITTI placebo-treated subjects, ITT placebo-treated subjects

Derived Variables: See Section 11.4.1 Time to Ability to Perform All Normal Activities

11.6.4. Proportion of Subjects Experiencing Complications of Colds by Symptom Relief Medication Use for Placebo-Treated Subjects

Repeat the analysis of proportion of subjects experiencing complications of colds for the ITTI and ITT populations comparing placebo-treated subjects who did not use symptom relief medication to placebo-treated subjects who did use symptom relief medication using a Fisher's exact test.

Analysis Population(s): ITTI placebo-treated subjects, ITT placebo-treated subjects

Derived Variables: None

11.7. Efficacy Listings

Efficacy listings will include data specified in Table 15 for all subjects presented by subject number and treatment group.

Table 15: 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)

Listing	Data Presented
Complications of Colds	MedDRA SOC MedDRA preferred term Reported term Onset date End date Severity Outcome Change in study drug dosage Treatment required
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 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 Quantitative PCR result

12. SAFETY

12.1. Safety Tables and Figures

Unless otherwise specified, all safety tables will be based on the ITT 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.

12.1.1. 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.

12.1.2. 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 (Note: unexpected TEAEs are those not listed in the Investigator's Brochure for the study);
- 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.

12.1.3. 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 Protocol 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.

12.1.4. 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 Protocol Appendix II). If two vital signs values are available in the database for one analysis time point (e.g., Day 7 Visit), the later value will be used for the analysis.

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.

12.1.5. 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.

12.2. Safety Listings

Safety listings will include data specified in Table 16 for all subjects presented by subject number and treatment group.

Table 16: 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)

Listing	Data Presented
Plasma Tizoxanide and Tizoxanide Glucuronide Results	Visit Date of visit Tizoxanide concentration (µg/mL) Tizoxanide glucuronide concentration (µg/mL)
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

APPENDIX 1. SCHEDULE OF ASSESSMENTS

Table 17: 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

APPENDIX 2. DATA DISPLAYS

Table 18: Data Displays

Display Number	Title	Type	Analysis Population
General Displays			
14.1.1.1	Disposition of Subjects	Table	ITT
14.1.1.2	Disposition of Subjects	Table	ITTI
14.1.2.1	Summary of Protocol Deviations	Table	ITT
14.1.2.2	Summary of Protocol Deviations	Table	ITTI
14.1.3.1	Summary of Baseline Demographic Characteristics	Table	ITT
14.1.3.2	Summary of Baseline Demographic Characteristics	Table	ITTI
14.1.4.1	Summary of Baseline Disease Characteristics	Table	ITT
14.1.4.2	Summary of Baseline Disease Characteristics	Table	ITTI
14.1.5.1	Summary of Prior Medication	Table	ITT
14.1.5.2	Summary of Prior Medication	Table	ITTI
14.1.6.1	Summary of Concomitant Medication	Table	ITT
14.1.6.2	Summary of Concomitant Medication	Table	ITTI
14.1.7.1	Summary of Medical History	Table	ITT
14.1.7.2	Summary of Medical History	Table	ITTI
14.1.8.1	Summary of Concomitant Diseases	Table	ITT
14.1.8.2	Summary of Concomitant Diseases	Table	ITTI
14.1.9.1	Summary of Treatment Exposure	Table	ITT
14.1.9.2	Summary of Treatment Exposure	Table	ITTI
Efficacy Displays			
14.2.1.1	Evaluation of Symptom Response Definitions	Table	ITTI
14.2.1.2	Analysis of Time to Symptom Response	Table	ITTI
14.2.1.3	Graphical Representation of Time to Symptom Response	Figure	ITTI
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	ITTI
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	ITTI
14.2.2.2.1	Sensitivity Analysis of Time to Symptom Response for the Per Protocol Population	Table	Per Protocol

Display Number	Title	Type	Analysis Population
14.2.2.2.2	Graphical Representation of Sensitivity Analysis of Time to Symptom Response for the Per Protocol Population	Figure	Per Protocol
14.2.2.3.1	Sensitivity Analysis of Time to Symptom Response Excluding NTZ-treated Subjects Reporting Chromaturia	Table	ITTI
14.2.2.3.2	Graphical Representation of Sensitivity Analysis of Time to Symptom Response Excluding NTZ-treated Subjects Reporting Chromaturia	Figure	ITTI
14.2.3.1	Analysis of Time to Ability to Perform All Normal Activities	Table	ITTI
14.2.3.2	Graphical Representation of Time to Ability to Perform All Normal Activities	Figure	ITTI
14.2.4	Analysis of Proportions Experiencing Complications of Colds	Table	ITTI
14.2.5.1.x	Analysis of Time to Individual Symptom Response	Table	ITTI
14.2.5.2.x	Graphical Representation of Time to Individual Symptom Response	Figure	ITTI
14.2.6.1.x	Analysis of Time to FLU-PRO Domain Response	Table	ITTI
14.2.6.2.x	Graphical Representation of Time to FLU-PRO Domain Response	Figure	ITTI
14.2.7.1	Analysis of Time to Return to Usual Health	Table	ITTI
14.2.7.2	Graphical Representation of Time to Return to Usual Health	Figure	ITTI
14.2.8	Analysis of Changes in EV/RV Virus Titer	Table	ITTI
14.2.9	Proportions of Subjects with Detectable EV/RV on Days 2, 3, and 7	Table	ITTI
14.2.10.1	Exploratory Evaluation of Symptom Response Definitions for All Subjects Treated	Table	ITT
14.2.10.2.1	Analysis of Time to Symptom Response for All Subjects Treated	Table	ITT
14.2.10.2.2	Analysis of Time to Ability to Perform All Normal Activities for All Subjects Treated	Table	ITT
14.2.10.2.3	Analysis of Proportions Experiencing Complications of Colds, All Subjects Treated	Table	ITT
14.2.10.3.1	Graphical Representation of Time to Symptom Response for All Subjects Treated	Figure	ITT
14.2.10.3.2	Graphical Representation of Time to Ability to Perform All Normal Activities for All Subjects Treated	Figure	ITT
14.2.11.x.1	Exploratory Evaluation of Symptom Response Definitions for Specific Virus Subgroups	Table	ITT
14.2.11.x.2	Analysis of Time to Symptom Response for Specific Virus Subgroups	Table	ITT
14.2.11.x.3	Graphical Representation of Time to Symptom Response for Specific Virus Subgroups	Figure	ITT
14.2.12.1.1	Analysis of Time to Symptom Response for Subjects Dosed ≤24 Hours from Symptom Onset	Table	ITTI
14.2.12.1.2	Graphical Representation of Time to Symptom Response for Subjects Dosed ≤24 Hours from Symptom Onset	Figure	ITTI
14.2.12.2.1	Analysis of Time to Symptom Response for Subjects Dosed >24 - ≤36 Hours from Symptom Onset	Table	ITTI
14.2.12.2.2	Graphical Representation of Time to Symptom Response for Subjects Dosed >24 - ≤36 Hours from Symptom Onset	Figure	ITTI
14.2.12.3.1	Analysis of Time to Symptom Response for Subjects Dosed >36 Hours from Symptom Onset	Table	ITTI

Display Number	Title	Type	Analysis Population
14.2.12.3.2	Graphical Representation of Time to Symptom Response for Subjects Dosed >36 Hours from Symptom Onset	Figure	ITTI
14.2.13	Analysis of Plasma Tizoxanide and Tizoxanide Glucuronide Concentrations	Table	ITTI
14.2.14.1.1	Analysis of Time to Symptom Response by Symptom Relief Medication Use, Placebo-Treated Subjects	Table	ITTI
14.2.14.1.2	Graphical Representation of Time to Symptom Response by Symptom Relief Medication Use, Placebo-Treated Subjects	Figure	ITTI
14.2.14.1.3	Analysis of Time to Symptom Response by Symptom Relief Medication Use, Placebo-Treated Subjects	Table	ITT
14.2.14.1.4	Graphical Representation of Time to Symptom Response by Symptom Relief Medication Use, Placebo-Treated Subjects	Figure	ITT
14.2.14.2.1	Analysis of Time to Ability to Perform All Normal Activities by Symptom Relief Medication Use, Placebo-Treated Subjects	Table	ITTI
14.2.14.2.2	Graphical Representation of Time to Ability to Perform All Normal Activities by Symptom Relief Medication Use, Placebo-Treated Subjects	Figure	ITTI
14.2.14.2.3	Analysis of Time to Ability to Perform All Normal Activities by Symptom Relief Medication Use, Placebo-Treated Subjects	Table	ITT
14.2.14.2.4	Graphical Representation of Time to Ability to Perform All Normal Activities by Symptom Relief Medication Use, Placebo-Treated Subjects	Figure	ITT
14.2.14.3.1	Analysis of Proportions Experiencing Complications of Colds by Symptom Relief Medication Use, Placebo-Treated Subjects	Table	ITTI
14.2.14.3.2	Analysis of Proportions Experiencing Complications of Colds by Symptom Relief Medication Use, Placebo-Treated Subjects	Table	ITT
Safety Displays			
14.3.1.1	Summary of All Treatment Emergent Adverse Events	Table	ITT
14.3.1.2	Number and Percent of Subjects with Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Table	ITT
14.3.1.3	Number and Percent of Subjects with Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Severity	Table	ITT
14.3.1.4	Number and Percent of Subjects with Grade ≥3 Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Table	ITT
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	ITT
14.3.1.6	Number and Percent of Subjects with Treatment Emergent Unexpected Adverse Events by System Organ Class and Preferred Term	Table	ITT

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

Display Number	Title	Type	Analysis Population
16.2.6.8	Listing of Time to Symptom Response for Specific Virus Subgroups	Listing	ITT
16.2.6.9	Listing of Virology Data	Listing	ITT
16.2.7	Listing of Adverse Events	Listing	ITT
16.2.8.1	Listing of Hematology Laboratory Results	Listing	ITT
16.2.8.2	Listing of Serum Biochemistry Laboratory Results	Listing	ITT
16.2.8.3	Listing of Urinalysis Laboratory Results	Listing	ITT
16.2.8.4	Listing of Plasma Tizoxanide and Tizoxanide Glucuronide Results	Listing	ITT
16.2.9.1	Listing of Vital Signs Data	Listing	ITT
16.2.9.2	Listing of Physical Examination Data	Listing	ITT