STATISTICAL ANALYSIS PLAN

A single-Centre, open-label, exploratory study of the effect of 20 mg ambroxol hydrochloride on cough reflex sensitivity in patients with acute cough

Study Number: IIT15419

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This study is to be performed in full compliance with International Conference on Harmonization (ICH) and all applicable local Good Clinical Practices (GCP) and regulations. All required study documentation will be archived as required by regulatory authorities.

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Compound:	Lysopain Ambr Mint™
Study Number:	IIT15419
Study Title:	A single-centre, open-label exploratory study of the effect of 20 mg ambroxol hydrochloride on cough reflex sensitivity in patients with acute cough.
Sponsor:	Hull and East Yorkshire Hospitals NHS Trust Hull Royal Infirmary, Anlaby Road, Hull, HU3 2JZ.
Plan Version:	Version 1.0
Version Date:	11/05/2018
Plan Created by:	Caroline Wright

The signature below indicates approval of the statistical analysis plan for this study

APPROVAL SIGNATURE

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Term	Definition
AE	adverse event
ATC	Anatomical Therapeutic Chemical
ATP	Adenosine Triphosphate
bpm	Beats per minute
C2	Concentration of cough challenge inducing at least 2 coughs
C5	Concentration of cough challenge inducing at least 5 coughs
CI	Confidence Interval
ECG	Electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
GCP	Good Clinical Practice
LN	Natural log
MedDRA	Medical Dictionary for regulatory Activities
PCI	Potentially Clinically Important
PPS	Per protocol set
SAE	serious adverse event
SAP	statistical analysis plan
SPSS	Statistical package for social sciences
TEAE	treatment-emergent adverse event
URTI	upper respiratory tract infection
UTC	urge-to-cough
VAS	Visual analogue scale
WHO	World Health Organization

1.0   INTRODUCTION

This statistical analysis plan (SAP) outlines the data and procedures used for assessing the safety and tolerability of ambroxol from Protocol IIT15419: A single-centre, open-label, exploratory study of the effect of 20 mg ambroxol hydrochloride on cough reflex sensitivity in patients with acute cough.

2.0   STUDY OBJECTIVE

2.1   Primary Objectives

The primary objective is to:

• To assess the effect of a single doses of 20 mg ambroxol hydrochloride on cough reflex sensitivity to citric acid, capsaicin, adenosine triphosphate (ATP) and distilled water in patients with acute cough related to upper respiratory tract infection.

2.2   Secondary Objectives

- To determine the effect of single dose of 20 mg ambroxol hydrochloride on cough severity visual analogue scale (VAS) and urge-to-cough VAS in subjects with acute cough related to upper respiratory tract viral infection.
- To evaluate changes in cough reflex sensitivity during acute cough associated with URTI in relation to the healthy (post-recovery) state.
- To evaluate changes in cough severity and urge to cough VAS during acute cough in relation to the post recovery state.
- To assess the safety and tolerability of ambroxol

3.0   STUDY DESIGN AND PROCEDURES

This was an open label, single centre exploratory study in patients with acute cough related to respiratory tract infection.

There was a Screening/Baseline and combined Treatment visit. Subjects return approximately 1-4 weeks after their treatment once all symptoms had subsided and the subject was no longer suffering from URTI, for a Follow-up Visit.

14 subjects with acute cough in relation to a URTI and who meet all entry criteria were be assigned to:

Treatment	p.o 20mg ambroxol lozenge
(Lysopain Ambr MintM)

At the Screening Visit/Baseline Visits and during Treatment phase, cough sensitivity (Section 5.1) was measured by standard clinical methodology incorporating four cough challenges (capsaicin, ATP, citric acid and distilled water).

Refer to the Schedule of Assessments and Procedures located at the end of the SAP (Appendix A) for additional details. The study includes the following study periods and scheduled visits:

Screening

The purpose of the Screening/baseline 1a visit, was to ensure that each subject met all the specified inclusion and none of the exclusion criteria.

Baseline Visit 1a

Subjects who satisfied all entry criteria at Screening continued in to the Baseline cough 1a visit for cough challenge assessments.

Treatment Phase 1b

Subjects who satisfied all entry criteria at baseline were then administered 20 mg ambroxol in the form of a lozenge, which was sucked until fully dissolved in the mouth.
The treatment was administered in the afternoon of the same visit day as the screening/baseline assessments.
The series of abbreviated cough challenges were conducted at 30 min and 90 min post dose.

Telephone Contact

Patients were contacted by phone once/week to establish if all symptoms associated with URTI has abated. Once this had been confirmed the patients were booked in for a follow-up visit.

Follow-Up/Early Withdrawal Visit

Subjects returned within 4 weeks after treatment for a Follow-Up Visit once symptoms of URTI had abated. A series of full cough challenges were performed at this visit to determine reduction in cough hypersensitivity.

3.1 Treatment Assignment

All subjects meeting inclusion/exclusion criteria were assigned to the 20mg ambroxol lozenge (Lysopain Ambr MintM).

4.0 INTERIM ANALYSES

No interim analysis was planned for this study.

5.0 EFFICACY ASSESSMENTS

5.1 Cough Reflex Sensitivity

Cough reflex sensitivity is measured by standard clinical methodology incorporating capsaicin, ATP, citric acid and distilled water cough challenges. The endpoints measured in cough challenge testing are concentrations of the challenge agents inducing 2 or more (C_2) and 5 or more coughs (C_5). In the case of distilled water the number of coughs generated in 1 minutes exposure will be recorded.

Cough challenges were carried out in accordance with the Study Site Standard Operating Procedures. Concentration range is pre-defined for each cough challenge agent and listed as below:

Distilled Water % Distilled Saline Control:, 20%, 40%, 60%, 80%, 100%

Citric Acid Saline: 1 mM, 3 mM, 10 mM, 30 mM, 100 mM, 300 mM, 1M, 3M

Capsaicin Saline: 0.3 μM, 1 μM, 3 μM, 10 μM, 30 μM, 100 μM, 300 μM, 1000 μM

ATP Saline: 0.1 mM, 0.3 mM, 1 mM, 3 mM, 10 mM, 30 mM, 100 mM, 300 mM

Capsaicin, Citric Acid, ATP, and Distilled Water cough challenges will be administrated 10 minutes apart and in a specific order for each subject (citric acid, capsaicin, ATP and distilled water) to determine C2 and C5. Cough reflex sensitivity testing will be conducted at Baseline, at treatment phase 1b, 30 min and 90 min post dosing and at the Follow-up visit..

5.2   Cough Frequency and Cough Severity

5.2.1 Visual Analogue Scales

Subjects were to complete two visual analogue scales (VAS) at screening, prior to dosing and at 30 min and 90 min post dose at treatment phase 1b. A further two VAS forms were recorded at the follow-up visit.

The first scale was a 100mm horizontal line representing a scale of cough severity from 'No Cough' at the left hand end of the line up to 'Worst Cough' at the right hand end. The participant was to be instructed to draw a single vertical line on the scale to indicate how severe they felt their cough was during the previous 30 and 90 min at Treatment phase 1b and over last 24hrs at baseline and follow-up.

A second 100 mm scale was used to record the severity of their urge to cough but marked at the extremes as 'No urge-to-cough' and 'Worst urge-to-cough'. Again, the participant was instructed to draw a single vertical line on the scale to indicate how severe their urge to cough was during the previous 30 and 90 min at Treatment phase 1b and over last 24hrs at baseline and follow-up.

5.3   Efficacy Endpoints

5.3.1 Primary Efficacy Endpoints

The co-primary endpoints are the average C2 and C5 across three time points (at baseline, 30 min, and 90 min post-dose) for capsaicin, ATP, citric acid and distilled water challenge: concentration inducing at least 2 coughs and at least 5 coughs. The primary endpoints include:

- Average C2 and C5 for capsaicin challenge
- Average C2 and C5 for ATP challenge
- Average C2 and C5 for citric acid challenge
- Average C2 and C5 for distilled water challenge

5.3.2 Secondary Efficacy Endpoints

Secondary endpoints that will be assessed include:

- Urge to cough (UTC) 30 min and 90 min post last cough challenge
- Cough severity Visual Analog Scale (VAS) 30 and 90 min post last challenge.
- To evaluate changes in cough reflex sensitivity during acute cough associated with URTI in relation to the healthy (post-recovery) state

6.0 SAFETY ASSESSMENTS

6.1 General Safety Assessments

Safety was assessed through monitoring of adverse events/serious adverse events, physical examinations, vital signs, and 12-lead ECGs,

7.0 ANALYSIS POPULATION

7.1 Full Analysis Set (FAS)

All subjects who have taken at least 1 dose of study medication and provided at least 1 post-dose primary endpoint observation.

7.2 Per protocol set (PPS)

All subjects who were fully compliant with the protocol completing all visits of the study.

7.3 Safety Set

All subjects who have received at least 1 dose of study drug.

8.0 DEFINITION OF VARIABLES

8.1 Baseline

Unless otherwise stated, baseline is defined as the last observation recorded on or prior to the first study treatment.

8.2   Study Day

Treatment phase 1b is the date of study medication.

8.3   Age

Age will be derived as date of informed consent minus date of birth then divided by 365.25 and presented in years.

8.4   Prior and Concomitant Medications

Prior medications are all those medications that are not concomitant (for which there is enough information to determine that the medication was stopped prior to the dose of study treatment). Concomitant medications are those medications that begin after the dose of study treatment or are present at baseline but continue after the dose of study treatment. Medications missing both start and stop dates or having a start date prior to the start of study treatment and missing stop date will be counted as concomitant medication.

9.0   GENERAL STATISTICAL CONSIDERATIONS

Cough challenge analyses, cough frequency and urge to cough analysis and recovery will be conducted using SPSS (version.22) and Excel.

9.1   General Analysis Considerations

For continuous variables, descriptive statistics will include the number of subjects reflected in the calculation (n), mean, standard deviation, median, minimum and maximum. categorical variables will be summarized using frequency counts and percentages.
Individual subject data obtained from the electronic case report forms (eCRFs), ECG data, cough challenge, and any derived data will be presented by subject in data listings to facilitate the investigation of tabulated values and to allow for the clinical review. In general, the disposition and demographics tables will be presented by time point sequence. All efficacy and safety tables will be presented by time point. In general, listings will be displayed by sorted unique subject identifier. A subject identification number is defined as a 3 digit sequentially assigned subject id number (xxx)..

The analyses described in this plan are considered a priori, in that they have been defined prior to database lock. Any analyses not described herein performed subsequent to database lock will be considered post hoc and exploratory.

9.2   Data Handling Rules for Cough Challenge Data

For cough reflex sensitivity testing, if a subject didn't reach 5 or more coughs at a specific concentration of the challenge agent (i.e., missing C5), 1.5 times the maximum concentration level for the challenge agent will be imputed for the subject.

9.3   Determination of Sample Size

Because the effects of ambroxol on these challenge solutions are currently unknown it is impossible to provide an accurate power calculation. However, previous studies done in subjects with acute cough have demonstrated a significant change in C_2 and C_5 using 14 subjects. Since these are essentially experimental studies rather than trials of drug efficacy, studies of smaller effects, requiring a larger number of subjects are inappropriate and would not support the hypotheses being tested.

10.0   STATISTICAL ANALYSES

10.1   Subject Disposition

The number and percentage of subjects who completed the study and the number and percentage of subjects who were withdrawn will be presented. Subjects who discontinued prematurely will be summarized by number and percentage by reason for discontinuation (subject withdrew consent, adverse event, lost to follow-up, non-compliance, study terminated by sponsor, other).

Protocol deviations will be listed and may be summarized.

10.2   Eligibility Assessments

Subject's eligibility assessments will be listed.

10.3.1 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be listed. Variables include gender, age, and race.

10.3.2 Medical History

Medical history will be listed.

10.3.3 Screening Laboratory Tests

Pregnancy tests data will be listed.

10.4   Prior and Concomitant Medication

Prior and concomitant medications will be coded to anatomical therapeutic class and preferred name using the WHO drug dictionary. Listings and tabular summary of prior and concomitant medications will be presented by subject health status (health or chronic cough) at baseline.
All medications will be classified using the Anatomical Therapeutic Chemical (ATC) classification codes and preferred drug names from the World Health Organization Drug Dictionary (WHO-DD), Q1March2014.

For the medications recorded on CRF page "Prior and Concomitant Medications", medications with a stop date before the date of study drug will be considered prior medications. Medications with start date or stop date on or after the first date of study drug dosing will be considered concomitant medications.

Prior medications and concomitant medications will be presented in tabular form using the ATC Level 2 (pharmacological subgroup) and preferred term (PT). Frequencies and percentages will be presented. The counts of medications will also be summarized. The tables will be sorted by overall descending frequency of ATC Level 2, and then, within an ATC Level, by overall descending frequency of PT. If a concomitant medication has a start date prior to dosing date (Day 1) and continues over the study, will be considered as concomitant medications to be summarized in a separate table from the other concomitant medications with start date on or after dosing.

Partial or Missing Medication Start and Stop Dates

If the medication start date is incomplete, then it will be imputed as follows for the purpose of determining concomitance only:

   • If the start date is completely missing, the start date will be equal to the dose date. However, if the stop date is not missing and is before the dose date, then the stop date will be used instead.

   • If the start day is missing, the first day of the month will be used.

   • If the start day and month are missing, then the first day of the first month (January) will be used.

If the medication stop date is partial, then it will be imputed as follows for the purpose of determining concomitance only:

   • If the stop date is completely missing and the medication is not ongoing, the stop date will be equal to the dose date or date of completion/withdrawal, whichever is the latest.

- If the stop day is missing, the last day of the month will be used.

- If the stop day and month are missing, then the last day of the last month (December) will be used.

The aforementioned missing or partial data handling rules will be applied to the summary tables. The recorded missing or partial date values will be presented in the listings.

10.5   Efficacy Analyses

All efficacy analyses will be based on FAS set.

10.5.1 Primary Endpoints

Cough reflex sensitivity is measured by standard clinical methodology incorporating capsaicin, citric acid, ATP, and distilled water cough challenges. The standard end points measured in cough challenge testing are the concentrations of the challenge agents inducing 2 or more (C2) and 5 or more coughs (C5). In the case of distilled water the number of coughs generated in 30 seconds exposure will be recorded.

A natural log (LN) C2 and C5 is generally regarded as normally distributed within a population, so the treatment comparison will be performed using an ANOVA repeated measures model with Bonferroni corrected post hoc measurements. The ANOVA model will use all available data at 30 min and 90 min post dose with LN C2 and LNC5 as dependent variables and time points as independent variable.
The mean (in log scale) with the associated standard errors will be displayed which will be the average of C2 or C5 across three timepoints. Estimated differences from baseline along with corresponding 95% CIs and p-values will also be presented.

10.5.2 Secondary Endpoints

The secondary endpoints for subjects will be analysed using a one way ANOVA for repeated measures model with Bonferroni corrected post hoc measurements. The ANOVA model will use all available data at 30 min and 90 min post dose with Vas Scores as dependent variables and time points as independent variable.
The mean VAS scores with the associated standard errors will be displayed, Estimated differences from baseline along with corresponding 95% CIs and p-values will also be presented.

To analyse post-recovery change in C_2 and C_5, a mixed linear model will be performed with change from recovery in C_2 and C_5 concentration modeled as the response.

10.6   Safety Analysis

The safety analysis will utilize the Safety Population. Unless stated otherwise, all safety parameters will be summarized by baseline health status.

10.6.1 Adverse Events

Adverse events will be mapped to system organ classes and preferred terms using the MedDRA dictionary version 17.1. All adverse event summaries will be restricted to treatment emergent adverse events (TEAE), which are defined as those AEs occurring on or after dosing and those existing AEs worsening during the study. Adverse events will be summarized at onset of TEAE. Each TEAE will be assigned to a single treatment according to the date and time of onset. If an AE occurred during the wash-out period, the event will be counted under the treatment in Period 1. Then, if an AE occurred during 14-day follow-up post the Period 2, it will be counted under the treatment in Period 2.

For all AE tables, counting will be by subject and not by event within each period. In other words, if a subject has more than one event with the same preferred term in a given period, the subject will be counted once for that preferred term in the treatment group corresponding to that period. If a subject has more than one event in the same system organ class in a given period, the subject will be counted once for that system organ class in the treatment group corresponding to that period.

An overall summary of AEs will be presented. The overall summary will include the number and percentage of subjects experiencing any AEs, study drug related AEs, AEs by maximum severity, SAEs, study drug related SAEs, discontinued due to AE, and deaths. The number and percentage of subjects experiencing each AE, study drug related AE, and SAEs will be summarized according to system organ class and preferred term. An additional table including only preferred term summarized by treatment for the number and percentage of subjects experiencing each AE will be presented as well.

Complete subject listings of all AEs will be provided. For each AE the following will be specified: treatment group, start and stop dates, severity grade, MedDRA system organ class and preferred term, frequency, relationship to study treatment, action taken, outcome of the adverse event and seriousness.

Missing Severity or Relationship

Missing severity or relationship to study drug will prompt queries to be sent to the investigator to provide the information. If an event with missing severity cannot be resolved via query, severity

will imputed as severe. AEs with a missing relationship will be considered possibly/probably related for this summary.

Partial or Missing AE Start and Stop Dates

If the AE start date is incomplete, then it will be imputed as follows for the purpose of determining the onset of AE only:

> In the event that the day portion (and only the day portion) of the AE onset date is missing:

- If the start date is completely missing, the start date will be equal to the first dose date. However, if the stop date is not missing and is before the first dose date, then the stop date will be used instead.

- If the start day is missing, the day of the first dose date will be used for the event occurring at the same month and year of the first dose date; otherwise, the first day of the month will be used.

- If the start day and month are missing, then the first day of the first month (January) will be used.

If the AE stop date is partial, then it will be imputed as follows for the purpose of determining the duration of AE only:

- If the stop date is completely missing and the event is not ongoing, the stop date will be equal to the last dose date or date of completion/withdrawal, whichever is the latest.

- If the stop day is missing, the day of the last study day will be used for the event stopping at the same month and year of the last study day; otherwise, the last day of the month will be used.

- If the stop day and month are missing, then the last day of the last month (December) will be used.

The aforementioned missing or partial data handling rules will be applied to the summary tables. The recorded missing or partial date values will be presented in the listings.

10.6.2 Physical Examination

Physical examination information will be listed.

10.6.3 Vital Signs

Vital signs were collected at Screening, and Follow-Up. Vital signs will be summarized by visit. Variables summarized include, systolic blood pressure, diastolic blood pressure, pulse rate, respiratory rate, and temperature.

At each post-baseline vital signs assessment, observed vital signs results will be categorized into the categories according to the following criteria (Table 2) of potentially clinically important (PCI) findings. Vital signs including PCI findings will be listed.

Table 2: Potentially Clinically Important (PCI) Criteria for Vital Signs

Parameter	Clinically significant value/change
Pulse rate	$\leq$50 bpm and decrease from baseline $\geq$20 bpm $\geq$110 bpm and increase from baseline $\geq$20 bpm
Systolic BP	$\leq$90 mmHg and decrease from baseline $\geq$20 mm Hg $\geq$160 mmHg and increase from baseline $\geq$20 mm Hg
Diastolic BP	$\leq$50 mmHg and decrease from baseline $\geq$20 mm Hg $\geq$105 mmHg and increase from baseline $\geq$20 mm Hg
Respiration rate	$\leq$8 breaths/min and $\leq$-30% change from baseline $\geq$25 breaths/min and$\geq$30% change from baseline

10.6.5 12-Lead Electrocardiograms

12-Lead electrocardiograms (ECG) evaluations were collected at Screening, and follow –up. The observed results will be summarized descriptively by visit. ECG variables include heart rate, PR, QT, QTcF, RR intervals and QRS duration. Baseline for ECG is defined as the assessment performed at Screening.

At each post-baseline ECG assessment, observed ECG results will be categorized into the categories according to the following criteria (Table 3) of potentially clinically important (PCI) findings. ECG data (including PCI findings) will be listed.

Table 3:   Potentially Clinically Important (PCI) Criteria for ECG

ECG Measures	Threshold
Heart Rate	Post-baseline value $\geq$ 110 bpm Post-baseline value $\leq$ 40 bpm Increase from baseline $\geq$ 20 bpm to < 30 bpm Increase from baseline $\geq$ 30 bpm
QTcF	Post-baseline value $\geq$ 480 msec to < 500 msec Post-baseline value $\geq$ 500 msec Increase from baseline $\geq$30 msec to < 60 msec Increase from baseline $\geq$ 60 msec

12.0   APPENDICES APPENDIX A: SCHEDULE OF ASSESSMENTS

Phase	Screening/ Baseline	Treatment phase#	Telephone contact	Telephone contact (if required)	Telephone contact (if required)	FU/EW
Duration (Days)	Days 1a	Day 1b	Day 8	Day 15	Day 22	Day 8 - Day 29
Procedure/Assessment						
Written Informed consent	X					
Demographics; medical and medication history	X					
Inclusion/exclusion Criteria	X	X				
Vital signs	X					X
Weight and height	X					
Physical exam	X					X
ECG (12 lead)	X					X
Spirometry	X					
Urine pregnancy test	X					X
Study drug administration		X				
Cough challenges citric acid, capsaicin, ATP, distilled water 30 min between challenges	X					X
Abbreviated cough challenge 10 min between challenges.		X[a]				
Urge-to-cough and cough severity VAS	X	X[b]				X
AE monitoring		X	X	X	X	X
Concomitant medications	X					X
Symptom monitoring			X	X		

a.   Cough challenges begin 30 min and 90 min after dosing / each challenge separated by 10 min and in the same order as Baseline for each subject
b.   Perfomed at 30 min and 90 min post treatment
#.   Occurs same day as the successful completion of baselin