MARCH EME

Is the mechanism of action of hypertonic saline and/or carbocisteine in the treatment of patients with acute respiratory failure due to an increase in mucus hydration?

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STATISTICAL ANALYSIS PLAN

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Contents

STATISTICAL ANALYSIS PLAN 1

ABBREVIATIONS **4**

1. Background and Design **5**
1.1 Background 5
1.2 Hypothesis 5
1.3 Aim 5
1.4 Objectives 5

2. Outcome measures **6**
2.1 Primary outcome measure 6
2.2 Secondary outcome measures 6

3. Data **7**
3.1 CRF Forms and variables 7
3.2 Management of datasets 7
3.3 Data completion schedule 7
3.4 Data verification 7
3.5 Data coding 7

4. Definition of terms **8**

5. Sample Size Calculations **9**

6. Randomisation and blinding **10**
6.1 Randomisation 10
6.2 Blinding and Allocation Concealment 10

7. Analysis Principles **11**

8. Analysis Details **13**
8.1 Recruitment and follow-up patterns 13
8.2 CONSORT Flow Diagram 13
8.3 Baseline Characteristics 14
8.4 Trial treatment 15
8.5 Trial Outcomes 15

9. Additional Information **17**
9.1 Trial Steering Committee (TSC) 17

10. References **18**

11. Signatures of Approval **19**

Appendix 1: Example Summary Tables **20**

ABBREVIATIONS

ABBREVIATION	DEFINITION
ABG	Arterial Blood Gas
AE	Adverse Event
ANCOVA	Analysis of Covariance
ARDS	Acute Respiratory Distress Syndrome
ARF	Acute Respiratory Failure
CI	Confidence Intervals
CRF	Case Report Form
CSR	Clinical Study Report
DMEC	Data Monitoring and Ethics Committee
EME	Efficacy and Mechanism Evaluation
eTMF	Electronic Trial Master File
ICH	International Council for Harmonisation
ICU	Intensive Care Unit
ITT	Intent-To-Treat
MARCH	Mucoactives in Acute Respiratory failure: Carbocisteine and Hypertonic saline
NICTU	Northern Ireland Clinical Trials Unit
PEEP	Positive end expiratory pressure
PP	Per-Protocol
PPIE	Patient and Public Involvement and Engagement
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedures
TSC	Trial Steering Committee
UACM	Usual Airway Clearance Management

1. BACKGROUND AND DESIGN

1.1 Background

Patients with acute respiratory failure (ARF) experience an increased risk of respiratory tract secretion retention due to altered secretion rheology and impaired mucociliary clearance. The use of carbocisteine and hypertonic saline are now being evaluated in patients with ARF as part of the MARCH clinical trial and in this study we will investigate the mechanistic effects of these two mucoactive agents.

1.2 Hypothesis

Treatment of critically ill patients with ARF with carbocisteine, hypertonic saline, or both, will lead to increased mucus hydration and changes in sputum viscosity and elasticity.

1.3 Aim

To determine if critically ill patients with ARF treated with carbocisteine, hypertonic saline, or both, experience increased hydration of airway mucus (reflected as a reduced percentage mucus solid content[1]).

1.4 Objectives

Primary objective
To measure percentage mucus solid content (dry-to-wet weight ratio) of sputum at Day 0 and Day 3 in critically ill patients with ARF following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone.

Secondary objectives
1. Measurement of percentage mucus solid content (dry to weight ratio) at Day 0 and Day 7 following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone
2. Measurement of yield stress (T_c) (derived from sputum viscosity (G') and elasticity (G'')) at Day 0 and Day 3 following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone
3. Measurement of sputum levels of IL-6, IL-8 and 8-isoprostane at Day 0 and Day 3 following commencement of treatment with carbocisteine, hypertonic saline, or both or usual airway clearance management alone (no mucoactive)
4. Measurement of yield stress (T_c) (derived from sputum viscosity (G') and elasticity (G'')) at Day 0 and Day 7 following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone
5. Measurement of sputum levels of IL-6, IL-8 and 8-isoprostane at Day 0 and Day 7 following commencement of treatment with carbocisteine, hypertonic saline, or both or usual airway clearance management alone (no mucoactive)

Full details of the background to the trial and its design are presented in the protocol.

2. OUTCOME MEASURES

2.1 Primary outcome measure

The primary outcome is to measure percentage mucus solid content (dry-to-wet weight ratio) of sputum at Day 0 and Day 3 following commencement of treatment with carbocisteine and/or hypertonic saline.

2.2 Secondary outcome measures

The secondary outcome measure post commencement of treatment will be to measure:

1. Percentage mucus solid content (dry-to-wet weight ratio) of sputum at Day 7 following commencement of treatment with hypertonic saline and/or carbocisteine
2. Sputum elasticity (G') and viscosity (G'') (and yield stress, T_c) at Day 3
3. Sputum IL-6, IL-8 and 8-isoprostane levels at Day 3
4. Sputum elasticity (G') and viscosity (G'') (and yield stress, T_c) at Day 7
5. Sputum IL-6, IL-8 and 8-isoprostane levels at Day 7

3. DATA

3.1 CRF Forms and variables

Full details of the data to be collected and the timing of data collection are described in the trial protocol.

3.2 Management of datasets

Laboratory data will be collated using MS Excel and transferred via email directly to the NICTU Trial Statistician. The data will be stored in the eTMF (Electronic Trial Master File).

3.3 Data completion schedule

Sputum (endotracheal secretions) samples will be collected at 3 time-points during the study; Day 0 (baseline - at randomisation), Day 3 and Day 7.

3.4 Data verification

Study specific data validation checks will be implemented. The process of data validation ensuring the accuracy and quality of the data will be carried out prior to transfer to NICTU. This will involve an independent cross-check of the data sheet against the raw data, discrepancies that be resolved will be amended, if a discrepancy is unable to be resolved it will be removed from the data sheet and reason for absence will be documented.

3.5 Data coding

The data received will be continuous and therefore data coding is not applicable. The units of each variable will be noted beside the variable name in the received data tables.

Data from the main clinical trial database (MACRO) may also be used throughout the analysis for this EME sub study. Please refer to the MARCH Statistical Analysis Plan for further detail.

4. DEFINITION OF TERMS

Term	Definition
Per protocol population	Per protocol analysis will be undertaken on the population who receive the complete treatment dose. Patients randomised to receive usual airway clearance management (UACM) will be excluded from the per protocol analysis if they commence a non-trial mucoactive.
Compliance	Patients who receive ≥90% available doses will be considered as compliant.
Yield Stress	Measure of the amount of stress applied by the rheometer at which the elastic (G') and viscous (G'') moduli cross. The amount of force needed to start a flow or break a solid.

5. SAMPLE SIZE CALCULATIONS

We plan to analyse sputum samples (at Day 0 and Day 3) collected from 340 patients enrolled in the MARCH trial from across participating ICUs. All consecutive patients recruited to MARCH will be approached to participate in the current study. The main MARCH clinical trial is recruiting a target of 1956 patients across the four arms of the study. Therefore, the proposed sample size for this study should be easily achieved given the anticipated recruitment for the MARCH study.

The sample size has been determined with statistical input from the Northern Ireland Clinical Trials Unit to generate significant differences in the primary outcome of percentage mucus solids in sputum samples of MARCH patients. A sample size of 85 in each of our treatment groups will have 90% power to detect a difference in means of 1.15% assuming that the common standard deviation is 2.3% using a two-group t-test with a 0.05 two-sided significance level. This is equivalent to an effect size of 0.5. Assuming approximately 5% dropout we will collect sputum samples from 360 patients recruited to MARCH trial (90 per arm). If the level of dropout is greater than anticipated, we will continue to collect sputum samples (matched Day 0 and Day 3) towards a target of 340 patients. Although there are no examples of mucus solids measurements in patients with ARF, the sample size generated above is based on mucus solid measurements evaluated in patients with cystic fibrosis and chronic obstructive pulmonary disease[1], the closest disease examples to ARF we could find in the literature.

Importantly, there are likely to be missing samples on day 7 due to extubation, discharge, or death. However, we intend analysing these samples as they will still provide important mechanistic information regarding the effect of both drugs on this day.

6. RANDOMISATION AND BLINDING

6.1 Randomisation

Patients will be screened and recruited according to MARCH trial procedures.

6.2 Blinding and Allocation Concealment

The MARCH trial is an open label trial.

7. ANALYSIS PRINCIPLES

The primary analysis will be conducted on outcome data from the per-protocol population. Analyses will be two-sided and tested at an *a priori* significance level of p=0.05.

We will describe baseline characteristics and follow-up measurements using suitable measures of central tendencies; means and medians with the associated standard deviations/95% confidence intervals (CI) and interquartile ranges for continuous data; and frequencies and proportions for categorical data (including binary data).

Primary outcome

To measure percentage mucus solid content (dry-to-wet weight ratio) of sputum at Day 0 and Day 3 following commencement of treatment with carbocisteine and/or hypertonic saline.

Analysis

Percent solids (wt%) of sputum will be assessed by measurement of pre- and post-desiccation weights of sputum aliquots. Higher percentage weight indicates greater mucus solid content and is associated with disease[2] and lower percentage mucus solid content i.e. more hydrated indicates lower solid content which is associated with a more normal phenotype.

We will assess the sputum percent solids in all 4 patient cohorts on the MARCH trial at day 3 and compare to sample measurements at baseline (before commencement of treatment).

Comparisons will be made between the groups. Analysis of Covariance (ANCOVA) (and post-hoc tests if significant differences are identified), or non-parametric equivalents, will be used to detect difference in means adjusting for baseline values.

Secondary outcomes

The secondary outcome measure post commencement of treatment will be to measure:

1. Percentage mucus solid content (dry-to-wet weight ratio) of sputum at Day 7 following commencement of treatment with hypertonic saline and/or carbocisteine
2. Sputum elasticity (G') and viscosity (G'') (and yield stress, T_c) at Day 3
3. Sputum IL-6, IL-8 and 8-isoprostane levels at Day 3
4. Sputum elasticity (G') and viscosity (G'') (and yield stress, T_c) at Day 7
5. Sputum IL-6, IL-8 and 8-isoprostane levels at Day 7

Analysis

Percent solids (wt%) of sputum will be assessed at day 7, as for the primary objective analysis, and compared to sample measurements at baseline (before commencement of treatment).

Comparisons will be made between the groups using ANCOVA (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values.

Sputum will also be characterized by its elastic (G') and viscous (G") moduli in order to determine the yield stress value, T_c, a marker of sputum motion. A high T_c, indicates a greater amount of obstruction in the sample and a lower T_c, indicates a decreased amount of obstruction in the sample.

We will assess the sputum elasticity, viscosity and yield stress in sputum samples obtained from all 4 patient cohorts on the MARCH trial at Day 3 and day 7 and compare to sample measurements at baseline (before commencement of treatment).

We will also measure sputum levels of IL-6, IL-8 and 8-isoprostane in sputum samples from all 4 patient cohorts on the MARCH trial at Days 3 and 7 and compare to sample measurements at baseline (before commencement of treatment).

Comparisons will be made between the groups using ANCOVA (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values.

In addition, all analyses will be undertaken by comparison (i.e. Carbocisteine vs No carbocisteine and Hypertonic saline vs No hypertonic saline).

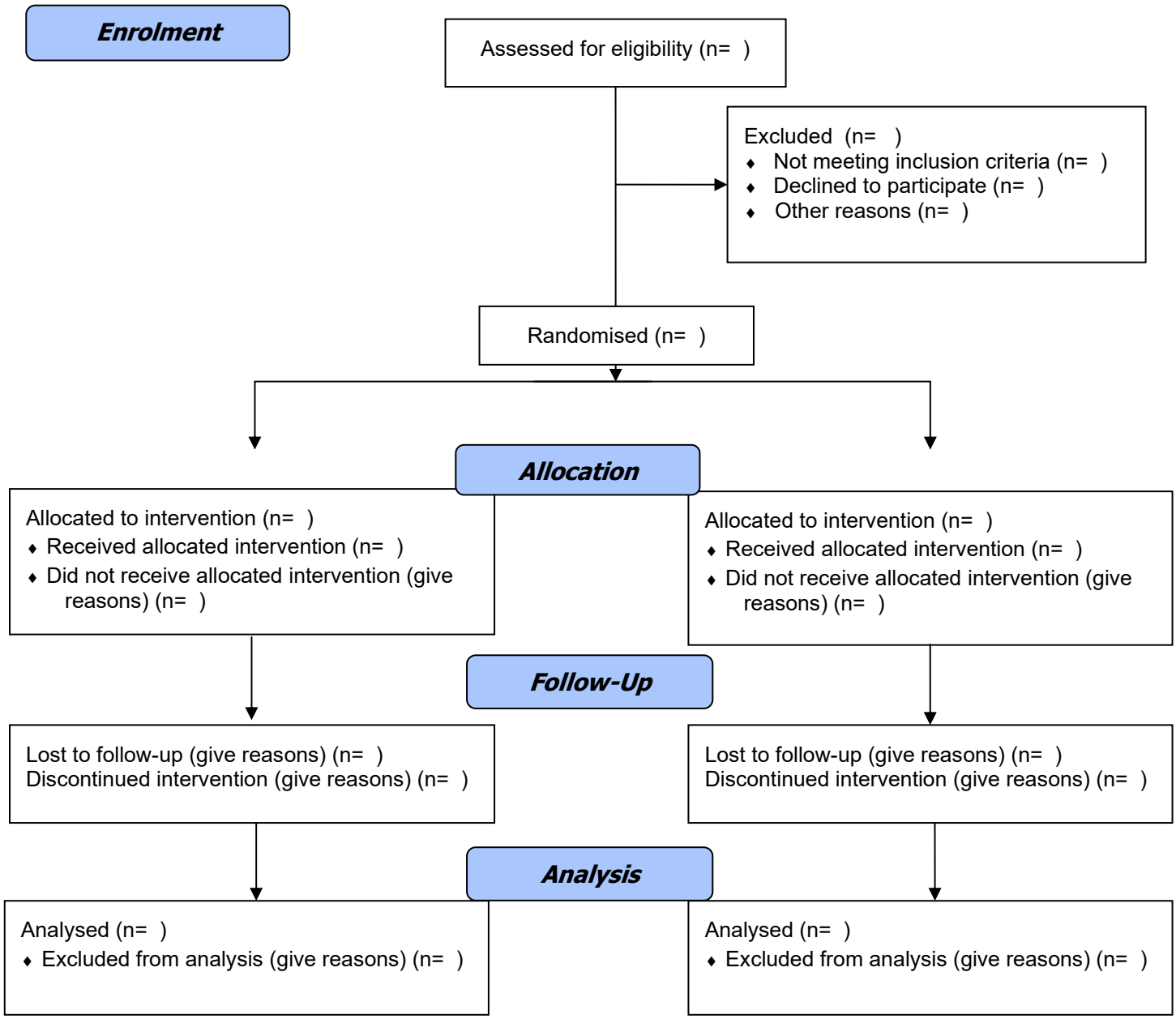
8. ANALYSIS DETAILS

The results of the analyses will be reported following the principles of the ICH E3 guidelines on the Structure and Content of Clinical Study Reports. Refer to the CONSORT Extensions for various trial designs.

8.1 Recruitment and follow-up patterns

- Recruitment by year, centre.
- Withdrawals by site - this should include the timing of withdrawals.

8.2 CONSORT Flow Diagram



8.3 Baseline Characteristics

- Age (years), mean(sd) by treatment arm
- Sex, n(%) by treatment arm
- Ethnicity, n(%) by treatment arm
- Temperature (°C), mean(sd) by treatment arm
- APACHE II Score, mean(sd) by treatment arm
- ABG (Worst)
    - PaO_2 (kPa), mean(sd) by treatment arm
    - FiO_2 %, mean(sd) by treatment arm
    - P/F Ratio (PaO_2/FiO_2), mean(sd) by treatment arm
- ABG (Closest to but prior to randomisation)
    - PaO_2 (kPa), mean(sd) by treatment arm
    - FiO_2 %, mean(sd) by treatment arm
    - P/F Ratio (PaO_2/FiO_2), mean(sd) by treatment arm
    - $PaCO_2$ (kPa), mean(sd) by treatment arm
    - Lactate (mmol/L), mean(sd) by treatment arm
    - pH, mean(sd) by treatment arm
    - H+ (only record if pH not available), mean(sd) by treatment arm
    - Bicarbonate (mmol/L), mean(sd) by treatment arm
- ARF Aetiology
    - Main admission diagnosis, n(%) by treatment arm
    - Pulmonary/Non Pulmonary Categorisation, n(%) by treatment arm
    - Presence of ARDS, n(%) by treatment arm
- Medical History
    - Is the patient currently receiving antibiotics to treat a pulmonary infection?, n(%) by treatment arm
    - Does the patient have a pre-existing chronic respiratory condition?, n(%) by treatment arm
    - Does the patient have a neurological diagnosis?, n(%) by treatment arm
    - Functional Co-morbidity Score, mean(SD) by treatment arm
    - Angina, n(%) by treatment arm
    - Anxiety or panic disorders, n(%) by treatment arm
    - Arthritis, n(%) by treatment arm
    - Asthma, n(%) by treatment arm
    - Chronic Obstructive Pulmonary Disease (COPD); Acquired Respiratory Distress Syndrome (ARDS); or Emphysema, n(%) by treatment arm
    - Congestive Heart Failure (or heart disease), n(%) by treatment arm
    - Degenerative Disc Disease (Back Disease, Spinal Stenosis or Severe Chronic Back Pain) , n(%) by treatment arm
    - Depression, n(%) by treatment arm
    - Diabetes Types I and II, n(%) by treatment arm
    - Hearing Impairments (very hard of hearing, even with hearing aids), n(%) by treatment arm
    - Heart Attack (Myocardial Infarction), n(%) by treatment arm
    - Neurological disease (such as Multiple Sclerosis or Parkinson's Disease), n(%) by treatment arm
    - Obesity and/or Body Mass Index > 30 (weight in kg/height in m^2), n(%) by treatment arm
    - Osteoporosis, n(%) by treatment arm
    - Peripheral Vascular Disease, n(%) by treatment arm

  - Stroke or Transient Ischaemic Attack, n(%) by treatment arm
  - Upper Gastrointestinal Disease (e.g. Ulcer, Hernia, Reflux), n(%) by treatment arm
  - Visual Impairment (e.g. Cataracts, Glaucoma, Macular Degeneration), n(%) by treatment arm
- Ventilation Parameters
  - Mode of ventilation, n(%) by treatment arm
  - Minute Volume (ml), mean(sd) by treatment arm
  - Total Respiratory Rate, mean(sd) by treatment arm
  - Plateau Pressure (cmH2O), mean(sd) by treatment arm
  - PEEP (cmH_2O), mean(sd) by treatment arm
  - Tidal Volume (Minute Volume/Total Respiratory Rate), mean(sd) by treatment arm
  - Respiratory Compliance (ml/cmH2O), mean(sd) by treatment arm
  - Driving Pressure (cmH_2O), mean(sd) by treatment arm
  - Was humidification used?, n(%) by treatment arm
  - Type of humidification, n(%) by treatment arm
- SOFA Score, mean(sd) by treatment arm

Will also be presented by comparison

8.4 Trial treatment

- Number of days on treatment, mean(sd) by treatment arm
- Number of doses, mean(sd) by treatment arm
- Treatment compliance, n(%) by treatment arm
- Reasons for termination of study drug, n(%) by treatment arm
- Did not receive allocated treatment, n(%) by treatment arm
- Received treatment of other group, n(%) by treatment arm
- Antibiotic Usage
  - Number of patients prescribed antibiotics at least once, n(%) by treatment arm
  - Number of days on antibiotics, mean(sd) by treatment arm
  - Overall dose (antibiotics), mean (sd) by treatment arm
- Usual Airway Clearance Management
  - Number of sessions, mean(sd) by treatment arm
- Non-trial mucoactive administration
  - No. patients with non-trial mucoactive administered, n(%) by treatment arm
  - Name of mucoactive, n(%) by treatment arm
  - Total number of days on non-trial mucoactive, mean(sd) by treatment arm
- Consent to Continue/Withdrawal of Consent , n(%) by treatment arm
- Protocol Deviations , no. events (%) by treatment arm, no. patients (%) by treatment arm

Will also be presented by comparison

8.5 Trial outcomes

Primary Outcome:

- Change from day 0 to day 3 in percentage mucus solid content (wt%), mean(SD) by treatment arm, difference in mean with 95% CI.

Comparisons will be made between the groups using ANCOVA (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values (Day 0 percentage mucus solid content (wt%)).

Secondary outcomes:

- Change from day 0 to day 7 in percentage mucus solid content (wt%), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 3 in Sputum elasticity (G'), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 3 in Sputum viscosity (G''), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 3 in yield stress (T_c), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 7 in Sputum elasticity (G'), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 7 in Sputum viscosity (G''), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 7 in yield stress (T_c), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 3 in Sputum IL-6, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 3 in Sputum IL-8, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 3 in Sputum 8-isoprostane, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 7 in Sputum IL-6, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 7 in Sputum IL-8, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from day 0 to day 7 in Sputum 8-isoprostane, mean(SD) by treatment arm, difference in mean with 95% CI.

Comparisons will be made between the groups using ANCOVA (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values.

Will also be presented by comparison

9. ADDITIONAL INFORMATION

9.1 Trial Steering Committee (TSC)

The Trial Steering Committee for the main MARCH clinical trial will also oversee this linked mechanistic study. This TSC provides oversight with respect to the conduct of the study on behalf of the Funder and Sponsor. An independent chair leads the TSC, with at least 75% independent membership. The TSC also includes the Chief Investigator, two Patient and Public Involvement and Engagement (PPIE) representatives, and a group of experienced critical care clinicians and trialists. The TSC meet at least annually, but more frequent meetings can be scheduled as required. Membership and roles of the TSC are listed in the TSC Charter. The TSC will take responsibility for monitoring and guiding overall progress, scientific standards, operational delivery and protecting the rights and safety of trial participants. Meetings will be formally minuted and stored in the Trial Master File. On occasion, observers may be invited and in attendance at TSC meetings.

10. REFERENCES

1. Hill DB, Vasquez PA, Mellnik J, et al. A Biophysical Basis for Mucus Solids Concentration as a Candidate Biomarker for Airways Disease. PLOS ONE 2014; 9(2):e87681. doi: 10.1371/journal.pone.0087681
2. Anand R, McAuley DF, Blackwood B, et al. Mucoactive agents for acute respiratory failure in the critically ill: a systematic review and meta-analysis. Thorax 2020; 75:623-631. doi: 10.1136/ thoraxjnl-2019-214355

11. SIGNATURES OF APPROVAL

Date: 26/11/2025
Version: 3.0
This document has completed a final review and is understood and approved by the following:

Cliff Taggart Chief Investigator Name	*Chief Investigator Signature*	Date dd/mm/yyyy
Christina Campbell Senior Statistician or designee Name	Campbell *Senior Statistician or designee Signature*	26/11/2025 Date dd/mm/yyyy
Christina Campbell Study Statistician Name	Campbell *Study Statistician Signature*	26/11/2025 Date dd/mm/yyyy

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Cliff Taggart Chief Investigator Name	*Chief Investigator Signature*	26/11/2025 Date dd/mm/yyyy
Christina Campbell Senior Statistician or designee Name	*Senior Statistician or designee Signature*	Date dd/mm/yyyy
Christina Campbell Study Statistician Name	*Study Statistician Signature*	Date dd/mm/yyyy

APPENDIX 1: EXAMPLE SUMMARY TABLES

Table x.x.x. Summary statistics for MARCH mucus hydration parameters

	Treatment Group			
	HTS	**Carbocisteine**	**HTS plus Carbocisteine**	**UACM**
	n=<n>	**n=<n>**	**n=<n>**	**n=<n>**
Sputum day 0				
Dry to wet ratio (wt %)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum elasticity (G') (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum viscosity (G'') (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Yield stress (Tc) (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum IL-6 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum IL-8 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum 8-isoprostane 9 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum day 3				
Dry to wet ratio (wt %)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum elasticity (G') (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum viscosity (G'') (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Yield stress (Tc) (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum IL-6 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum IL-8 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum 8-isoprostane 9 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum day 7				
Dry to wet ratio (wt %)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum elasticity (G') (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum viscosity (G'') (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Yield stress (Tc) (Pa)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum IL-6 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)

Sputum IL-8 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Sputum 8-isoprostane 9 (pg/mL)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)

Table x.x.x. Baseline Characteristics at trial entry

		HTS	Carbocisteine	HTS plus Carbocisteine	UACM
		n=	n=	n=	n=
Age (years)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
Sex	**Male**	n(%)	n(%)	n(%)	n(%)
	Female	n(%)	n(%)	n(%)	n(%)
Ethnicity	**White**	n(%)	n(%)	n(%)	n(%)
	Black	n(%)	n(%)	n(%)	n(%)
	Asian	n(%)	n(%)	n(%)	n(%)
	Unknown	n(%)	n(%)	n(%)	n(%)
	Other*	n(%)	n(%)	n(%)	n(%)
Temperature(°C)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
APACHE II Score		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
ABG (Worst)	**PaO_2 (kPa)**	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	FiO_2 %	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	P/F Ratio (PaO_2/FiO_2)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
ABG (Closest but prior to randomisation)	**PaO_2 (kPa)**	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	FiO_2 %	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	P/F Ratio (PaO_2/FiO_2)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	$PaCO_2$ (kPa)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Lactate (mmol/L)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	pH	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	H+ (nmol/L)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Bicarbonate (mmol/L)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
Admission Diagnostic Category	**Central nervous system**	n(%)	n(%)	n(%)	n(%)
	Cardiovascular system	n(%)	n(%)	n(%)	n(%)
	Respiratory system	n(%)	n(%)	n(%)	n(%)
	Gastrointestinal	n(%)	n(%)	n(%)	n(%)
	Hepatobiliary	n(%)	n(%)	n(%)	n(%)
	Renal and urinary	n(%)	n(%)	n(%)	n(%)
	Toxicology/poisoning	n(%)	n(%)	n(%)	n(%)

		HTS	Carbocisteine	HTS plus Carbocisteine	UACM
		n=	n=	n=	n=
	Haematology	n(%)	n(%)	n(%)	n(%)
	Orthopaedics/Trauma	n(%)	n(%)	n(%)	n(%)
	Infection	n(%)	n(%)	n(%)	n(%)
	Surgical and medical procedures	n(%)	n(%)	n(%)	n(%)
	Other*	n(%)	n(%)	n(%)	n(%)
ARDS Aetiology	Smoke/toxin inhalation	n(%)	n(%)	n(%)	n(%)
	Gastric content aspiration	n(%)	n(%)	n(%)	n(%)
	Near - drowning	n(%)	n(%)	n(%)	n(%)
	Thoracic trauma	n(%)	n(%)	n(%)	n(%)
	Pneumonia	n(%)	n(%)	n(%)	n(%)
	Sepsis	n(%)	n(%)	n(%)	n(%)
	Cardiopulmonary bypass	n(%)	n(%)	n(%)	n(%)
	Pancreatitis	n(%)	n(%)	n(%)	n(%)
	Non-thoracic trauma	n(%)	n(%)	n(%)	n(%)
	COVID-19				
	Other*	n(%)	n(%)	n(%)	n(%)
Currently receiving antibiotics to treat a pulmonary infection?		n(%)	n(%)	n(%)	n(%)
Pre-existing chronic respiratory condition?	COPD	n(%)	n(%)	n(%)	n(%)
	Asthma	n(%)	n(%)	n(%)	n(%)
	Lung Cancer	n(%)	n(%)	n(%)	n(%)
	Bronchiectasis	n(%)	n(%)	n(%)	n(%)
	Cystic Fibrosis	n(%)	n(%)	n(%)	n(%)
	Interstitial Lung Disease	n(%)	n(%)	n(%)	n(%)
	Sarcoidosis				
	Other*	n(%)	n(%)	n(%)	n(%)
Neurological diagnosis?	Head Injury	n(%)	n(%)	n(%)	n(%)
	Brain Tumor	n(%)	n(%)	n(%)	n(%)
	Epilepsy	n(%)	n(%)	n(%)	n(%)
	Stroke	n(%)	n(%)	n(%)	n(%)

		HTS	Carbocisteine	HTS plus Carbocisteine	UACM
		n=	n=	n=	n=
	Guillain-Barre Syndrome	n(%)	n(%)	n(%)	n(%)
	Other*	n(%)	n(%)	n(%)	n(%)
Functional Co-morbidity Score (Components)	**Angina**	n(%)	n(%)	n(%)	n(%)
	Anxiety or Panic Disorders	n(%)	n(%)	n(%)	n(%)
	Arthritis *(Rheumatoid or Osteoarthritis)*	n(%)	n(%)	n(%)	n(%)
	Asthma	n(%)	n(%)	n(%)	n(%)
	Chronic Obstructive Pulmonary Disease (COPD); Acquired Respiratory Distress Syndrome (ARDS); or Emphysema	n(%)	n(%)	n(%)	n(%)
	Congestive Heart Failure *(or heart disease)*	n(%)	n(%)	n(%)	n(%)
	Degenerative Disc Disease *(Back Disease, Spinal Stenosis or Severe Chronic Back Pain)*	n(%)	n(%)	n(%)	n(%)
	Depression	n(%)	n(%)	n(%)	n(%)
	Diabetes Types I and II	n(%)	n(%)	n(%)	n(%)
	Hearing Impairments *(very hard of hearing, even with hearing aids)*	n(%)	n(%)	n(%)	n(%)
	Heart Attack *(Myocardial Infarction)*	n(%)	n(%)	n(%)	n(%)
	Neurological disease *(such as Multiple Sclerosis or Parkinson's Disease)*	n(%)	n(%)	n(%)	n(%)
	Obesity and/or Body Mass Index > 30 (weight in kg/height in m^2)	n(%)	n(%)	n(%)	n(%)
	Osteoporosis	n(%)	n(%)	n(%)	n(%)
	Peripheral Vascular Disease	n(%)	n(%)	n(%)	n(%)
	Stroke or Transient Ischaemic Attack	n(%)	n(%)	n(%)	n(%)
	Upper Gastrointestinal Disease *(e.g. Ulcer, Hernia, Reflux)*	n(%)	n(%)	n(%)	n(%)
	Visual Impairment *(e.g. Cataracts, Glaucoma, Macular Degeneration)*	n(%)	n(%)	n(%)	n(%)
Functional Co-morbidity Score (Total)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)

			HTS	Carbocisteine	HTS plus Carbocisteine	UACM
			n=	n=	n=	n=
Ventilation Parameters	Mode of Ventilation	SIMV	n(%)	n(%)	n(%)	n(%)
		APRV	n(%)	n(%)	n(%)	n(%)
		HFOV	n(%)	n(%)	n(%)	n(%)
		BIPAP	n(%)	n(%)	n(%)	n(%)
		PC	n(%)	n(%)	n(%)	n(%)
		PRVC	n(%)	n(%)	n(%)	n(%)
		PS	n(%)	n(%)	n(%)	n(%)
		CPAP/ASB	n(%)	n(%)	n(%)	n(%)
		Other*	n(%)	n(%)	n(%)	n(%)
		None	n(%)	n(%)	n(%)	n(%)
	Minute Volume (ml)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Total Respiratory Rate		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Plateau Pressure (cmH_2O)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	PEEP (cmH_2O)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Tidal Volume (Minute Volume/Total Respiratory Rate)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Respiratory Compliance (ml/cmH_2O)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Driving Pressure (cmH_2O)		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Type of Humidification	None	n(%)	n(%)	n(%)	n(%)
		Heated Humidification	n(%)	n(%)	n(%)	n(%)
		Heat Moisture Exchange	n(%)	n(%)	n(%)	n(%)
		Other*	n(%)	n(%)	n(%)	n(%)
SOFA Score			xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)

Mean (SD) presented for continuous variables and no. (%) for all categorical variables.
*Other reasons may be summarised

Table x.x.x. Treatment after Trial Entry

		HTS	Carbocisteine	HTS plus Carbocisteine	UACM
		n=	n=	n=	n=
No. of days on treatment*		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
No. of doses		xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
Treatment Compliance	**Compliant**	n(%)	n(%)	n(%)	n(%)
	Not Compliant	n(%)	n(%)	n(%)	n(%)
	Compliance Rate (%)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
Did not receive allocated treatment		n(%)	n(%)	n(%)	n(%)
Received treatment of other group		n(%)	n(%)	n(%)	n(%)
Reasons for termination of study drug	**28 days elapsed since randomisation**	n(%)	n(%)	n(%)	n(%)
	First successful unassisted breathing (maintained for 48 hours)	n(%)	n(%)	n(%)	n(%)
	Study mucoactive related serious adverse event	n(%)	n(%)	n(%)	n(%)
	Discharge from ICU	n(%)	n(%)	n(%)	n(%)
	Death	n(%)	n(%)	n(%)	n(%)
	Discontinuation of active medical treatment	n(%)	n(%)	n(%)	n(%)
	Request from Legal Representative or Patient to withdraw from the trial	n(%)	n(%)	n(%)	n(%)
	Decision from the attending ICU physician that the study drug should be discontinued on safety grounds	n(%)	n(%)	n(%)	n(%)
	Other Reason**	n(%)	n(%)	n(%)	n(%)
Non-trial Mucoactive Administration	**Number of patients with non-trial mucoactives administered at least once**	n(%)	n(%)	n(%)	n(%)
	Carbocisteine	*n(%)*	*n(%)*	*n(%)*	*n(%)*
	Hypertonic Saline	*n(%)*	*n(%)*	*n(%)*	*n(%)*
	Heparin (non-anticoagulation purposes)	*n(%)*	*n(%)*	*n(%)*	*n(%)*
	N-acetylcysteine	*n(%)*	*n(%)*	*n(%)*	*n(%)*
	Dornase Alfa	*n(%)*	*n(%)*	*n(%)*	*n(%)*
	Other*	*n(%)*	*n(%)*	*n(%)*	*n(%)*
	Number of days on non-trial mucoactive	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
Antibiotic Usage	**Number of patients prescribed antibiotics at least once**	n(%)	n(%)	n(%)	n(%)

		HTS	Carbocisteine	HTS plus Carbocisteine	UACM
		n=	n=	n=	n=
	Number of days on antibiotic	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
	Overall dose	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
Usual Airway Clearance Management	**Number of sessions**	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)	xx.x(xx.x)
Post-randomisation withdrawal	**Mucoactive administration if ongoing**	n(%)	n(%)	n(%)	n(%)
	Ongoing data collection during hospital admission	n(%)	n(%)	n(%)	n(%)
	On-going data collection following hospital discharge	n(%)	n(%)	n(%)	n(%)
	Confirmation of vital status	n(%)	n(%)	n(%)	n(%)
	Use of samples collected to date	n(%)	n(%)	n(%)	n(%)
	Use of data collected to date	n(%)	n(%)	n(%)	n(%)
	Other**	n(%)	n(%)	n(%)	n(%)

*Mean (SD) no. of days on treatment

**Other reasons include <specify>

Table x.x.x Primary Outcome (Change from day 0 to day 3 in percentage mucus solid content (wt%))

	Treatment Group				p-value
	HTS	Carbocisteine	HTS plus Carbocisteine	UACM	
	n=<n>	n=<n>	n=<n>	n=<n>	
Percentage mucus solid content (wt%)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx

Comparisons will be made between the groups using ANCOVA (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values.

Table x.x.x Secondary Outcomes

	Treatment Group				p-value
	HTS	Carbocisteine	HTS plus Carbocisteine	UACM	
	n=<n>	n=<n>	n=<n>	n=<n>	
Change from day 0 to day 3					
Sputum elasticity (G')	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum viscosity (G'')	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Yield stress Tc (derived from G' and G'')	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum IL-6	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum IL-8	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum 8-isoprostane	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Change from day 0 to day 7					
Percentage mucus solid content (wt%)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum elasticity (G')	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum viscosity (G'')	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Yield stress Tc (derived from G' and G'')	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum IL-6	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum IL-8	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx
Sputum 8-isoprostane	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	0.xxx

Comparisons will be made between the groups using ANCOVA (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values.