CLEAR
(EME Sub Study)

Is the mechanism of action of hypertonic saline and/or carbocisteine in the treatment of patients with bronchiectasis due to a decrease in sputum viscoelasticity, inflammation and bacterial load?

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STATISTICAL ANALYSIS PLAN

Final v1.0 18/12/2024

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This document and all preceding versions will be stored in the Trial Master File for this trial

Contents

ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
CRF	Case Report Form
NICTU	Northern Ireland Clinical Trials Unit
CSR	Clinical Study Report
DMEC	Data Monitoring and Ethics Committee
ITT	Intent-To-Treat
PDRA	Postdoctoral Research Assistant
PP	Per-Protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOP	Standard Operating Procedures
TSC	Trial Steering Committee

1. BACKGROUND AND DESIGN

Research question:
Does treatment with hypertonic saline and/or carbocisteine decrease sputum viscosity, elasticity, inflammation and bacterial load in the lungs of patients with bronchiectasis?

Aims and objectives:
We will assess sputum samples obtained from the CLEAR trial for reductions in sputum viscosity and elasticity, reductions in inflammation and changes in bacterial abundance/composition for patients receiving Hypertonic Saline and/or Carbocisteine . Any changes in these sputum indices will be related to reductions in exacerbation frequency.

Primary Objective:
The primary objective is to measure sputum elasticity (G') and viscosity (G'') (which combined give a single summary measure of sputum viscoelasticity called the yield stress, T_c) at the initial visit and 2 weeks following commencement of treatment with Hypertonic Saline and/or Carbocisteine.

Secondary Objectives:
1). Measure sputum viscoelasticity (yield stress, T_c), at 8 weeks following commencement of treatment with HS and/or CS.
2). Evaluate sputum inflammation as measured by IL-6, IL-8 and 8-isoprostane levels at the initial visit, 2 and 8 weeks following commencement of treatment with Hypertonic Saline and/or Carbocisteine.
3). Evaluate sputum bacterial load/composition at the initial visits, 2 and 8 weeks following commencement of treatment with Hypertonic Saline and/or Carbocisteine.

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

2. OUTCOME MEASURES

2.1 Primary outcome measure(s)

The primary outcome measure at the initial visit and 2 weeks post commencement of treatment will be:

- Sputum elasticity (G')
- Sputum viscosity (G'')
- Yield stress T_c (derived from G' and G'')

2.2 Secondary outcome measures

The secondary outcome measure at the initial visits and 2 weeks post commencement of treatment will be:

- Sputum bacterial density (qPCR)
- Sputum bacterial composition (Next generation sequencing – NGS)
- Sputum IL-6
- Sputum IL-8
- Sputum 8-isoprostane

The secondary outcome measure at 8 weeks post commencement of treatment will be:

- Sputum elasticity (G')
- Sputum viscosity (G'')
- Yield stress T_c (derived from G' and G'')
- Sputum bacterial density (qPCR)

- Sputum bacterial composition (Next generation sequencing – NGS)
- Sputum IL-6
- Sputum IL-8
- Sputum 8-isoprostane

The overall aim of this study is to provide mechanistic insight into the action of Hypertonic Saline and/or Carbocisteine in bronchiectasis

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 will be collected at 3 time-points during the study – at the initial visit when patients are randomised to treatment (baseline), week 2 after commencement of treatment and week 8 after the commencement of treatment.

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 CLEAR Statistical Analysis Plan for further detail

4. DEFINITION OF TERMS

Term	Definition
Per Protocol	
Yield Stress Tc	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.
Compliance	

5. SAMPLE SIZE CALCULATIONS

The proposed study is a prospective, multi-centre, exploratory, cohort observational study embedded within the CLEAR trial. It will be conducted in a minimum of 14 Respiratory Clinics participating in CLEAR.

We will aim to recruit 72 patients sequentially enrolled to the CLEAR trial, at sites participating in the main CLEAR Trial. All consecutive patients recruited to CLEAR will be approached to participate in the current study. The Postdoctoral Research Assistant (PDRA) recruited to carry out sputum lab analysis (rheometry, bacterial and inflammation) will be blinded to the treatment allocation (Hypertonic Saline, Carbocisteine, Hypertonic Saline and Carbocisteine and Usual Airway Clearance Management) of each sample

6. RANDOMISATION AND BLINDING

6.1 Randomisation

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

6.2 Blinding and Allocation Concealment

The CLEAR trial is an open label trial. .

7. ANALYSIS PRINCIPLES

The primary analysis will be on the per-protocol population as we want to determine differences between groups of patients who adhered to the study drugs and at a significance level of 0.05 unless adjustment for multiple testing is needed.

Primary objective: The primary objective is to measure sputum elasticity (G') and viscosity (G'') (which combined give a single summary measure of sputum viscoelasticity called the yield stress, T_c) at the initial visit and 2 weeks following commencement of treatment with Hypertonic Saline and/or Carbocisteine.

Analysis:

Sputum will characterized by its elastic (G') and viscous (G") moduli in order to determine the yield stress value, Tc, 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 CLEAR trial at the initial visit and 2 weeks.

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

Secondary objectives:

1). Measure sputum elasticity (G') and viscosity (G'') (and yield stress, $T_{c)}$, at 8 weeks following commencement of treatment with Hypertonic Saline and/or Carbocisteine.
2). Evaluate sputum inflammation as measured by IL-6, IL-8 and 8-isoprostane levels at the initial visit, 2 and 8 weeks following commencement of treatment with Hypertonic Saline and/or Carbocisteine.
3). Evaluate sputum bacterial load/composition at the initial visit, 2 and 8 weeks following commencement of treatment with Hypertonic Saline and/or Carbocisteine.

Analysis:

Differences in sputum elasticity (G'), viscosity (G") and yield stress value, T_c, will be determined at 8 weeks, as for the primary objective analysis, and compared to sample measurements at baseline (before commencement of treatment). Further comparisons will be made between the groups using ANCOVA.

For bacterial abundance, and IL-6, IL-8 and 8-isoprostane measurements, comparisons will be made to sample measurements at baseline (before commencement of treatment). Further, comparisons between the groups will also be made using ANCOVA.

The following analyses will be conducted outwith CTU and are therefore not detailed in this SAP.

Bacterial density and microbiota data will be combined with clinical and biomarker data and analysed using network and linear regression (univariate and multivariate regression modelling) and principal component analysis (PCA).

Intra-sample similarities/differences will be assessed through a number of ecological indices, such as sample richness (number of observed taxa), diversity (Shannon-Wiener index), community evenness and dominance. Factors explaining differences among microbial communities will be assessed through principal coordinates analysis (PCoA) of UniFrac distance values

(β-diversity measure used for comparing biological communities based on phylogenetic distance information), with the axes scaled by the percentage of the variance. The amount of variation explained will be presented for the first 2 principal coordinates. Furthermore, potential overlap of taxa within communities will be assessed through the construction of co-occurrence/ co-exclusion networks (31). The relationship between presence/absence of potentially pathogenic bacteria, the main ecological indexes of the metacommunity (i.e., taxonomic richness, Shannon-Wiener index of diversity, evenness, dominance) and 1º/2º endpoints will be assessed by regression analysis (coefficients of determination; residuals; significance) and by the correlation between relevant variables. Statistical analyses and graphical representations will be carried out in GraphPad Prism (version 8.00) and R version 3.4.2. (https://www.r-project.org/).

We will investigate associations between the rheometry, microbiology and inflammatory data and the clinical outcomes in CLEAR such as mean number of exacerbations and lung function.

Depending on the nature of the data, we will use appropriate methods of regression analysis and calculate estimates of effect and 95% Confidence Intervals to analyse the data and correlate between the study and the CLEAR outcomes.

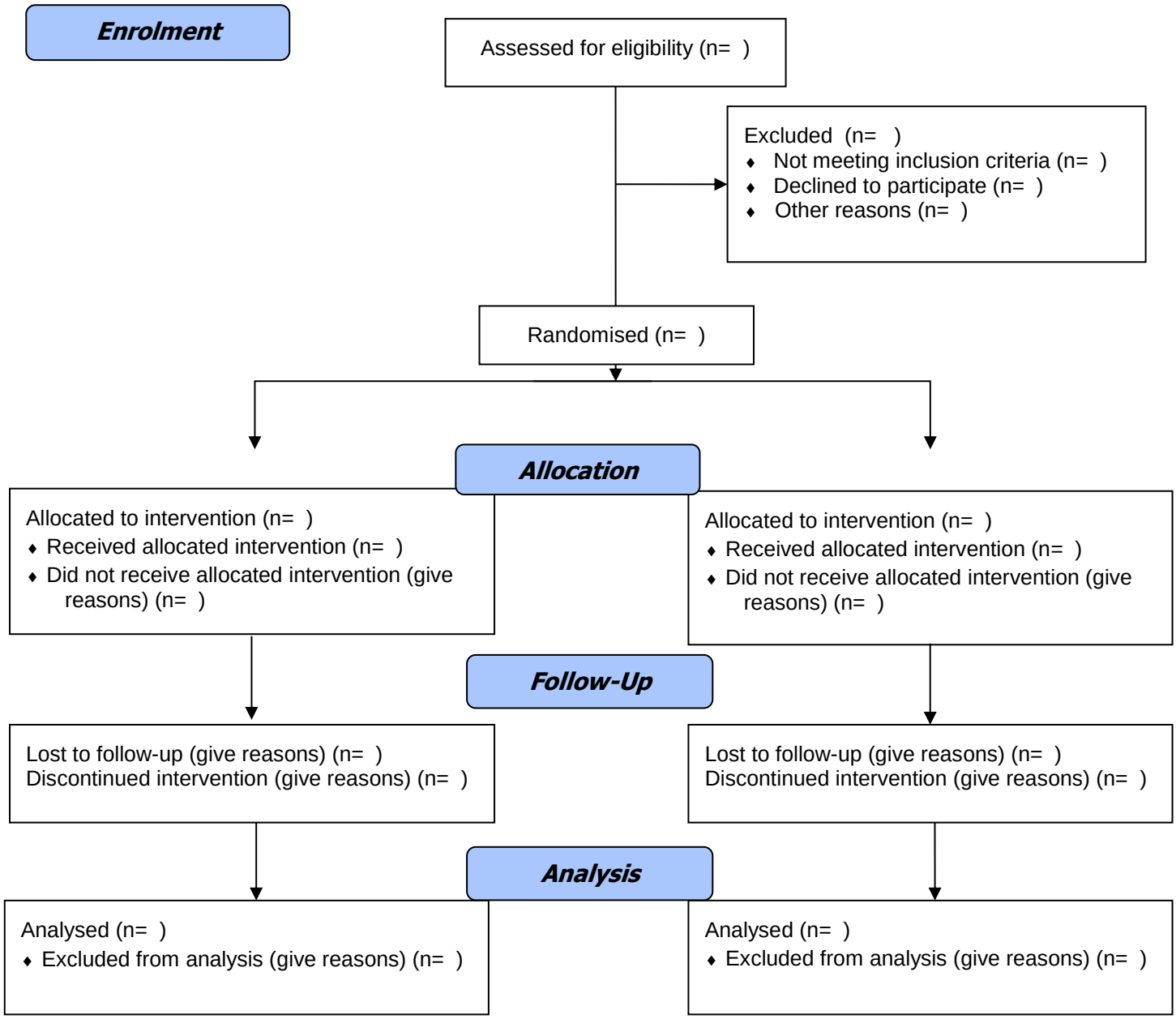
8. ANALYSIS DETAILS

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

- Gender, n(%) by treatment arm
- Age, mean(sd) by treatment arm
- Ethnicity, n(%) by treatment arm
- Cigarette Use, n(%) by treatment arm
- Pack Years, mean(sd) by treatment arm
- E-cigarette use, n(%) by treatment arm
- Height, mean(sd) by treatment arm
- Weight, mean(sd) by treatment arm
- Temperature, mean(sd) by treatment arm
- Blood Pressure, mean(sd) by treatment arm
- Pulse, mean(sd) by treatment arm
- Respiratory Rate, mean(sd) by treatment arm
- SpO2, mean(sd) by treatment arm

8.4 Trial treatment

- Study drug dispensed, n(%) by treatment arm
- Time from randomisation to start of treatment (days), mean(sd) by treatment arm
- No. of doses received, mean(sd) by treatment arm
- Adherence(Good (>=79%); Moderate (<79% to >=50%); Low (>50% to >=21%) and Very Low (<21%)), n(%) by treatment arm
- Did not receive allocated treatment, n(%) by treatment arm
- Received treatment of other group, n(%) by treatment arm
- Reasons for termination of study drug, n(%) by treatment arm
- Post-randomisation withdrawal, n(%) by treatment arm
- Protocol violations, no. events (%) by treatment arm, no. patients (%) by treatment arm

8.5 Trial Outcomes

Primary Outcome:

- Change from baseline to week 2 in Sputum elasticity (G'), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 2 in Sputum viscosity (G''), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 2 in Yield stress Tc (derived from G' and G''), mean(SD) by treatment arm, difference in mean with 95% CI.

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

Secondary outcomes:

- Change from baseline to week 8 in Sputum elasticity (G'), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 8 in Sputum viscosity (G''), mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 8 in Yield stress Tc (derived from G' and G''), mean(SD) by treatment arm, difference in mean with 95% CI.

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

- Change from baseline to week 2 in Sputum IL-6, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 2 in Sputum IL-8, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 2 in Sputum 8-isoprostane, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 8 in Sputum IL-6, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 8 in Sputum IL-8, mean(SD) by treatment arm, difference in mean with 95% CI.
- Change from baseline to week 8 in Sputum 8-isoprostane, mean(SD) by treatment arm, difference in mean with 95% CI.

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

9. ADDITIONAL INFORMATION

9.1 Trial Steering Committee (TSC)

A group of experienced clinicians, trialists and lay people will act as a TSC. The TSC will provide oversight with respect to the conduct of the study on behalf of the Funder and Sponsor.

The TSC will have at least 75% independent member ship. It will include the CI, independent clinicians (1 of whom will act as chair) and lay representatives. The TSC will meet during the course of the trial and observers may be invited and be in attendance at TSC meetings, such as the Sponsor or Funder representatives or the Trial Manager to provide input on behalf of the CTU.

A TSC charter will be drawn up to detail the terms of reference of the TSC including membership and roles/responsibilities.

9.2 Data Monitoring and Ethics Committee (DMEC)

The role of the DMEC is to safeguard the rights, safety and wellbeing of trial participants, monitor data and make recommendations to the TSC on whether there are any ethical or safety reasons why the trial should not continue and monitor the overall conduct of the study to ensure the validity and integrity of the study findings. The DMEC will meet annually.
The DMEC will comprise independent members with at least one statistician and two respiratory specialists. A DMEC charter will be drawn up to detail the terms of reference of the DMEC including membership and roles/responsibilities.

10. REFERENCES

List any references used.

11. SIGNATURES OF APPROVAL

Date: 18th December 2024
Version: 1.0
This document has completed a final review and is understood and approved by the following:

See attached email

Cliff Taggart Chief Investigator Name	*Chief Investigator Signature*	Date dd/mm/yyyy
Clíona McDowell Senior Statistician or designee Name	*Senior Statistician or designee Signature*	18/12/2024 Date dd/mm/yyyy
Christina Campbell Study Statistician Name	*Study Statistician Signature*	18/12/2024 Date dd/mm/yyyy

APPENDIX 1: EXAMPLE SUMMARY TABLES

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

Baseline Characteristics			Treatment Group			
			HTS	Carbocisteine	HTS plus Carbocisteine	Standard Care
			n=<n>	n=<n>	n=<n>	n=<n>
Gender	Male		n(%)	n(%)	n(%)	n(%)
	Female		n(%)	n(%)	n(%)	n(%)
Age (years)			xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Ethnicity	White		n(%)	n(%)	n(%)	n(%)
	Non-White		n(%)	n(%)	n(%)	n(%)
Cigarette Use	Never Smoked		n(%)	n(%)	n(%)	n(%)
	Ex-Smoker					
		Cigarettes	n(%)	n(%)	n(%)	n(%)
		No. smoked per day	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
		No. years smoking	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
		Pack years	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
		No. years stopped	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
		Pipe	n(%)	n(%)	n(%)	n(%)
		No. smoked per day	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
		No. years smoking	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
		Pack years	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
		No. years stopped	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
E-cigarette use	Never vaped		n(%)	n(%)	n(%)	n(%)
	Ex-vaper		n(%)	n(%)	n(%)	n(%)
	Current Vaper		n(%)	n(%)	n(%)	n(%)
Height (m)			xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Weight (kg)			xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Temperature (°C)			xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Blood Pressure			xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Pulse			xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Respiratory Rate			xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
SpO2			xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)

- Mean (SD) (or median[IQR] if appropriate) presented for continuous variables and no. (%) for all categorical variables.

- **Table x.x.x. Treatment after Trial Entry**

		Treatment Group			
		HTS	**Carbocisteine**	**HTS plus Carbocisteine**	**Standard Care**
		n=<n>	**n=<n>**	**n=<n>**	**n=<n>**
Treatment Adherence					
Study drug dispensed		n(%)	n(%)	n(%)	n(%)
Time from randomisation to start of treatment (days)		xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
No. of doses received*		xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Adherence	**Good (≥79 %)**	n(%)	n(%)	n(%)	n(%)
	Moderate (<79 % and ≥50%)	n(%)	n(%)	n(%)	n(%)
	Low (<50 % and ≥21%)	n(%)	n(%)	n(%)	n(%)
	Very Low (<21 %)	n(%)	n(%)	n(%)	n(%)
Did not receive allocated treatment		n(%)	n(%)	n(%)	n(%)
Received treatment of other group		n(%)	n(%)	n(%)	n(%)
Reasons for termination of study drug					
Patient has completed 52 weeks of intervention		n(%)	n(%)	n(%)	n(%)
Adverse Event		n(%)	n(%)	n(%)	n(%)
Serious Adverse Event		n(%)	n(%)	n(%)	n(%)
Protocol violations or non compliance as determined by the PI		n(%)	n(%)	n(%)	n(%)

Intercurrent significant illness	n(%)	n(%)	n(%)	n(%)
Occurrence of intolerable side effects	n(%)	n(%)	n(%)	n(%)
Patient request	n(%)	n(%)	n(%)	n(%)
Decision by the PI the study drug should be discontinued on safety grounds	n(%)	n(%)	n(%)	n(%)
Death	n(%)	n(%)	n(%)	n(%)
Complexity of study	n(%)	n(%)	n(%)	n(%)
Increased hypertonic intolerance	n(%)	n(%)	n(%)	n(%)
Other	n(%)	n(%)	n(%)	n(%)
Post-randomisation withdrawal				
Withdrawal of consent	n(%)	n(%)	n(%)	n(%)
Refused use of data already collected	n(%)	n(%)	n(%)	n(%)
Refused data collection from NHS records	n(%)	n(%)	n(%)	n(%)

Table x.x.x Primary Outcome (change from baseline to week 2)

	Treatment Group				**p-value**
	HTS	**Carbocisteine**	**HTS plus Carbocisteine**	**Standard Care**	
	n=<n>	**n=<n>**	**n=<n>**	**n=<n>**	
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

Table x.x.x Secondary Outcomes

	Treatment Group				**p-value**
	HTS	**Carbocisteine**	**HTS plus Carbocisteine**	**Standard Care**	
	n=<n>	**n=<n>**	**n=<n>**	**n=<n>**	

Change from baseline to week 8					
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
Change from baseline to week 2					
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 baseline to week 8					
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