Supplemental Online Content

Wilson AM, Clark AB, Cahn T, Chilvers ER, Fraser W, Hammond M, Livermore DM, Maher TM, Parfrey H, Swart AM, Stirling S, Thickett DR, Whyte M, . Effect of Co-Trimoxazole (Trimethoprim-Sulfamethoxazole) vs Placebo on Death, Lung Transplant, or Hospital Admission in Patients With Moderate and Severe Idiopathic Pulmonary Fibrosis. *JAMA.* Published online December 8, 2020. doi:10.1001/jama.2020.22960

Supplement 2. Statistical analysis plan

This supplemental material has been provided by the authors to give readers additional information about their work.

NORWICH CTU

Statistical Analysis Plan (SAP)

The Efficacy and Mechanism Evaluation of Treating Idiopathic Pulmonary Fibrosis with the addition of Co-trimoxazole (EME-TIPAC)

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Version of SAP: 3.0

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1. Trial Background:

Background

EME-TIPAC is designed to evaluate the efficacy and mechanisms of action of Co-trimoxazole in individuals diagnosed with Idiopathic Pulmonary Fibrosis The intervention, Co-trimoxazole, is a broad spectrum antibiotic with bactericidal effects against respiratory pathogens. It is envisaged that this could lead to outcomes in terms of survival, but other outcomes include lung function and quality of life. The mechanism of action of the intervention is uncertain, it could act through anti-microbial effects by fighting infection or other have non-anti-microbial effects by inhibiting neutrophil activation and reducing neutrophil-derived oxidative stress. Part of the study is designed to test for a difference in the primary and secondary outcomes, but also in differences in various laboratory measurements to assess how the intervention might improve survival.

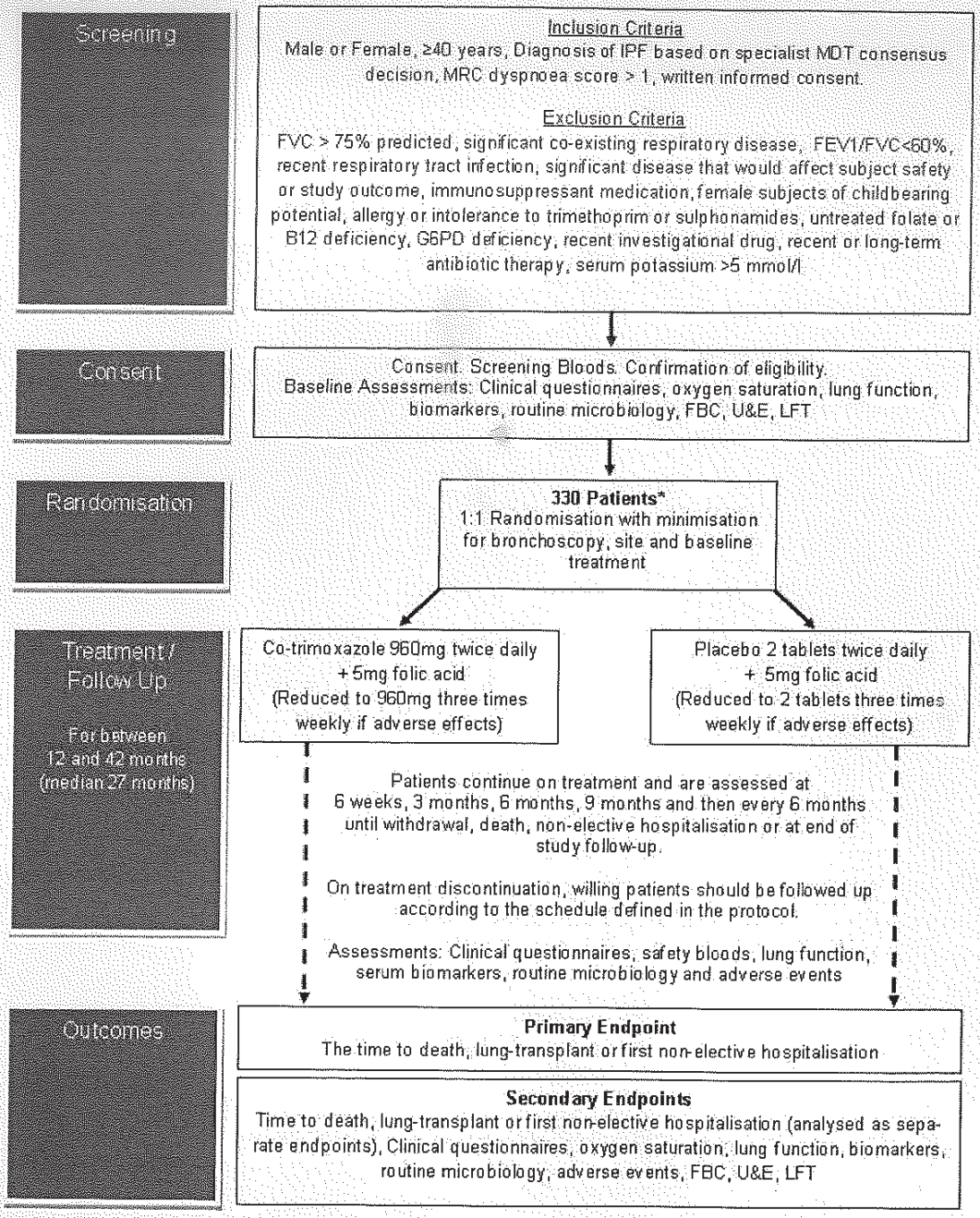
Full details of the background to the trial and the mechanism of action are given in the protocol.

Trial design

The study is a Phase III double blind, parallel group, 1 to 1 randomised placebo controlled multi-centre clinical superiority trial of oral co-trimoxazole versus placebo in 330 patients with moderate to severe (FVC <=75% predicted) IPF, with outcomes assessed during a median treatment period of 27 (range 12-42) months.

Trial diagram

The Efficacy and Mechanism Evaluation of Treating Idiopathic Pulmonary Fibrosis with the addition of Co-trimoxazole (EME-TIPAC)



2. Populations:

The primary analysis will use an Intention To Treat (ITT) population which includes all randomised individuals regardless of adherence to the study medication.

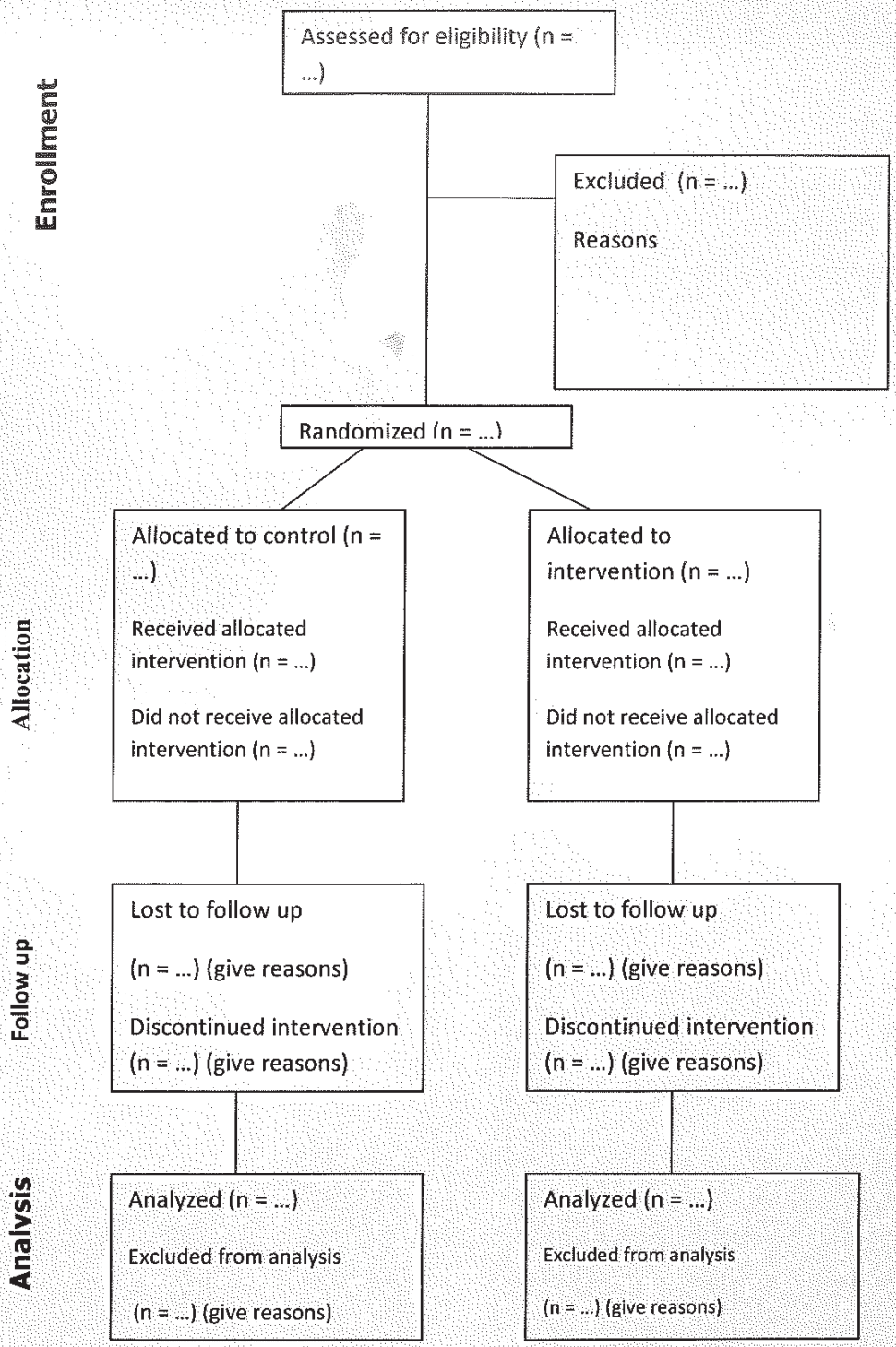
Further analyses will be performed using the following populations:

(a) Per-protocol: all randomised individuals who adhere to the study medication to within 80% (based on pill counts)
(b) Modified-per-protocol: all randomised individuals who adhere to the high-dose regime to within 80% (based on pill counts)
(c) Safety population: all patients randomised who received at least one dose of the study treatment

Patients who fail to take even one dose of medication will be included in the ITT analysis but excluded from further analyses (a), (b) and (c). Patients Randomised in Error (PRE) will be dealt with on a case by case basis and agreed prior to the start of analysis.

Individuals who have met the primary endpoint or have withdrawn consent for collection of any outcome will be censored at the last observation point for example data on the time until first hospitalisation will be censored at the time of death (if death occurs).

3. Flow of participants.



4. Objectives and Data

The primary objective is to compare the time to death (all causes), lung transplant or first non-elective hospital admission between co-trimoxazole and placebo arms in patients with moderate to severe (forced vital capacity (FVC) ≤ 75% predicted) IPF during a median treatment period of 27 months (range 12 to 42 months). Secondary objectives are to compare between co-trimoxazole and placebo arms: clinical efficacy in terms of respiratory-related hospital admission, death, health-related quality of life (King's Brief Interstitial Lung Disease questionnaire), quality of life adjusted years, cough score and quality of life, lung function and oxygen saturations. Exploratory mechanistic outcomes will include blood biomarkers such as those of infection / inflammation (C-reactive protein), disease progression (surfactant protein (SP– D) and Matrix Metalloproteinase (MMP)-7)) or neutrophil activity (myeloperoxidase (MPO), and blood will be taken for genetic testing to determine whether treatment response or adverse event profile is related to any genetic marker.

The schedule of data collection is summarised below

Table 1: Data collection schedule

	Enrolment	Randomisation	Post-allocation[1]						Close-out
TIMEPOINT	*-28 to -1 days*	*0*	*6 weeks*[2]	*3 months*	*6 months*	*9 months*[2]	*12 months*	*every 6 months.*	*End of study or first non-elective admission*
Informed consent	X								
Demographics etc	X								
Entry criteria	X								
Allocation		X							
Investigational Medicinal Product dispensed		X		X	X		X	X	
Safety bloods[3] (FBC, U&Es, LFTs)	X		X	X	X	X	X	X	X
B12, Folate, G6P4[4]	X								
DNA	X								
Biomarkers	X			X	X		X		X
K-BILD, MRC Breathlessness Score, EQ5D, Cough Score, Global Rating of Concept Scale	X			X	X		X	X	X
Leicester Cough Questionnaire, Global Rating of Change – QOL	X						X		
Full lung function	X			X	X		X	X	X
Microbiology (as clinically indicated)	X		X	X	X	X	X	X	X

Adverse events			X	X	X	X	X	X	X
BALF (subgroup) [5]	X			X					X

[1] Visits within the first 6 months should be within ±2 weeks, after 6 months visits should be within ±1 month of the schedule. Where possible, visits should be arranged prior to the time-point to ensure patients have sufficient supply of IMP available.

[2] Unless the patient is otherwise due to attend a clinic visit at the 6 week and 9 month time-points as part of their standard care, the safety bloods for these visits can be performed at the patients GP surgery and the patient followed up via telephone (to check for adverse events and any change in concomitant medication).

[3] Patients over 66 years old, with an initial Potassium between 4.7 and 5.0 mmol/L who are taking potassium sparing diuretics (including angiotensin converting enzyme inhibitors or angiotensin receptor blockers) are required to have an extra safety blood test 1 week after starting treatment (see Section 6.4.5 for more information)

[4] G6P is only required for patients of African, Asian or Mediterranean descent.

[5] BALF only performed by selected centres. 3 month BALF can be performed from 10 to 17 weeks after treatment

NB: Shaded cells are normally part of routine clinical care

Full details are provided in the protocol.

5. Outcomes/Endpoints

Other than the time-to-event outcomes, the outcomes are measured at the time points specified in section 4. Any outcomes measured out with the time interval will be treated as missing data.

Primary outcome

- Time from randomisation to death (all causes), lung transplant or first non-elective hospital admission for any reason.

Secondary efficacy outcomes

- Time from randomisation to respiratory related death;
- Time from randomisation to first respiratory related non-elective hospital admission;
- Time from randomisation to respiratory related death, first non-elective hospital admission for any reason or lung transplant.
- Time from randomisation to death (all causes)
- Time from randomisation to first non-elective hospital admission for any reason
- Time from randomisation to lung transplantation.
- Quality of life measured using
    - the King's Brief Interstitial Lung Disease (K-BILD) health related quality of life questionnaire;
    - the MRC Breathlessness Score;
    - the EQ5D quality adjusted life year's assessment;
    - cough score;
    - quality of life (Leicester cough questionnaire (LCQ));
- Lung function including assessment by spirometry and total lung diffusing capacity of carbon monoxide (DLCO). These will be the percent predicted based on scoring using the CRAPO equations.

Secondary outcome measures for safety (measured at local hospital laboratories)

- Full blood count
- Urea and electrolytes
- Liver function
- Adverse Events including SAEs

Further exploratory and mechanistic outcomes

The analysis of exploratory and mechanistic outcomes from the bloods will be detailed in a separate document. This is due to these being more exploratory and the actual measures extracted from the blood samples is yet to be decided.

Copies of the CRFs are available in the TMF.

6. Baseline data presentation and analyses

Descriptive statistics of baseline data will be reported by randomised group. No formal hypothesis tests will be undertaken. A skeleton table is given below, however it is noted that an edited version might be required for publication.

	Active Treatment	Placebo
Baseline characteristics		
Number in group		
Male participants: n (%)		
Age in years: mean (SD)		
Smoking status: never smoked: n (%)		
ex-smoker: n (%)		
current smoker: n (%)		
Comorbidities		
COPD: n (%)		
Bronchiectasis: n (%)		
Ischaemic Heart or Angina: n (%)		
GORD: n (%)		
Diabetes Mellitus: n (%)		
Osteoporosis: n (%)		
Pulmonary Hypertension: n (%)		
Anxiety or Depression: n (%)		
Lung Tests		
Absolute value of		
FVC: mean(SD)		
FEV1: mean(SD)		
DLCO: mean(SD)		
DLCO/VA: mean (SD)		
Percent predicted		
FVC: mean(SD)		
FEV1: mean(SD)		
DLCO: mean(SD)		

DLCO/VA: mean (SD)		
Minimisation factor		
Number(%) on licensed IPF medication		
Study Site		
Outcome measures:		
K-BILD: mean (SD)		
MRC: n(%)		
1		
2		
3		
4		
5		
EQ-5D utility: mean (SD)		
Cough score: mean (SD)		
Leicester cough questionnaire (LCQ): mean (SD)		
Global Rating of concept		
Global Change		

7. Treatment allocation

The allocated treatment for a patient will be generated via computer written code using minimisation. Minimisation will be performed using Taves' method with the factors measured at baseline: i) study site, ii) Bronchoscopy (yes/no); iii) and licensed medication for IPF (yes/no). In order to decide on the treatment allocation the code will calculate the number of patients in each group that have the same characteristics as the patient awaiting allocation; they will be allocated to the intervention with the smaller number with a high probability. If the numbers are the same then simple randomisation is used.

Full details of the minimisation algorithm (including the probability of allocation) were documented in a separate document (called Randomisation plan for EME-TIPAC) stored in a shared file accessible to only the study statistician and database manager.

It should be noted that the TSC agreed to stop recruiting participants who would agree to undergo Bronchoscopy on 1st August 2017.

8. Treatment Received (where applicable)

Returned tablet counts

The number of tablets returned will be summarised as the mean of the total over all follow-up visits and the percentage of individuals who are deemed to have taken more than 80% of the allocated tablets. For individuals who do not return pills this will be treated as missing data.

The full dose is 2 tablets, twice a day, which amounts to 124 tablets per month and 372 tablets per three months. The tablets are dispensed in bottles of 124 tablets. The modified dose is 2 tablets once a day three times a week which amounts to 26 tablets per month; 78 tablets per 3 months. Once a participant has switched to the modified dose the medication is dispensed in bottles of 78 tablets. Participants receive the first set of medications at randomisation which lasts for 3 months and receive a new set at 3 months follow-up and then at 6 months and then every subsequent 6 months. At each visit participants are asked to return any un-used medication.

The adherence is calculated per person over the whole of the study follow-up as:

$$Adherence = 100 \text{ x} \left(\frac{\text{number of pills taken}}{\text{expected number pills taken}} \right)$$

The expected number pills of that a participant is expected to have taken over the trial period is calculated as:

$$expected\ number = number\ of\ days\ full\ dose \text{ x } 4 + number\ of\ days\ reduced\ dose \text{ x } \frac{6}{7}$$

The number of pills taken is the number dispensed over the trial minus the number returned. It is recognised that this adherence measure could be greater than 100%, but the estimate will be capped at 100%.

Dose modification

The number (and percentage) of individuals who reduce dose from 2 tablets twice a day to 2 tablets once a day 3 times a week will be given in tabular form as follows:

	Active Treatment		Placebo	
	Number of participants	Number (%) of dose modifications	Number of participants	Number (%) of dose modifications
3 month visit				
6 month visit				

9. Efficacy

The outcome measures will be summarised by group at each time-point for questionnaires and non-time-to-event data. For time-to-event data the number of events and the estimated rate of events will be presented.

The primary outcome will be analysed using a Cox proportional hazards model adjusted for the variables included in the minimisation algorithm (bronchoscopy, baseline licensed IPF medication, site). The results will be presented as the Kaplan-Meier estimate of the survival function for each treatment arm separately and if appropriate the median will be estimated. The treatment effect size will be the hazard ratio and estimated with 95% confidence intervals and p-value.

The time until death and time until non-elective hospital admission will be analysed using Cox proportional hazards models adjusted for the variables included in the minimisation algorithm, (bronchoscopy, baseline licensed IPF medication, site). The results will be presented as the cumulative incidence function survival functions for each treatment arm separately and if appropriate the medians will be estimated; this is due to the Kaplan-Meier estimate being biases when competing risks are present (Austin et al 2016). The treatment effect sizes will be the hazard ratios and estimated with 95% confidence intervals and p-value.

The time until respiratory related death and time until first respiratory related hospitalisation and time until first respiratory related event will be analysed using the same models as before. The proportional hazards assumptions will be formally tested using a Therneau and Grambsch test, if evidence of non-proportional hazards is found then a flexible parametric hazards model will be fitted (Royston and Parmar - SIM 2011)and the difference between groups will be estimated via the restricted mean survival time (RMST) at a suitably chosen time-point. The choice of time-point will be made without reference to the size of the difference only in terms of maximising power and being reasonably close to the last observed event time.

At 12 months post randomisation, the K-BILD, EQ5D, LCQ, spirometry (FVC per cent predicted, FEV_1 per cent predicted, FVC absolute value, FEV_1 absolute value and FVC/FEV_1 ratio) and DLCO will be analysed using linear models to compare the average values between the treatment arms adjusted for the variables included in the minimisation algorithm., Due to the potential of a small number of individuals from some sites, site will be included as a random effect. The effect size will be the mean difference and will be presented with 95% confidence intervals and p-values. Baseline values of the measure will also be used to give an adjusted analysis.

In addition to the above a repeated measures model will be done including all post-randomisation observations for all individuals, a time variable will also be included in the analysis. An additional random effect for patient will be included in the model. If there is a significant interaction between time-point and the difference between arms then the difference at each time-point will be reported, if there is no significant interaction then the overall difference will be reported.

The MRC Breathlessness Score and cough score will be analysed using a Mann-Whitney test to compare the distribution of the score between the treatment arms. A generalized effect size will be estimated and presented with 95% confidence intervals and a p-value.

Missing data that occur in secondary and mechanistic outcomes will be multiply imputed to increase precision of the treatment effect estimates. Sensitivity analyses will be conducted to assess the impact of the multiple imputations on the complete case analysis will also be conducted. All imputations will be examined to ensure sensible values are being generated. Imputation models will contain baseline measures, outcome measures and factors predictive of missing data. The total number of imputations approximately the same as the percentage of cases that are incomplete up to a maximum of 20 imputations.

Individuals who have met the primary endpoint or have withdrawn consent for collection of any outcome will be censored at the last observation point for example data on the time until first hospitalisation will be censored at the time of death (if death occurs).

Censoring

For all of the time-to-event outcomes the following defines the censoring rules that will be applied

Outcome	Censoring dates if it occurs before event date
Time from randomisation to death (all causes), lung transplant or first non-elective hospital admission for any reason.	Date of withdrawal
Time from randomisation to death (all causes)	Date of withdrawal Date of first hospitalisation Date of lung transplant
Time from randomisation to first non-elective hospital admission for any reason	Date of withdrawal Date of lung transplant
Time from randomisation to lung transplantation.	Date of withdrawal Date of first hospitalisation
Time from randomisation to respiratory related death	Date of withdrawal Date of first hospitalisation Date of lung transplant
Time from randomisation to first respiratory related non-elective hospital admission	Date of withdrawal Date of lung transplant
Time from randomisation to respiratory related death, first non-elective hospital admission for any reason or lung transplant	Date of withdrawal

Additional analysis

In addition to the intention-to-treat analysis described above and compliance adjusted causal effect analysis will be conducted using instrumental variables (ivreg in Stata).

A subgroup analysis by disease duration (new – within 2 years of diagnosis vs old – more than 2 years of diagnosis) will be undertaken for the primary outcome by including an interaction in the selected model. The results will be summarised by the estimated effect in each subgroup.

10. Safety Analyses

For each Medra (version 22.0) term the number of individuals with at least one adverse event and the number of adverse events will be tabulated by treatment group as defined for the safety population. The percentage of individuals with at least one adverse event, by Medra term, will be compared using Fisher's exact test if the rate is low, or a logistic regression model adjusting for the factors used in the minimisation algorithm. The rate of adverse events, by Medra term, will be compared using either s Poisson or negative binomial regression model adjusting for the factors

used in the minimisation algorithm. The same analyses will be conducted for the serious adverse events.

The other safety outcomes (full blood count, urea, electrolytes and liver function), will be analyzed at each time-point separately using linear regression adjusting for the factors used in the minimisation algorithm.

11. Changes made to SAP at after first sign-off

This section will detail any changes to the SAP.

Change	Rationale	Date
It was noticed on review that the pre-specified outcomes of a) time from randomisation until a respiratory-related event; b) time from randomisation until first respiratory-related death; c) time from randomisation until first respiratory-related hospitalisation; were not fully included in the first SAP.	These were detailed in the protocol and published protocol paper as outcome measures and have now been added here.	22nd July 2019
After review of the results it was noticed that obvious errors were present in the lung function data, namely the DLCO. As such we implemented a rule to exclude DLCO data where the values for DLCO, percent predicted DLCO and DLCO/VA were excluded if the participant had a DLCO value of 15 or greater.	As stated in the file note NCTU_Q_TaT_4_v1.1File Notes – Post data lock DLCO exclusion these values are not physiologically possible for this participant group.	13th September 2019
Due to model convergence issues the use of 'site' as a random effect in the modelling of survival data was not done.	This was done to allow the model to run. It does not introduce any bias into the analysis.	13th September 2019
The biomarkers referred to in section 5 were MPO, SPD, MMP7, CRP, CA-125, CA19.9, PRO-BNPO, ORG, CCL18, TRAIL, MCP1 for full details see the appropriate lab analysis plan. The analysis was to compare the groups on the full ITT analysis only using a Mann-	The biomarker data was made available after the database lock, but during analysis period so it was decided to include them in the analysis.	15th November 2019

Whitney test at 12 months and a Mann-Whitney test on the change from baseline.		
The subgroup analysis of disease duration was not completed.	After discussion with the TMG is was noted that the date of diagnosis was not uniformly collected.	During analysis.
It was decided that the significant MEDRA groups identified in the safety analysis would have the most common subgroup analysed.	To try and help identify the important safety concerns.	February 2020
Global rating of concept and global rating of change were removed from section 5	These were never meant to be outcome measures, but rather used for secondary analyses of the questionnaire data.	During analysis

REFERENCES:

Austin, PC., Lee DS., and Fine JP (2016) . Introduction to the analysis of survival data in the presence of competing risks. *Circulation*. 133:601-609.

Royston, P. and Parmar, M.K.B. (2011). The use of restricted mean survival time to estimate the treatment effect in randomized clinical trials when the proportional hazards assumptions is in doubt. *Statistics in Medicine*, **30**, 2409-2421. https://doi.org/10.1002/sim.4274|