WP4 Statistical Analysis Plan

15/1/2019

The following provides the statistical analysis plan for Work Package 4 (WP4) trial. Its purpose is (i) to clarify the primary analyses, and (ii) to avoid misleading inferences that would arise from post-hoc analyses. Thus the statistical analysis plan should be drawn up in advance of looking at the outcome data.

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Background to WP4

The principal research question to be addressed by the trial is as follows:

"Can patients with incompletely recovered COPD exacerbations experience significantly increased time until next exacerbation if treated with a 1-week course of ciprofloxacin?"

The study is a multi-centre randomised controlled trial conducted in two hospitals, the Royal Brompton Hospital in London, University Hospital Aintree in Liverpool, St. Mary's Hospital in London, St. George's Hospital London.

Inclusion/exclusion criteria

Main Inclusion Criteria:

- Diagnosis of COPD confirmed spirometrically at screening.
- COPD exacerbation with treatment commenced 14 days prior to study enrolment and treated with 5-14 days of a non-quinolone antibiotic. Exacerbation here will be defined as an episode of symptomatic worsening of COPD that was treated by the patient's attending clinician. Confirmation of the initial exacerbation diagnosis will be provided from the case notes, referral letter, or directly from the treating clinician, and will be documented in the CRF.
- Age: ≥ 45 years of age at screening.

- Persistent symptoms and/or a CRP≥8mg/dL when assessed 2 weeks after exacerbation onset.
- Able to complete questionnaires for health status and symptoms and keep written diary cards.
- Severity of disease: Patients with a measured FEV1<80% of predicted normal values at 2 weeks post exacerbation.
- Able and willing to give signed and dated written informed consent to participate.

Main Exclusion Criteria:

- Other clinically predominant chronic respiratory disease.
- Intubated and receiving mechanical ventilation.
- Patients with known hypersensitivity to the antibiotic under evaluation, to other quinolones or any excipients of the IMP/placebo.
- Patients with a prior history of tendonopathy or tendon rupture.
- Elderly patients taking long term systemic corticosteroids.
- Patients on long term antibiotics for other conditions.
- Patient too unwell for randomisation, i.e. requiring retreatment in the judgment of the study doctor.
- Female patients who are pregnant or planning on becoming pregnant during the study, or are breastfeeding.
- Patient taking clinically significant contraindicated medication as per the SmPC, such as use of concomitant tizanidine or methotrexate.

Consent

The informed consent discussion will take place at the approved sites. The delegated individuals (as documented in the delegation log at sites) will be GCP trained, suitably qualified and experienced.

When patients present with an exacerbation and are treated with standard antibiotics, the patient's treating clinician will briefly discuss the study with the patient and obtain the patient's permission for the study team to approach them. This will be documented in the patient's medical records or source document. The study team

will then contact the patient, discuss the study in more detail, give or send the patient a PIS and ensure they have a minimum of 24 hours to consider this information before agreeing to take part. Patients expressing an interest in participation will be offered a screening appointment at the hospital 2 weeks after the start of treatment for the initial exacerbation

Prior to obtaining written consent at the screening visit, the delegated research staff will explain of the aims, methods, anticipated benefits and potential hazards of the study and give the patient opportunity to ask questions. It will also be explained to the patient that they are under no obligation to enter the trial and that they can withdraw at any time from the trial, without having to give a reason and without impact upon their usual clinical care.

Written informed consent will include consent for all trial related procedures, access to all written and electronic medical records and databases, and contact with the patient's usual medical team and general practitioner. A copy of the signed informed consent will be given to the participant. The original signed form will be retained at the study sites and placed in the TMF/SMF; a third copy will be kept with the paper CRF.

If new safety information results in significant changes in the risk/benefit assessment, the consent form will be reviewed and updated as necessary and subjects will be re-consented as appropriate.

No clinical screening or trial procedures will be conducted prior to taking consent from the participant. Consent will not denote enrolment into trial. All patients screened will be recorded on the screening log, along with a screening number. Those patients who are enrolled into the trial will be allocated a formal trial identifier at the point of randomisation, and this will be the study number used to identify them for all subsequent study procedures.

Randomisation and Blinding

- Randomisation will take place according to the trial specific randomisation SOP.

- Sealed Envelope™ (www.sealedenvelope.com) will be responsible for the randomisation process including production of the randomisation list. The randomisation list will be held by Sealed Envelope™ until data lock.
- Randomisation of patients will be conducted by delegated member of the research team at the sites using an internet randomisation service provided by Sealed Envelope, at visit 1 (screening visit) (if patient is eligible for the study following assessment of results), and after the patient has provided informed consent and baseline data (Class A method of randomisation).
- Patients will be randomised equally into the treatment (ciprofloxacin) or control (placebo) group (1:1 randomisation) using a computer-generated permuted block system of variable sizes, stratified by site and presence or absence of symptoms (as defined by any one or more of persisting breathlessness, increased sputum volume, increased sputum colour. This will be assessed by the standardised symptom questionnaire detailed in section 8.4).
- Upon randomisation, patients will be allocated a trial number and will be given a study specific patient card, which will have the study title, IMP details, patient trial number and the contact details of the PI/CI/research team and out of hours contact details in cases of emergency.
- Randomisation details will also be entered on to the trial subject log held in the site file.
- Following the randomisation process and the generation of a randomisation number, pharmacy at the research sites will be notified by an automatic email. The research fellow will give the study medication to the patient at the screening visit.

Study variables and endpoints

(i) Each centre provides information on patients not randomised into the trial (overall number, reasons for exclusion).

(ii) For randomised patients, baseline data are collected for a variety of characteristics, including

- Post-bronchodilator spirometry: Specifically the measurement of volume and flow of air inhaled and exhaled. The parameters measured will be the Forced vital

capacity (FVC) and Forced expiratory volume (FEV) at 1 second. The FEV1/FVC ratio and FEV1 as a percentage of predicted normal value will be computed to assess FEV1 and FVC. A minimum of three spirometry readings will be taken to assess FEV1 and FVC. The highest individual FEV1 and FVC readings will be used to calculate the FEV1/FVC ratio and for comparison with the predicted normal value. This will be taken at screening visit (V1), first follow up (V2), second follow up (V3), end of study (V4) and if patient presents with exacerbation during study (EX).

The spirometry will be considered post-bronchodilator if the patient has taken their usual long-acting bronchodilator medication OR if they have had a short-acting bronchodilator administered in clinic 10-15 minutes prior to spirometry testing.

- Diary Cards: These cards will be used to measure changes in symptoms/side effects, as well as in defining exacerbations for the primary endpoint, and therefore will be carefully explained to the patient at this time, and at every visit (as spirometry).
- General baseline characteristics: Age, gender, smoking status, self-reported exacerbation frequency in the previous year, Smoking history (pack years)
- Basic physiological measurements and clinical examination: These include blood pressure, oxygen saturations, pulse oximetry, respiratory rate, pulse rate, height, weight, BMI and a full clinical exam. These will all be performed in the usual manner, and measured at V1 and at exacerbation visit (EX).
- Pill count: This will be taken at V2.
- CRP Blood samples: Will be collected and analysed for CRP levels to measure presence of inflammation in the body. This is measured in milligrams per litre of blood (mg/L), and will be measured at every visit (as spirometry).
- Serum samples: will be taken at every visit (as spirometry) and stored at -80C for future analysis of inflammatory markers.
- Sputum samples: If spontaneously expectorated, the sputum samples will be collected and analysed to measure bacterial load in the lung. Concentrations will be log transformed and expressed as log10 CFU (colony forming units)/ml. Collection of sputum is not a requirement for study entry and this will therefore form a subgroup analysis. The sputum samples will be collected from patients at

every visit (as spirometry) and processed as clinical samples in the hospital microbiology laboratories for:

  - Analysis for bacterial numbers by quantitative culture methods.
  - Evaluation of any bacterial resistance by susceptibility testing +/- gene sequencing.

- Questionnaires (study specific symptom questionnaire, EQ5D, CAT, SGRQ) will be given to the patient to complete while in the clinic room. The study doctor will be on hand to answer any questions and give any necessary assistance.
- SGRQ: This is a standardized self-completed questionnaire for measuring impaired health and perceived well-being ('quality of life') in airways disease. It has been designed to allow comparative measurements of health between patient populations and quantify changes in health following therapy. It will be measured at V1 and V4.
- EQ5D: This is a standardised instrument for use as a measure of health outcome. It provides a simple descriptive profile and a single index value for health status. Health outcome at treatment end will be compared to baseline data to assess change in health status. It will be measured at V1 and V4.
- CAT: This is a standardised self-completed symptom questionnaire, and will be measured at every visit (as spirometry).

The flow of patients through the trial will be summarised in a CONSORT diagram, showing all exclusions including reasons for non-randomisation and drop-outs/lost to follow-up statistics. The CONSORT diagram will be stratified by randomised group.

Discontinuation/withdrawal of participants and 'stopping rules'

Patients will be withdrawn from the study only when they drop out, with reasons recorded wherever possible.

Patients will be discontinued from study medication but still followed-up in the case of:

- AE/SAE of any type which prevents continued participation in the study, with reasons recorded.

- Patient commences on medication that is contraindicated with the IMP (e.g. tizanidine). In this case the IMP will be discontinued and the patient will continue study procedures as per intention-to-treat protocol.
- SUSAR.

In all cases, when patients are discontinued from study medication, the following minimum information will be recorded in the CRF by the research fellow:

- Date of discontinuation
- Date last study medication taken
- Pill count/drug count
- A sputum sample taken if possible
- Any SAE/AE

Subjects who cease taking their randomised treatment (non-compliers) will continue to be followed up at the end of study visit (day 90), i.e. intention to treat analysis. They will not be "replaced" with a further randomised patient.

It is unlikely that the trial will be stopped prematurely as the study IMP is routinely used for a similar duration to that here. However If a number of SAEs occur that in the opinion of the TSC are deemed unacceptable (if IMP behaving outside the normal safety profile) then the trial will be prematurely stopped.

Compliance to the IMP

- Patients will be asked to complete a daily diary card for the whole period of the trial (i.e. 90 days) to record daily symptoms, patient status, and the need for further therapy. This will also include documentation of IMP compliance, and any missed doses of study medication, during the week of IMP therapy.

Sample size

Over the two centres HES data suggest that it is possible to identify at least 350 hospital admission or ambulatory exacerbations over the 18-month recruitment

period (http://www.hscic.gov.uk/hes). Based on data from the London COPD Cohort, 33% of exacerbating patients have a CRP of 8 or above at 2 weeks. We therefore expect that approximately 115 of the hospital admission or ambulatory exacerbations will be eligible for inclusion due to high CRP. Furthermore, our pilot data suggest that of patients with a normal CRP (<8mg/L), at least 60% of patients report symptoms at day 14; thus of all sampled patients we expect approximately 115+0.6(350-115)=256 to be eligible for inclusion into the trial. Assuming that 90% of eligible patients will agree to take part we initially hoped to randomise 231 patients in total.

Pilot data from our group suggests that at a level of CRP>10mg/l at 14 days, there will be 36% recurrent exacerbations within 50 days. Assuming a constant hazard of exacerbation this translates to around a 55% recurrent exacerbation rate at 90 days. A similar proportion of recurrent exacerbations are seen in the London COPD cohort (for both CRP>10 and CRP<10). An observational database study by Roede et al. (2008) reported a hazard ratio of 0.62 when antibiotics were given in addition to usual treatment.

Due to relocation from the Royal Free Hospital to the Royal Brompton Hospital, a more realistic sample size of 138 is now expected. We do not anticipate a change in the expected proportion of exacerbation-free survival at 90 days of 0.45, and we still allow for 5% dropout. This would result in:

- 90% power to detect a HR of 0.39 – an increase in survival at 90 days from 0.45 to 0.73;
- 80% power to detect a HR of 0.46 – an increase in survival at 90 days from 0.45 to 0.70.

The recruitment period for this trial is estimated to be 18 months, requiring approximately 20 exacerbations per month to be identified across both sites. It is likely that 2/3 of these will come from RFH and 1/3 from UHA.

Statistical Analysis Plan

Timing of Analyses

- Recruitment of the 138 patients began in the London site in July 2014, and should commence shortly at the Liverpool site.

- Data for completed patients should begin to become available in October 2014.

Descriptive analyses

(i) Should include CONSORT diagram describing patient flow with exclusions (including reasons for exclusions) and total numbers randomised to each treatment arm. This will include attendance at each visit and loss to follow-up (including follow-up time).

(ii) Baseline comparability of randomised groups:

- By December 2015 (approximately), recruitment should be completed, at which point tables of summary statistics will be produced. These tables will include (but are not limited to) the following baseline variables; age (continuous), sex, BMI, smoking status, smoking pack year history, centre, bacterial load, CRP (from blood sample), spirometry measurements, exacerbation history (number experienced in previous year) and questionnaire scores for each randomised group.
- Continuous variables will be summarised using the following statistics; n (non-missing sample size), mean, standard deviation, median, inter-quartile range, maximum and minimum. The number of missing observations will also be reported.
- The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. The number of missing observations will also be reported.

Primary outcome analysis

The primary outcome is the time to the next exacerbation within the 90-day study period. This will analysed using Cox proportional hazards regression, ~~stratified by centre~~. The estimated hazard ratio between the groups will be reported, along with its 95% CI. A Kaplan-Meier plot will be produced. Patients who do not suffer a recurrent exacerbation during follow-up will be treated as censored observations at their end of follow-up time. As analysis is intention to treat, non-compliers will be

treated as belonging to the treatment group originally randomised to. Where patients withdraw from follow up, every effort will be made to ascertain their primary outcome (i.e. date of next exacerbation OR exacerbation-free survival to 90 days) by telephone. ~~We will adjust only for number of exacerbations in previous year and centre in this primary analysis.~~

Variables:

- **Time until event or censoring:** *excn.or.cens.d*

  surv1 <- coxph(Surv(time, cens) ~ arm

A supporting analysis will be undertaken that adjusts for site and patient self-reported history of exacerbations in the previous year.

- **site:** *site*
- **Excns in previous year:** *prev.excns*
- **Arm:** [not yet released]

surv1 <- coxph(Surv(time, cens) ~ arm + strata(site) + prev.excns)

An additional supporting analysis will be undertaken, additionally adjusting for baseline covariates age; sex; FEV1% predicted; SGRQ total score; number of exacerbations in previous year; whether hospitalised for index exacerbation; whether index exacerbation was treated with steroids in addition to antibiotics; whether included due to symptomatic or inflammatory criteria, and smoking status. This will be for hypothesis generation only. It will not be used to come to overall conclusions regarding treatment efficacy; this will be treated as a secondary analysis.

- **Age (continuous):** *age.cts*
- **Sex:** *sex*
- **FEV1% predicted at baseline:** *fev.pred*
- **SGRQ total score at baseline:** *sgrq1.total*
- **Hospitalised for index excn (y/n):** *hospital.yn*
- **Index excn treated with steroids (y/n):** *steroids.given.yn*
- **Included due to symptomatic criteria or inflammatory criteria [to be queried]**
- **Smoking status:** *curr.smok*

surv2 <- coxph(Surv(time, cens) ~ arm + strata(site) + prev.excns + age.cts + sex + fev.pred + sgrq1.total + hospital.yn + steroids.given.yn + XXX[inclusion crit.] + curr.smok, data=wp4)

The percentage of missing data will be summarised and spurious data double checked as far as possible. Primary analysis will be intention to treat, and so every effort will be made to collect all follow-up data from non-compliers and patients who discontinue treatment. All patients will be analysed from the date of randomisation until end of follow-up period or the date of drop-out. The primary analysis will therefore assume patients who drop-out of the trial do so 'at random'.

Secondary outcome analysis

For each secondary outcome, two analyses will be undertaken – the first adjusting for centre and the baseline value of the endpoint being analysed (where available), and the second also taking into account the additional baseline characteristics specified in the supporting analysis of the primary outcome above.

When modelling continuous data, transformations will be undertaken as necessary to normalise the data. Where appropriate, analyses will take account of length of follow up as an offset, and also account for differences in at-risk periods. Summary measures of the secondary outcomes will be presented (mean, standard deviation, median, interquartile range for continuous variables, percentages for categorical variables) by treatment group and for both baseline and end of trial follow-up visits. The percentage of missing data will be summarised.

Secondary outcomes

- Time until *resolution* of first exacerbation will be compared between the active treatment and placebo groups, using Cox proportional hazards regression, stratified by centre.
    - **Duration of first exacerbation:** *dur*
- The number of treatment-related adverse events will be analysed by using negative binomial regression. Negative binomial regression models collapse to Poisson regression if there is no overdispersion.
    - **Number of treatment-related AEs:** *trt.rltd.aes*

- Exacerbation frequency: The rate of exacerbations experienced during the study period per person-year will be analysed by using negative binomial regression.

    - **Number of exacerbations:** *excns*

- Frequency of antibiotic courses: The rate of antibiotic courses received for patients in the active treatment and placebo groups will be compared using negative binomial regression.

- The rate of hospital attendances during the study period will be analysed by negative binomial regression. .

- Changes in respiratory healthcare status, assessed using SGRQ. SGRQ (score between 0 and 100from baseline to day 90 will be modelled as a normally distributed random variable. Linear regression will be used,

- Changes in respiratory healthcare status, assessed using CAT. CAT (score between 0 and 40) from baseline to day 90 will be modelled as a normally distributed random variable. Repeated measures analysis will be used to assess treatment effects over time during the follow-up period.

- Lung function: Changes in FEV1, FEV1 as % predicted, and FEV1/FVC ratio and FVC at day 90 from day 0 will be modelled separately as continuous outcomes and assumed to be normally distributed. Repeated measures analysis will be used to assess treatment effects over time during the follow-up period.

- Change in CRP from study entry to 90 days will be modelled as a normally distributed random variable. Repeated measures analysis will be used to assess treatment effects over time during the follow-up period.

- Resistance patterns (not resistant, resistant) will be analysed using logistic regression.

- The change in (log10) bacterial load from trial beginning to end will be analysed using parametric survival regression(linear regression with censored outcome data). Values below limits of detection will be treated as left censored.

- Adherence as assessed by pill counts. The number of pills taken over total number dispensed will be modelled using logistic. The difference in percent adherence will be assessed between treatment groups.

- Cost-effectiveness will be assessed as part of a future study (WP5)

This study is not powered to test these secondary outcomes – only the primary outcome. As such, analyses of the secondary outcomes will be hypothesis generating only.

Subgroup analyses

Relevant subgroups may be analysed (including patients who gave sputum samples), allowing the incorporation of any important new information and methodological concerns identified between now and the time of writing the analysis plan. However, such analyses should be undertaken before unblinding, and keeping in mind that any analyses will be hypothesis generating only.

Sensitivity analysis

In the primary analysis, sensitivity analyses will be undertaken: The efficacy of the IMP will be investigated using a per-protocol analysis. Such an analysis includes only patients who comply fully.. This is in contrast to the main intention to treat analysis, which includes as much patient data as possible, regardless of loss to follow up or ending treatment. If there is great discrepancy between both sets of results, then further investigation will be undertaken using inverse probability-of-censoring weights (IPCW).

Interim analysis

An interim analysis will not be required on the primary end point. The IMP has a well-established safety record and is being administered for a short course only; all treatment-related adverse events will be reviewed with the PI and trial steering committee during the trial. If there is suspicion that the IMP is acting outside its normal safety profile then further analysis will be undertaken.

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, the procedure for an early termination or temporary halt will be arranged after consultation by all involved parties. This will be recorded in the CRF and documented in the final report. Unblinding at this point is unlikely to be necessary.

The sponsor will submit a written notification to the Regulatory Authority concerned and Ethics Committee/Institutional Review Board providing the justification of premature ending or of the temporary halt.

Other statistical considerations

Any deviations from the statistical plan will be discouraged. However, any unforeseen changes will be described and justified in the final report, as appropriate. The sponsor, CI, TMG and or DMC will be notified of these deviations. Where required the sponsor and Chief Investigator will notify REC, Regulatory Authorities and the funder.

References

1. Wilkinson TMA, Wilks M, Patel IS, Lloyd Owen, Donaldson GC, Wedzicha JA. Airway bacterial colonisation and decline in lung function in COPD. Am J Resp Crit Care Med 2003 167: 1090-95.
2. Patel IS, Seemungal TAR, Wilks M, Lloyd Owen S, Donaldson GC, Wedzicha JA. Relationship between bacterial colonisation and the frequency, character and severity of COPD exacerbations. Thorax 2002:57:759-64.
3. Seemungal TAR, Donaldson GC, Bhowmik A, Jeffries DJ, Wedzicha JA Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000; 161: 1608-13
4. Davies L, Angus RM, Calverley PMA. Oral corticosteroids in patients admitted to hospital with exacerbations of chronnic obstructive pulmonary disease: a prospective randomised controlled trial. Lancet 1999; 354: 456-60
5. Niewoehner DE, Erbland ML, Deupree RH et al. Effect of systemic glucocorticoids on exacerbations of chronic obstuctive pulmonary disease. N Engl J Med 1999; 340: 1941-7
6. Anthonisen, NR., Manfreda J, Warren CPW, Hershfield ES, Harding GKM, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann. Intern. Med. 1987;106:196-20
7. Roede BM, Bresser P, Bindels PJE, Kok A, Prins M, Rietter G, Geskus RB, Herings RMC, Prins JM. Antibiotic treatment is associated with reduced risk of a subsequent exacerbation in obstructive lung disease: an historical population based cohort study. Thorax 2008; 63: 968-973.
8. Hurst JR, Donaldson GC, Quint JK, Goldring JJP, Baghai-Ravary B, Wedzicha JA. Temporal Clustering of Exacerbations in Chronic Obstructive Pulmonary Disease Am. J. Respir. Crit. Care Med. 2009; 179**:** 369-374.
9. Roberts CM, Lowe D, Bucknall CE, Ryland I, Kelly Y, Pearson MG. Clinical audit indicators of outcome following admission to hospital with acute exacerbation of chronic obstructive pulmonary disease. Thorax 2002;57:137-41

10. Perera WR, Hurst JR, Wilkinson TMA, Sapsford RJ, Müllerova H, Donaldson GC, Wedzicha JA. Inflammatory changes and recurrence at COPD exacerbations. Eur Resp J 2007; 29:527-34

11. Rabe K. F., et al. 2007. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am. J. Respir. Crit. Care Med. 176:532–555.

12. Woodhead M., et al. 2005. Guidelines for the management of adult lower respiratory tract infections. Eur. Respir. J. 26:1138–1180.

13. Balter M. S., et al. 2003. Canadian guidelines for the management of acute exacerbations of chronic bronchitis. Can. Respir. J. 10(Suppl.):3B–32B.

14. Kontou P, Chatzika K, Pitsiou G, Stanopoulos I, Argyropoulou-Pataka P, Kioumis I. Pharmacokinetics of ciprofloxacin and its penetration into bronchial secretions of mechanically ventilated patients with chronic obstructive pulmonary disease. Antimicrob Agents Chemother. 2011 Sep;55 (9):4149-53. Epub 2011 Jun 13.

15. GOLD Executive committee. Global Strategy for Diagnosis, Management, and Prevention of COPD (Revised 2006). http://www.goldcopd.com/Guidelineitem.asp

Signed

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Professor J.A. Wedzicha Date

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Professor Gavin Donaldson Date