**REVISED AMAZES ANALYSIS PLAN**

## Study objectives

To conduct a large-scale, multicentre, double-blind, placebo controlled randomised trial assessing the efficacy (and safety) of the addition of oral azithromycin, compared with placebo, for 48 weeks to fixed dose maintenance therapy on the incidence of asthma exacerbations and clinical asthma status in people with persistent asthma.

The specific hypotheses that will be addressed are:

1. That among patients with non-eosinophilic asthma, the addition of azithromycin, compared with placebo, will result in significant reduction in the cumulative incidence of asthma exacerbations; and
2. Azithromycin treatment will significantly improve health status and reduce asthma symptom scores in patients with non-eosinophilic asthma.

### Asthma exacerbations

The primary outcome for hypothesis 1 will be asthma exacerbations.

Asthma exacerbations will be defined as ‘severe’ or ‘moderate’ according to the ATS/ERS Asthma Outcomes Taskforce guidelines. 40

A ‘severe’ exacerbation will be defined as a participant who requires:

* Use of systemic corticosteroids, or an increase from a stable systemic corticosteroid maintenance dose, for at least 3 days. (Courses of corticosteroids separated by 1 week or more will be treated as separate severe exacerbations);
* Hospitalisation or an emergency department visit requiring systemic corticosteroids.

A ‘moderate’ exacerbation will be defined as a participant who has/requires:

* Emergency department visit for asthma, not requiring systemic corticosteroids OR;
* A temporary change in preventer treatment (ICS, OR ICS/LABA) or commencement of antibiotics

AND at least one of the following:

* Deterioration in asthma symptoms for at least 2 days;
* Deterioration in lung function for at least 2 days;
* Increased rescue bronchodilator use for at least 2 days

Total number of exacerbations will constitute ‘severe’ plus ‘moderate’ exacerbations. Asthma exacerbation data will be collected at 6-weekly clinical assessments, follow-up phone calls, patient diary and by review of medical records.

## Asthma inflammatory Phenotype

Non-eosinophilic asthma is defined as sputum eosinophils < 3%, or if sputum is not available, blood eosinophils <0.3x10E9/l.

Eosinophilic asthma will be defined by sputum eosinophils >/= 3%, or if sputum is not available, blood eosinophils >/=0.3x10E9/l.

## Sample size

**AMAZES ANALYSIS UPDATE 01 FEB 2016**

The AMAZES analysis strategy has been modified based on data that has been published since the grant submission (Brusselle GG, Thorax 2013, AZISAST Study). These data show that the effect of AZM on reduction in asthma exacerbations is restricted to the group with non-eosinophilic asthma (NEA).

Q. What is the sample size required in AMAZES to detect a reduction in severe asthma exacerbations in patients with non-eosinophilic asthma(NEA), of the same magnitude as observed in AZISAST (0.62 to 0.26)?

A. With 79 per group, 158 in total, AMAZES has 90% power to detect a reduction in severe asthma exacerbations in participants with NEA

AMAZES NEA classification

|  |  |
| --- | --- |
|  | n/N(%) |
| Randomisation sputum (<3%) | 158/350 (51.8%) |

**AMAZES power calculations for NEA (randomisation sputum) analysis based on varying reductions (59% to 46%)**

AMAZES Non eosinophilic average severe exacerbation rate =0.43

AZISAST severe rate 0.62 vs 0.26 (59% reduction)

With an AMAZES NEA average exacerbation rate=0.43 (68 severe exacerbations in n=158), a 59% reduction in severe exacerbations between placebo & AZM is equivalent to a placebo exacerbation rate=0.61 and AZM severe exacerbation rate=0.25, while a 49% reduction is equivalent to a placebo event rate=0.57 and AZM event rate=0.29.

With 80% power, alpha=0.05, AMAZES requires 58 per group to detect a 59% reduction, 72 per group to detect a 54% reduction and 93 per group to detect a 49% reduction

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Non eosinophilic average severe exac rate =**0.43,** | | | | |  |  |
| alpha | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| power | 80% | 80% | 80% | 80% | 80% | 80% |
| reduction | **59%** | **57%** | **54%** | **52%** | **49%** | **46%** |
| Control event rate | 0.61 | 0.6 | 0.59 | 0.58 | 0.57 | 0.56 |
| Intervention event rate | 0.25 | 0.26 | 0.27 | 0.28 | 0.29 | 0.3 |
|  |  |  |  |  |  |  |
| n | **57.6** | **64.1** | **71.9** | **81.4** | **92.9** | **107.2** |

This exacerbation rate could vary but a worst case scenario is a severe exacerbation rate=0.4 (68 severe exacerbations in 168 NEA subjects)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Non eosinophilic average severe exacerbation rate =0.4,** | | | | |
| alpha | 0.05 | 0.05 | 0.05 | 0.05 |
| power | 80% | 80% | 80% | 80% |
| reduction | **58%** | **55%** | **52%** | 49% |
| Control event rate | 0.57 | 0.55 | 0.54 | 0.53 |
| Intervention event rate | 0.24 | 0.25 | 0.26 | 0.27 |
|  |  |  |  |  |
| n | **64.2** | **76.0** | **86.7** | 99.9 |

**Power calculations for a reduction in the proportion experiencing a severe exacerbation**

The average AMAZES proportion experiencing a severe exacerbation in the NEA group=36/134 (27%)

|  |  |  |  |
| --- | --- | --- | --- |
| Reduction % | Placebo % exacerbator | AZM % exacerbator | Power |
| 50% | 36 | 18 | 92 |
| 41% | 34 | 20 | 74 |
| 31% | 32 | 22 | 45 |

### Health Status (Quality of Life, AQLQ)

The effect of azithromycin on health status will be examined in the 2 strata of EA and NEA. Based on (32), 20% of the control group have a clinically significant improvement in QOL (i.e. a > 0.5 unit improvement in their AQLQ score) the study will have 80% power to detect a clinically significant improvement in 33% of patients in the active treatment group (i.e. a 13% difference).32 In order to detect the same effect on health status

## Analysis (supervised by Associate Investigator Dr. Patrick McElduff)

Hypothesis 1 will be examined using a negative binomial model.46 This is a flexible extension of the Poisson regression model with allowance for extra Poisson variation (over dispersion) that is usually observed in count data, including over dispersion of counts of exacerbations among asthma patients. The outcome of interest in the model will be the number of exacerbations experienced by each patient. The predictor of interest in the model will be treatment group with length of follow-up included as an offset. The model assumes that the event rates do not vary with time and therefore patients can be included in the analysis even if they only contribute data for part of the follow-up period. The offset variable adjusts for the difference in follow-up times.

Additional analysis will explore differences in the time to first exacerbation between patients treated with Azithromycin and those treated with placebo. The data will be presented graphically using Kaplan-Meier plots and differences in survival curves tested using log-rank test. The hazard ratio between treatment groups will be estimated using a Cox proportional hazards model.

Hypothesis 2 will be tested using analysis of covariance. Separate models will be fitted with health status (AQLQ) and asthma symptom score at 48 weeks as the outcome of interest. The predictor variable of interest in the analysis of variance models will be treatment with baseline level of health status or asthma symptom score included as a covariate. To examine if there is a greater benefit of treatment among patients with NEA, NEA status and an interaction term between treatment and NEA status will be included in the model. The p‑value of the interaction term will indicate if there is a statistically significant difference in the treatment effect between eosinophilic and non-eosinophilic patients.

Other outcomes of interest such as ICS dosage, 2-agonist use, and questionnaire results will be also be analysed using analysis of covariance. Data will be analysed on an intention-to-treat basis using 2-sided tests with p values <0.05 considered statistically significant. The analysis will be repeated on the per protocol population and all analyses will be repeated by NEA status.