AstraZeneca

Clinical Study Report Synopsis

Drug Substance	AZD9668
Study Code	D0520C00009
Edition Number	1
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A Phase II, Randomised, Double-Blind, Placebo-Controlled, Parallel Group Study to Assess the Efficacy of 28 Day Oral Administration of AZD9668 in Patients with Cystic Fibrosis

Study dates: First patient enrolled: 30 October 2008
Last patient last visit: 04 August 2009

Phase of development: Therapeutic exploratory (II)

This study was performed in compliance with Good Clinical Practice, including the archiving of essential documents.

Study centres

The study was conducted at 15 centres in 6 countries (UK, Germany, Sweden, Poland, Denmark, Russia). The first patient was enrolled on 30 October 2008, and the last patient was enrolled on 5 June 2009. The data cut-off date for the final analysis was 04 August 2009 based on last subject last visit.

Publications

None at the time of writing this report.

Objectives and criteria for evaluation

Table S1 Primary and secondary objectives and outcome variables

Objectives	Outcome variables	Type
Primary	**Primary**	
1. Absolute and percentage neutrophil counts in sputum	Cytospin slides produced for assessment of absolute and differential cell counts at Visits 1a, 2, 3a and 4.	Efficacy
2. BronkoTest© diary card	The BronkoTest© diary card was completed daily by patients and taken to study visits (excluding Visit 2a) to record signs and symptoms of CF (breathing, sputum colour, sputum amount, sputum type, wellbeing, cough, chest pain, cold or flu, , antibiotics or steroids, sleep disturbances, peak expiratory flow [PEF, morning and evening], use of reliever medication).	PRO
3. Quittner Questionnaire	The Quittner Questionnaire for CF patients (CFQ-R) was completed at Visits 2 and 4 to measure the impact of CF on Quality of Life (physical, role/school, vitality, emotion, social, body image, eating, treatment burden, health perceptions) and symptoms (weight, respiratory and digestion).	PRO
4. 24 hour sputum collection weight	Patients were asked to collect sputum for a 24 hour period before Visit 1a and Visit 4.	Efficacy
5. Lung function tests	FEV_1 (forced expiratory volume in 1 second), SVC (slow vital capacity), forced expiratory flow between 25 and 75% of forced vital capacity (FEF_{25-75}) and FVC (forced vital capacity) were measured pre-bronchodilator at Visits 1, 2, 4 and 5.	Efficacy
Secondary	**Secondary**	
6. NE activity in sputum	Assay of NE activity in induced sputum collections at Visits 1a, 2, 3a and 4	Pharmaco-dynamic
7. Inflammatory markers in sputum	Assay of induced sputum collections at Visits 1a, 2, 3a and 4 for the following markers: (including, but not limited to) TNF-α, IL-6, IL-1β, RANTES, MCP-1, (exploratory non-GLP assays) LTB_4 and IL-8 (validated assays)	Pharmaco-dynamic
8. Inflammatory markers in blood	Assay of blood samples taken at Visits 2 and 4 for the following markers: (including, but not limited to) absolute and differential neutrophil cell count serum amyloid-A and CRP[a] (validated assays) and plasma TNF-α, IL-6, IL-8 and IL-1β (exploratory non-GLP assays)	Pharmaco-dynamic
9. Safety and tolerability of AZD9668	Reported adverse events (AEs), haematology, clinical chemistry, urinalysis, physical examination, resting 12-lead ECG, vital signs, sputum culture	Safety

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Objectives	Outcome variables	Type
10. Pharmacokinetic parameters (plasma and sputum supernatant)	Concentration of AZD9668 in plasma was analysed in samples taken at Visits 2 and 4, using a validated method of liquid chromatography and mass-spectrometry (LC-MS/MS) after protein precipitation. The lower limit of quantification (LLOQ) of AZD9668 in plasma was 1.00 nM. Concentration of AZD9668 in sputum supernatant was analysed in samples taken at Visits 2, 3a and 4, using a method of LC-MS/MS based on that validated for human plasma. The method was not formally validated, but was supported by investigations into the stability of AZD9668 in this matrix and each batch of samples included quality control samples to demonstrate the performance of the method. The lower limit of quantification (LLOQ) of AZD9668 in sputum supernatant was 2.00 nM.	Pharmaco-kinetic
11. Markers of tissue degradation	Assay of desmosine from 24 hour urine collections at Visits 1a and 4	Pharmaco-dynamic

CF Cystic Fibrosis; CRP C-reactive protein; ECG Electrocardiogram; IL Interleukin; LTB-4 Leukotriene B 4; MCP-1 Monocyte chemoattractant protein-1; NE Neutrophil elastase; RANTES Regulated on activation, normal T cell expressed and secreted; TNF-α Tumour necrosis factor alpha.

[a] Where CRP is reported in the synopsis, this refers to CRP data generated using a 'normal' CRP (nCRP) assay and not the 'high sensitivity' CRP (hsCRP) assay as stated in the Clinical Study Protocol. The nCRP assay has a lower limit of quantification of 0.2 mg/L.

Exploratory objectives/variables/data are included in the Clinical Study Report but not in this synopsis

Study design

This was a multi-centre, randomised, double-blind, placebo-controlled, parallel group study to assess the efficacy of 28 days of dosing with AZD9668 (60 mg bd) in CF patients. AZD9668 was to be taken bd with approximately 12 hours between each dose. It was planned that 40 patients with CF would be randomised at Visit 2 to AZD9668 or placebo (ratio of 1:1).

Target patient population and sample size

Male and female patients aged ≥16 years with a clinical diagnosis of CF with a forced expiratory volume in 1 second (FEV_1) ≥40%. Female patients must be surgically sterile or post-menopausal.

The sample size for this study was selected to be consistent with the research hypothesis. The research hypothesis was that the neutrophil elastase (NE) inhibitor AZD9668 shows an effect on the inflammatory markers, signs and symptoms (including quality of life [QoL]) in patients with CF and is safe and well tolerated when given orally at a dose of 60 mg bd for 28 days. This study was exploratory and the sample size in this study was not based on obtaining power to detect specific effects. Adequate data are not available to perform such a powering. However, assuming that the variability is such that a 50% decrease in neutrophil numbers in the sputum would not be missed, a sample size of 40 subjects (20 per group) was considered sufficient.

Investigational product and comparator(s): dosage, mode of administration and batch numbers

AZD9668 was manufactured and supplied by AstraZeneca as white to off-white, plain oblong biconvex tablets at 400mg (batch numbers H 2029-01-01-01 and H 2029-01-01-02). The

composition of the tablets was 30 mg AZD9668 (corresponding to 39.5 mg AZD9668 tosylate) and two 30 mg tablets were taken to achieve the 60 mg dose. Placebo tablets manufactured by AstraZeneca matched AZD9668 tablets (batch numbers H 2030-01-01-01 and H 2030-01-01-02). All doses of study medication were to be taken with 100 mL of water.

Duration of treatment

Twice daily oral treatment with AZD9668 tablets 60 mg (2 x 30 mg) or placebo tablets continued for 28 days (±2 days) unless discontinuation criteria were met.

Statistical methods

The primary outcome variables were compared between AZD9668 and placebo using an analysis of variance model with fixed factors treatment and country and using baseline as a covariate. As the study was exploratory in nature, a p-value of <0.1 was considered significant. A 2-sided 90% confidence interval was constructed for the treatment difference and p-values given. For variables with a skewed distribution, data may be log-transformed prior to analysis or a non-parametric test (Wilcoxon rank sum) used instead.

The analyses were carried out on the Efficacy Analysis Set (all patients randomised into the study who received at least 1 dose of study medication and had at least 1 piece of evaluable data). All other data were summarised and listed. There was no interim analysis for this study.

Patient population

In total, 70 patients were enrolled into the study and 56 were randomised (29 patients on placebo and 27 on AZD9668). More patients have been recruited and randomised than initially planned due to a rapid increase in recruitment and a lower than projected screen failure rate towards the end of the recruitment period.

Patients were recruited from 15 centres in 6 countries: UK, Germany, Sweden, Poland, Denmark and Russia and randomisation was reasonably well balanced in each centre and country. A total of 51 (91%) patients completed the study. There were 5 patients who withdrew. Two patients in the placebo group discontinued due to treatment emergent adverse events. Three patients in the AZD9668 group withdrew. One withdrew due to an adverse event before starting treatment, 1 prematurely discontinued due to incorrect enrolment and a third discontinued voluntarily after taking AZD9668 for 14 days.

One patient was excluded from the Efficacy Analysis set due to a lack of efficacy data post-dosing. Another patient did not receive any study medication following randomisation and was excluded from the Efficacy and Safety Analysis Sets. No patients were excluded from either analysis set due to protocol deviations. There were 55 patients included in the Safety Analysis Set and 54 in the Efficacy Analysis Set.

The mean age of the patients in the placebo group was 27 years (range 17 years to 53 years), and in the AZD9668 group the mean age was 29 years (range 20 years to 51 years); 53 (98%) of the patients were male. All patients were white, mean weight, height and BMI were similar

in the 2 groups. Percent predicted FEV_1 at screening was 79.3% (range 42.4% to 128.3%) in the placebo group and 67.4% (range 41.5% to 102%) in the AZD9668 group. Mean lung function measurements (FEV_1, SVC, FVC and FEF_{25-75}) at baseline were also lower in the AZD9668 group than the placebo group. The most frequently used antibiotics were macrolides (azithromycin), taken by more patients on AZD9668 (15, 58%) than on placebo (8, 28%). The use of other antibiotics was similar in both treatment groups.

Summary of efficacy results

Sputum neutrophils

Absolute and percent neutrophil cell counts were lower in the placebo group than the AZD9668 group. The ratio of the geometric mean of the sputum cell count between AZD9668 and placebo was 0.97 with a wide confidence interval for absolute neutrophil counts. The analysis of covariance did not show a statistically significant difference between AZD9668 and placebo for the absolute or % sputum neutrophils.

Signs and symptoms of cystic fibrosis (including effects on quality of life)

24 hour sputum weight: Sputum weight was lower in the placebo group than the AZD9668 group. During the study, both treatment groups showed decreases in sputum production. The placebo group had a mean decrease of 4.34g, in the AZD9668 group sputum weight decreased by 5.19g. Analysis of covariance did not show a statistically significant difference between AZD9668 and placebo.

Lung function: Mean baseline measurements were higher in the placebo group compared to AZD9668. For FEV_1, SVC, FVC and FEF_{25-75} the mean changes from baseline in the placebo group were -0.01 L, -0.12 L, -0.01 L and 0.08 L/s, respectively, and in the AZD9668 group the changes were 0.00 L, 0.04 L, 0.00 L and -0.04 L/s, respectively. Percent predicted FEV_1 mean changes from baseline were -0.15% in the placebo group and -0.26% in the AZD9668 group. The changes from baseline in these lung function variables were small on both treatments; there were no statistically significant differences between treatment groups.

Peak expiratory flow (PEF) - BronkoTest© diary card: The mean baseline morning and evening PEF were both higher in the placebo group than the AZD9668 group, there were small changes in both groups during the study. The LS (Least Squares) mean difference between the treatment groups was –15.22 (-32.37 to 1.93 90% CI, p=0.143) for morning PEF and –5.72 (-20.39 to 8.95 90% CI, p=0.516) for evening PEF. This represented a deterioration in peak expiratory flow in the AZD9668 group compared to placebo at both times.

Daily symptom scores and use of reliever medication - BronkoTest© diary card: Scores for all variables were similar although sputum volume was higher in the AZD9668 group during the study. There were small, variable daily mean changes in all symptom scores. There were no statistically significant differences between treatment groups for symptom scores or use of reliever medication.

Quittner questionnaire (CFQ-R): A deterioration on AZD9668 compared to placebo was seen for the overall score. The LS mean difference in the overall score between treatment groups was –24.4 (-62.9 to 14.1 90% CI, p=0.293). The changes in individual categories were not statistically significant with the exception of 'emotion' and 'eat'. The LS mean differences in these scores represented a deterioration on AZD9668 and were –6.1(-10.4 to –1.9 90% CI, p=0.02) and –4.0 (-7.8 to -0.2 90% CI, p=0.081) respectively.

Summary of pharmacokinetic results

Plasma concentrations were measured pre-dose and 3 to 4 h post-dose on Day 1 in the AZD9668 group and at the same time points in all subjects that completed the study on Day 28. The plasma concentrations 3 to 4 h post-dosing on Day 28 were slightly higher than on Day 1, this is consistent with pharmacokinetic properties of AZD9668.

AZD9668 concentrations in sputum supernatant samples were measured in the AZD9668 at pre-dose on Day 1, on Day 21 to 26 and at pre-dose in all subjects that completed the study on Day 28. The concentration of AZD9668 was below the lower limit of quantification in all pre-dose sputum supernatant samples on Day 1 with the exception of 2 samples in which AZD9668 was detected (reasons for this were not established). From these 2 patients there were measurable concentrations of AZD9668 in sputum supernatant samples taken from the other 2 sampling periods (Day 21 to 26 and Day 28).

Summary of pharmacodynamic results

NE activity in sputum: The NE activity at baseline and the changes from baseline varied markedly between patients in both treatment groups. The geometric mean baseline NE activity was higher in the AZD9668 group than the placebo group. The ratios of the NE activity at end of treatment to baseline showed a 51% increase in the placebo group and a 5% decrease in the AZD9668 group. There was a 37% reduction in NE activity in the AZD9668 group compared to the placebo group, this was not statistically significant.

Inflammatory markers in induced sputum (TNFα, IL-6, IL-8, IL-1β, LTB_4, RANTES, MCP1): The inflammatory markers at baseline, and the changes from baseline, varied between patients in both treatment groups. The difference between AZD9668 and placebo was significant only for IL-6 (ratio AZD9668:placebo 0.59, 90% CI 0.44 to 0.80, p=0.006) and RANTES (ratio AZD9668:placebo 0.77, 90% CI 0.59 to 1.00, p=0.1).

Inflammatory markers in blood (neutrophil cell count, TNFα, IL-6, IL-8, IL-1β, CRP, amyloid A): The inflammatory markers at baseline, and the changes from baseline, varied between patients in both treatment groups. The difference between AZD9668 and placebo was significant only for IL-1β, however, the majority of measurements were below the lower limit of quantification and this may have impacted the result of the statistical analysis.

Marker of tissue degradation in urine – desmosine: Free desmosine increased during the study by 17% in the placebo group and decreased by 20% in the AZD9668 group. Total desmosine decreased in both groups, by 2% in the placebo group with a larger 35% decrease in the AZD9668 group. When normalised for creatinine, the ratio AZD9668:placebo were

0.70 and 0.69 for free and total desmosine and this was statistically significant, p=0.002 and p=0.044, respectively. These ratios were also statistically significant without normalisation for creatinine.

Summary of safety results

There were no deaths during the study. Two treatment emergent serious adverse events occurred, both were in the placebo group. Two patients in the placebo group discontinued due to an adverse event. The frequency of adverse events reported was similar in both treatment groups although the total number of adverse events was greater in the placebo group. The adverse event reported by most patients was headache, this was reported by 5/29 (17%) patients on placebo and 7/26 (27%) patients on AZD9668. All headaches were mild apart from one of moderate intensity in each treatment group; none were considered to be related to study drug. The headache of moderate intensity reported in the AZD9668 group was not treatment emergent.

One patient on AZD9668 had a raised creatinine phosphokinase (CK), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) at Visit 2a following unaccustomed heavy exercise on the preceding day. The Investigator attributed this elevation to strenuous exercise and did not exclude the patient from the study. A second peak in CK, ALT, AST and LDH was noted for this patient at Visit 4, however there is insufficient information regarding the patient's exercise pattern at this point to attribute this peak to exercise, and therefore a relationship to study drug cannot be excluded. The patient was asymptomatic, in good health, did not abuse alcohol, had no evidence of liver disease and no family history of liver disease. Overall, no clinically relevant changes in other haematology, clinical chemistry, urinalysis, vital signs, ECG, physical examination or sputum bacteriology were observed.