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Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis

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Summary
Neutrophil elastase (NE) activity is increased in bronchiectasis and may play a role in this condition. We wished to determine the effect of AZD9668, a selective oral inhibitor of NE.

Efficacy and safety of AZD9668 60 mg twice daily over 4 weeks were evaluated in a randomised, double-blind, placebo-controlled, parallel-group, Phase II, signal-searching study in patients with bronchiectasis. Outcome measures included: waking and post-waking sputum neutrophil counts; lung function tests; 24-h sputum weight; BronkoTest® diary card data; St George's Respiratory Questionnaire for COPD patients (SGRQ-C); sputum NE activity; inflammatory biomarker levels; desmosine levels; adverse events, safety haematology and biochemistry. AZD9668 levels in plasma and sputum were measured to confirm exposure.

Thirty-eight patients were randomised: 16 to placebo and 22 to AZD9668. There was no change in sputum neutrophils with AZD9668. Forced expiratory volume in 1 s improved by 100 mL in the AZD9668 group compared with placebo ($p = 0.006$). Significant changes (defined *a priori* as $p < 0.1$) in favour of AZD9668 were also seen in slow vital capacity, plasma

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interleukin-8, and post-waking sputum interleukin-6 and Regulated on Activation, Normal T-cell Expressed and Secreted levels. Non-significant changes in favour of AZD9668 were seen in other lung function tests, sputum weight and the SGRQ-C. AZD9668 was well tolerated.

In this small signal-searching study, 4 weeks' treatment with AZD9668 improved lung function in patients with bronchiectasis and there were trends for reductions in sputum inflammatory biomarkers. Larger studies of longer duration would be needed to confirm the potential benefits of this agent in bronchiectasis.

Registration: NCT00769119.

Introduction

Bronchiectasis is a disease characterised by localised, irreversible dilatation of parts of the bronchial tree, caused by destruction of the structural components of the bronchial wall resulting from a vicious cycle of transmural infection and inflammation.[1] It is associated with varying degrees of airway obstruction and impaired clearance of secretions.

Neutrophils play a key role in the inflammation in bronchiectasis. Airway neutrophilia results in high concentrations of neutrophil proteases, a phenomenon implicated in the pathogenesis of several inflammatory lung diseases including bronchiectasis associated with cystic fibrosis[2,3] and non-cystic fibrosis bronchiectasis.[4,5] Neutrophil elastase (NE) is a serine protease found in high concentrations in neutrophils[6] that is able to degrade extracellular matrix and proteins, damaging the lung parenchyma and airway walls.[7–9] Control of NE activity might help to down-regulate proteolytic lung destruction and slow disease progression in bronchiectasis. NE also has pro-inflammatory effects[10,11] and stimulates mucus secretion[12–14] as well as inhibiting mucociliary clearance.[3] Hence, it is plausible that an NE inhibitor could also reduce inflammatory mediators and improve symptoms and health-related quality of life (HRQoL) in bronchiectasis.

AZD9668 is a novel, orally active reversible inhibitor of human NE.[15] The safety, tolerability and pharmacokinetics of AZD9668 have previously been investigated in healthy volunteers and patients with chronic obstructive pulmonary disease (COPD).[16] AZD9668 at a dose of 60 mg twice daily (bid) for 14 days was well tolerated and achieved blood levels adequate to cause >90% inhibition of zymosan-stimulated NE activity in whole blood *ex vivo*.

The aim of this signal-searching study (NCT00769119) was to assess the efficacy and safety of AZD9668 in patients with bronchiectasis, and to examine its effect on biomarkers of inflammation and tissue damage.

Methods

Design

This was a randomised, double-blind, placebo-controlled, parallel-group, Phase II signal-searching study (NCT00769119) to investigate the efficacy of 28 days' dosing with oral AZD9668 in patients with bronchiectasis. After a run-in period lasting up to 3 weeks, patients were randomised to receive oral AZD9668 or matching placebo. For assessments of sputum weight and biomarkers, a baseline 24-h sputum collection and two waking (first expectoration on rising, brought to study centre as soon as possible) and two post-waking (subsequent expectorant collected over up to 2 h) sputum samples were obtained during the week prior to randomisation. Sputum sampling was repeated during the last week on treatment and at the end of Visit 4. The study design is shown in Fig. 1 of the Supplementary Material and sample collection and assessment information at each visit are detailed in the Supplementary Methods. The study was conducted at four centres in the UK and six in Canada.

Patients

Patients were included in the study if they: were male, or female of non-child bearing potential; were aged between 18 and 80 years, with a clinical diagnosis of idiopathic or post-infective bronchiectasis; had a history of chronic expectoration on most days of most weeks of the year; were clinically stable for 6 weeks prior to study entry; and had normal laboratory values at enrolment, unless the investigator considered an abnormality to be clinically irrelevant.

Exclusion criteria included: participation (defined as administration of at least one dose of investigational product) in another clinical study within 12 weeks of enrolment; bronchiectasis of other aetiologies.

Treatments

Patients were randomised using a 1:1 computer-based randomisation scheme (block size 4) to receive either AZD9668 (60 mg bid) or matching placebo tablets for 28 days. Patients were given sufficient tablets to allow dosing at 60 mg bid until Visit 3 (Day 14 ± 2 days). At Visit 3, the remaining study drug was dispensed, to allow 60 mg bid until Visit 4 (Day 28 ± 2 days).

Assessments

The primary efficacy variables for this study were: absolute and percentage neutrophil counts in waking and post-waking sputum samples; change from baseline (Visit 1a or 1b) in 24-h sputum weight; change from randomisation in pre-bronchodilator (in order to detect any beneficial or detrimental effect of the drug) forced expiratory volume in 1 s [FEV_1], slow vital capacity [SVC], forced vital capacity [FVC], forced expiratory flow between 25 and 75% [$FEF_{25-75\%}$] of FVC; BronkoTest® diary card data (including

morning and evening peak expiratory flow [PEF], symptom scores and reliever medication use); and the change from randomisation in HRQoL measured using the St George's Respiratory Questionnaire for COPD patients (SGRQ-C). The SGRQ has been previously validated for bronchiectasis.[17]

Sputum was processed at 4 °C within 2 h of expectoration, where possible (see Supplementary Material for further details). Preparation of the cytospin slides and an assessment of their viability and quality were conducted at each local investigation site. All slides were subsequently shipped to the Institute for Lung Health, Glenfield Hospital, Leicester, UK and analysed for absolute and percentage neutrophil counts.

Secondary efficacy variables included: NE activity in spontaneous sputum; inflammatory biomarkers in sputum (tumour necrosis factor-alpha [TNFα], interleukin [IL]-6, IL-8 [CXCL8], IL-1 beta [IL-1β], leukotriene B4 [LTB-4], Regulated on Activation, Normal T-cell Expressed and Secreted [RANTES (CCL5)] and monocyte chemoattractant protein-1 [MCP-1 (CCL2)]); blood inflammatory biomarkers (including TNFα, IL-6, IL-8 and IL-1β); urinary (total and free) desmosine (creatinine normalised)[18]; safety (reported adverse events [AEs], clinical laboratory evaluations, vital signs, electrocardiogram [ECG] monitoring, physical examinations and sputum cultures); and pharmacokinetic parameters (AZD9668 concentrations in plasma and sputum). Plasma (total) desmosine was measured as an exploratory outcome.

NE activity and desmosine and creatinine levels were analysed by Clinical Pharmacology & DMPK, AstraZeneca R&D, Charnwood, UK. All other sputum and plasma inflammatory biomarkers, except for sputum IL-8 and LTB-4, were analysed at Biosciences, AstraZeneca R&D, Lund, Sweden. For biomarker analyses, the supernatant from processed sputum was stored frozen at −20 °C until transported and analysed using an AstraZeneca custom-made 5-plex immunoassay, purchased from Meso Scale Discovery, Gaithersburg, MD, USA. Sputum IL-8 and LTB4 were analysed at Quotient Bioresearch Ltd, Fordham, Cambridgeshire, UK using an R&D Systems ELISA kit and a Cayman Chemicals Enzyme Immunoassay kit, respectively. For assessment of plasma biomarkers, plasma was collected in appropriate tubes and samples were stored at or below −20 °C. Assay of plasma biomarkers was performed using the Human ProInflammatory-4 II Ultra-Sensitive Kit #K11025C-2, which was purchased from Meso Scale Discovery, Gaithersburg, MD, USA. High sensitivity-CRP was analysed using an immuno-turbidimetric test using an Olympus System CRP latex kit. Amyloid-A was assessed at Quotient Bioresearch Ltd, Fordham, Cambridgeshire, UK using an Anogen ELISA kit (Cat No. EL10015).

Haematology, clinical chemistry and urinalysis were conducted for all UK investigational sites by Covance Central Laboratory Services SA, Geneva, Switzerland. For Canadian sites, these parameters were assessed by Covance Central Laboratory Services, Inc., Indianapolis, IN, USA.

AZD9668 concentrations in sputum supernatant were analysed by Clinical Pharmacology & DMPK, AstraZeneca R&D, Lund, Sweden. The concentration of AZD9668 in plasma was assessed by York Bioanalytical Solution, York, UK, on behalf of Clinical Pharmacology & DMPK, AstraZeneca.

All sample analysis was undertaken and the data locked prior to unblinding the study.

Statistical analyses

This study was exploratory and therefore the sample size was not based on obtaining power to detect specific effects. However, a sample size of 40 patients (20 per treatment group) was judged to be sufficient to detect a 50% decrease in neutrophil numbers with a power of 80%, assuming a standard deviation of 1 on logged data. As this was a signal-searching study, a 2-sided p-value of <0.1 was considered significant. There was no adjustment for multiplicity.

Analysis of variance (ANOVA) was used to compare AZD9668 with placebo for the neutrophil count and biomarker data. Baseline data (on a log scale) and country were included as covariates. For the other efficacy outcomes, ANOVA was used, where treatment and country were fixed factors and baseline values were the covariate.

The primary efficacy analysis population included all patients who received at least one dose of study drug and for whom post-randomisation efficacy data were available. The safety analysis set included all patients who received at least one dose of study drug and for whom any post-dose data were available.

Post-hoc analyses

Sputum biomarker data were subjected to post-hoc analyses to determine if adjusting for sputum weight (biomarker concentration × sample weight) would affect the variability of the results. Analyses were initially of sputum biomarkers that were significantly changed in the pre-specified analyses and were then extended to all sputum biomarkers. Further post-hoc analyses were performed to determine whether pooling the data from the waking and post-waking samples would also reduce sample variability. Pooled data were not adjusted for sputum weight.

Ethical aspects

The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation/Good Clinical Practice and received independent ethics committee approval. All patients provided written informed consent to participate in the study.

Results

Patients

The first patient was enrolled on 25 September 2008, and the last patient completed on 20 April 2009. Patient disposition is shown in Fig. 1. Of 69 patients enrolled, 38 patients were randomised to treatment: 16 patients received placebo and 22 patients received AZD9668 60 mg bid. (Thirty-one patients were not randomised: 4 due to AEs, 9 due to voluntary withdrawal and 18 did not meet the eligibility
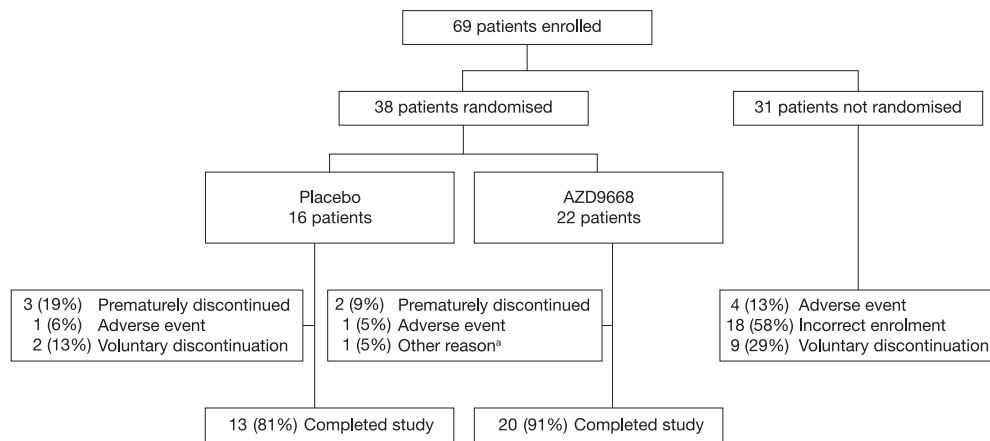
Figure 1 Patient disposition during the study. [a]Use of restricted antibiotic.

criteria during the 3-week run-in period.) Treatment compliance was >80% in both groups. Thirty-three (87%) patients completed the study and no patient was excluded from any analysis. One patient in the AZD9668 group used a prohibited antibiotic, but a sensitivity analysis showed that exclusion of this patient from the efficacy analysis set had no effect on study outcome (data not shown).

Despite the block randomisation process, the two treatment groups differed in certain demographic and patient characteristics (Table 1), with a higher proportion of males (59%) in the AZD9668 group than in the placebo group (31%) and fewer patients in the placebo group taking long-acting muscarinic antagonists (LAMA) (placebo, 6% vs AZD9668, 41%), inhaled corticosteroid/long-acting beta

Table 1 Patient demographic and clinical characteristics at enrolment (safety analysis set).

	Treatment group	
	Placebo (*n* = 16)	AZD9668 60 mg bid (*n* = 22)
Age, years, median (range)	62 (54−73)	61 (42−79)
Gender, *n* (%)		
Male	5 (31)	13 (59)
Female	11 (69)	9 (41)
Race, *n* (%)		
White	14 (88)	19 (86)
Black or African American	1 (6)	0 (0)
Asian	0 (0)	3 (14)
Other	1 (6)	0 (0)
Weight, kg, median (range)	76 (63−99)	75 (48−111)
Height, cm, median (range)	163 (149−177)	168 (155−188)
BMI, kg/m^2, median (range)	29.1 (23.5−34.7)	24.7 (17.6−46.2)
Smoking history		
Current, *n* (%)	0 (0)	1 (5)
Former, *n* (%)	5 (31)	11 (50)
First diagnosis of bronchiectasis, *n* (%)		
≤24 months	3 (19)	4 (18)
>24 months	13 (81)	17 (77)
No date given	0 (0)	1 (5)
Type of bronchiectasis sputum		
Mucoid	3 (19)	5 (23)
Purulent with non-Pseudomonas bacteria	7 (44)	9 (41)
Purulent with Pseudomonas bacteria	6 (38)	8 (36)
Concomitant medication, *n* (%)		
LAMA	1 (6)	9 (41)
ICS/LABA	5 (31)	10 (45)
Azithromycin	1 (6)	4 (18)

bid = twice daily; BMI = body mass index; LAMA = long-acting muscarinic antagonist; ICS/LABA = inhaled corticosteroid/long-acting beta agonist.

Table 2 Measurements for primary outcome variables and sputum NE activity (efficacy analysis set).

	Baseline measurements		End of treatment measurements		Comparison between AZD9668 and placebo at end of treatment (ANCOVA)		
	Placebo	AZD9668	Placebo	AZD9668			
Sputum neutrophils	$n = 11$ (waking) $n = 10$ (post-waking)	$n = 19$ (waking) $n = 18$ (post-waking)	$n = 11$ (waking) $n = 10$ (post-waking)	$n = 19$ (waking) $n = 18$ (post-waking)	Ratio	90% CI	p-value
Waking sample, 10^6/g geometric mean (CV, %)	5.63 (100.4)	6.57 (123.8)	5.34 (297.5)	6.5 (141.1)	1.06	0.59, 1.91	0.872
Post-waking sample, 10^6/g geometric mean (CV, %)	5.19 (368.1)	6.69 (284.4)	5.75 (270.8)	7.05 (224.9)	1.07	0.55, 2.07	0.862
24-h sputum weight, g mean (SD)	$n = 13$	$n = 20$	Change from baseline $n = 13$	Change from baseline $n = 20$	LSM difference (SEM)	90% CI	p-value
Weight, g mean (SD)	15.48 (9.663)	37.06 (39.61)	5.01 (18.37)	−8.27 (18.72)	−5.22 (5.693)	−14.9, 4.46	0.367
Lung function tests	$n = 13$	$n = 20$	Change from baseline $n = 13$	Change from baseline $n = 20$	LSM difference (SEM)	90% CI	p-value
FEV_1, L mean (SD)	1.77 (0.377)	2.07 (0.798)	−0.04 (0.082)	0.06 (0.100)	0.10 (0.034)	0.04, 0.16	0.006
SVC, L mean (SD)	2.74 (0.687)	3.38 (1.071)	−0.06 (0.173)	0.06 (0.200)	0.13 (0.074)	0.01, 0.26	0.079
FVC, L mean (SD)	2.67 (0.680)	3.37 (0.994)	−0.01 (0.174)	0.05 (0.227)	0.03 (0.080)	−0.10, 0.17	0.674
$FEF_{25-75\%}$, L/s mean (SD)	1.08 (0.416)	1.16 (0.939)	0.01 (0.165)	0.08 (0.134)	0.07 (0.049)	−0.01, 0.15	0.157
BronkoTest® diary card variables	$n = 16$	$n = 21$	Change from baseline $n = 16$	Change from baseline $n = 21$	LSM difference (SEM)	90% CI	p-value
PEF, L/min mean (SD)							
Morning	312.8 (74.92)	364.4 (124.6)	−2.06 (35.22)	2.11 (22.70)	9.05 (N/A)	−7.14, 25.23	0.351
Evening	311.0 (73.09)	372.4 (132.7)	0.99 (35.79)	2.24 (23.38)	5.71 (N/A)	−11.0, 22.46	0.568
Night time symptoms,[a] mean (SD)	0.76 (0.966)[b]	0.74 (1.035)	−0.28 (0.920)[b]	−0.15 (0.657)	0.11 (0.215)	−0.25, 0.48	0.598
Breathing,[a] mean (SD)	2.08 (0.299)	2.07 (0.301)	−0.01 (0.340)	−0.07 (0.518)	−0.05 (0.131)	−0.27, 0.17	0.700
Sputum colour,[a] mean (SD)	3.44 (1.939)	3.85 (1.454)	−0.13 (0.752)	−0.17 (1.285)	0.12 (0.330)	−0.44, 0.68	0.713
Sputum amount,[a] mean (SD)	2.17 (0.712)	2.57 (0.655)	−0.13 (0.488)	−0.12 (0.626)	0.09 (0.199)	−0.25, 0.42	0.666
How do you feel?,[a] mean (SD)	2.22 (0.225)	2.03 (0.305)	−0.17 (0.447)	0.04 (0.531)	0.05 (0.153)	−0.21, 0.31	0.731
How often do you cough?,[a] mean (SD)	1.57 (0.716)	1.30 (0.689)	−0.16 (0.520)	−0.02 (0.571)	0.11 (0.184)	−0.20, 0.42	0.549
Reliever medication use,[a] mean (SD)	0.96 (1.979)	1.94 (3.816)[c]	−0.04 (0.143)	0.16 (0.581)[c]	0.22 (0.152)	−0.04, 0.47	0.165
SGRQ-C score	$n = 13$	$n = 19$	Change from baseline $n = 13$	Change from baseline $n = 19$	LSM difference (SEM)	90% CI	p-value
Symptoms, mean percent (SD)	68.91 (13.80)	66.18 (16.13)	−1.8 (11.49)	−7.2 (18.60)	−6.02 (5.947)	−16.1, 4.10	0.320
Activity, mean percent (SD)	52.20 (29.96)	37.75 (24.63)	0.6 (13.31)	−3.5 (14.06)	−5.55 (5.278)	−14.5, 3.43	0.302
Impact, mean percent (SD)	38.17 (23.15)	31.22 (19.68)	−2.3 (13.87)	−6.3 (13.84)	−5.74 (5.001)	−14.2, 2.77	0.261
Overall, mean percent (SD)	47.93 (21.22)	39.42 (17.90)	−1.3 (11.04)	−5.6 (13.15)	−5.64 (4.651)	−13.5, 2.27	0.236
Sputum NE activity	$n = 12$	$n = 20$	$n = 12$ (waking) $n = 12$ (post-waking)	$n = 20$ (waking) $n = 19$ (post-waking)	Ratio	90% CI	p-value

Table 2 (*continued*)

	Baseline measurements		End of treatment measurements		Comparison between AZD9668 and placebo at end of treatment (ANCOVA)
	Placebo	AZD9668	Placebo	AZD9668	
Waking sample, μmol/L AMC/h geometric mean (CV, %)	7.28 (12,302)	24.54 (14,185)	7.49 (26,228)	15.92 (29,962)	0.95 0.31, 2.93 0.941
Post-waking sample, μmol/L AMC/h geometric mean (CV, %)	4.17 (18,238)	22.21 (18,772)[c]	13.46 (8216)	16.80 (10,156)	0.51 0.18, 1.49 0.296

ANCOVA = analysis of covariance; CI = confidence interval; CV = between subject coefficient of variation; SD = standard deviation; LSM = least squares mean; FEV_1 = forced expiratory volume in 1 s; SVC = slow vital capacity; FVC = forced vital capacity; $FEF_{25-75\%}$ = forced expiratory flow between 25% and 75% of forced vital capacity; PEF = peak expiratory flow; SGRQ-C = St George's Respiratory Questionnaire for COPD patients; NE = neutrophil elastase; AMC = 7-amino-4-methylcoumarin.

[a] The diary card symptom scores are recorded on a scale of 0 (no symptom) to 4 or 5 (worst symptom); a decrease is an improvement.

[b] n = 15.

[c] n = 19.

agonists (ICS/LABA) combinations (placebo, 31% vs AZD9668, 45%) and/or azithromycin (placebo, 6% vs AZD9668, 18%). Baseline lung function, neutrophil counts, 24-h sputum weight and NE activity were all lower in the placebo group than in the AZD9668 group (Table 2), and percentage predicted FEV_1 values at the enrolment and randomisation visits were slightly higher in the placebo group (mean [standard deviation]% predicted FEV_1 for placebo, 76.1 [22.1] vs 72.0 [23.9] for AZD9668).

Efficacy

Primary efficacy variables
Absolute and percentage neutrophil cell count in sputum
For patients who completed the study, analysis of the absolute and percentage sputum neutrophil cell counts showed that there was no significant difference between AZD9668 and placebo at Day 28 for either the waking or post-waking samples (Table 2 & Fig. 2). Individual patient data showed that the changes from baseline in both neutrophil counts and percentage neutrophils were variable in magnitude and direction (data not shown).

24-h sputum weight
The mean sputum weight in the AZD9668 group decreased from 37.06 g by 8.27 g, versus an increase of 5.01 g from baseline of 15.48 g in the placebo group, although the changes were not statistically significant due to the wide confidence intervals (least squares means [LSM] difference: −5.22, 90% CI −14.9, 4.46; $p = 0.367$) (Table 2).

Lung function tests
In the AZD9668 group (n = 20) there were increases from randomisation in the mean values for FEV_1, SVC, FVC and $FEF_{25-75\%}$ at Day 28, whereas in the placebo group (n = 13) (except for $FEF_{25-75\%}$), there were decreases from randomisation (Table 2).

ANCOVA indicated that the improvements from baseline in FEV_1 and SVC were significantly greater for AZD9668 than placebo ($p = 0.006$ for FEV_1 and $p = 0.079$ for SVC, Table 2). FVC and $FEF_{25-75\%}$ increased on AZD9668 and
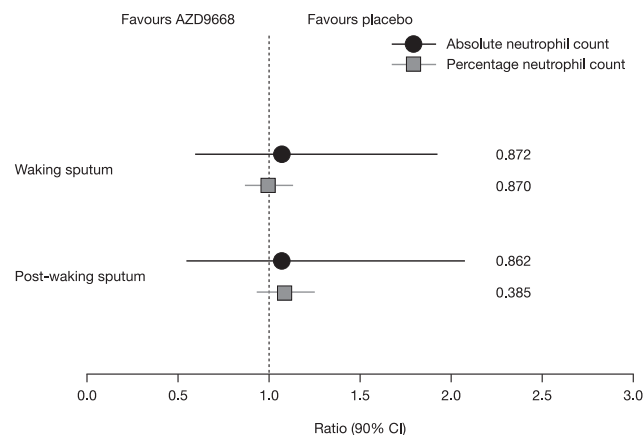

Figure 2 Ratio of absolute and percentage neutrophil cell counts for AZD9668 versus placebo at Day 28. CI = confidence interval.

declined on placebo, but these differences were not statistically significant (Table 2).

BronkoTest® diary card symptoms

While small increases in morning PEF did occur for AZD9668, there was no statistically significant difference between AZD9668 and placebo (Table 2). There were also no statistically significant differences between treatments for the diary card symptom scores or use of reliever medication (Table 2).

St George's Respiratory Questionnaire scores

Mean changes from randomisation to end of treatment in SGRQ-C scores in the AZD9668 and placebo groups are shown in Table 2. The LSM difference between AZD9668 and placebo exceeded 4 units for the SGRQ-C overall score and for each individual domain, indicating changes of clinical relevance.[19] However, due to wide CIs, these differences were not statistically significant.

Secondary efficacy variables

AZD9668 exposure in plasma and sputum

AZD9668 was measurable in plasma on Day 28 from all treated patients both pre-dose and 1–2 h after dosing (pre-dose: geometric mean 274.6 nM, covariate [CV] 98.2%; 1–2 h post-dose: geometric mean 1377.6 nM, CV 28.5%). These data are summarised in Fig. 3. G_{mean} values for AZD9668 in sputum at Day 28 pre-dose and 1–2 h after dosing were 62.9 nM (CV 151%) and 96.5 nM (CV 179%), respectively (Fig. 3).

NE activity in sputum

NE activity in both the waking (placebo $n = 12$, AZD9668 $n = 20$) and post-waking (placebo $n = 12$, AZD9668 $n = 19$) sputum samples decreased from baseline in individuals receiving AZD9668 compared with an increase in individuals receiving placebo; however, the CI was wide and overall differences between AZD9668 and placebo were not statistically significant (Table 2). The NE activity at baseline and the changes from baseline varied markedly between patients in both groups, for both the waking and post-waking samples.

Inflammatory and tissue degradation markers

Values at randomisation and the changes from randomisation in inflammatory and tissue degradation markers
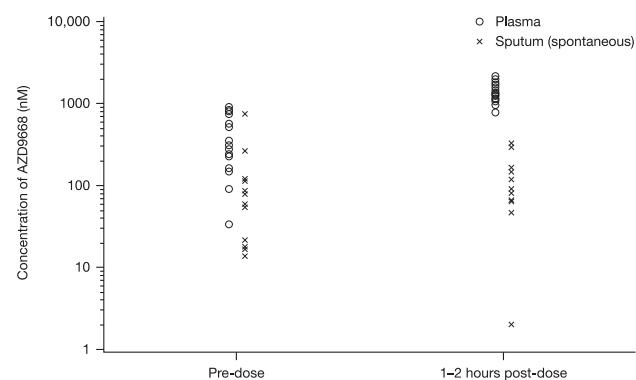
varied markedly between patients in both the placebo ($n = 12$) and AZD9668 groups ($n = 20$). For sputum biomarkers, IL-6 and RANTES in the post-waking sputum sample decreased with AZD9668 (ratio AZD9668 to placebo: 0.72, 90% CI 0.52, 1.00; $p = 0.098$ and 0.63, 90% CI 0.46, 0.86; $p = 0.018$ respectively). For blood biomarkers, IL-8 showed a decrease with AZD9668 (ratio: 0.74, 90% CI 0.56, 0.99; $p = 0.085$). There was no significant difference between AZD9668 and placebo in urine desmosine levels (free or total) or plasma desmosine levels (Table 2 of the Supplementary Material).

Post-hoc analyses

Adjusting for sputum weight had no effect on the post-waking results for change over the course of the study in IL-6 and RANTES: IL-6 adjusted (ratio: 0.63, 90% CI 0.40, 0.99; $p = 0.094$); RANTES adjusted (ratio: 0.58, 90% CI 0.38, 0.90; $p = 0.042$) (Fig. 4). Indeed, in most cases CVs (variability) were slightly higher for values adjusted for sputum weight. A similar lack of impact of adjusting for sputum weight was seen for the other biomarkers. In addition, variability was only slightly reduced when the results of the waking and post-waking samples were pooled, and this had no effect on the interpretation of the data.

Safety

Overall, the incidence of AEs was higher in the placebo group (94%) than the AZD9668 group (68%). There were no deaths or treatment-related serious AEs. One patient in each treatment group discontinued due to AEs: low mood, lethargy, neck swelling, sleep disorder and increased appetite (placebo) and exacerbation of bronchiectasis (AZD9668).

There were more respiratory symptoms reported on placebo and more nervous system symptoms and infections (mostly nasopharyngitis) on AZD9668. Table 3 shows that the most commonly reported AEs were headache (2/16 [13%] placebo and 7/22 [32%] AZD9668), diarrhoea (4/16 [25%] placebo and 2/22 [9%] AZD9668) and nasopharyngitis (0/16 [0%] placebo and 4/22 [18%] AZD9668).

No clinically relevant changes in clinical chemistry, haematology, urinalysis, vital signs, ECG, physical examination or sputum bacteriology were observed. In one patient, transaminases increased during treatment with AZD9668, began to improve prior to last dose of study drug and returned to baseline on further monitoring once off study medication; there were no associated symptoms and bilirubin remained normal, but a relationship to study drug could not be excluded.

Discussion

This was a signal-searching study of AZD9668, a novel orally active NE inhibitor, in patients with bronchiectasis. Bronchiectasis was chosen due to the high and persistent levels of NE activity in sputum in this condition.[20] A number of clinical and biomarker variables that could potentially reflect the result of NE inhibition were assessed. We did not demonstrate that 28 days of AZD9668 (60 mg bid) altered the sputum neutrophil counts, although the sputum neutrophil elastase activity had decreased numerically (not
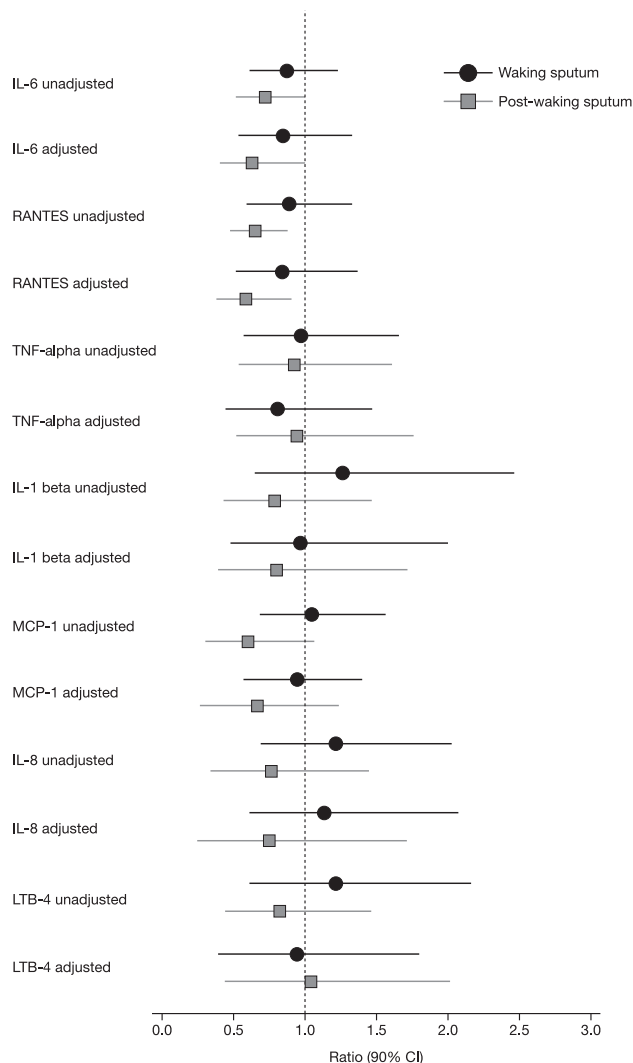

Figure 3 Pre-dose and 1–2 h post-dose concentration of AZD9668 in plasma and sputum at Day 28 for patients in the AZD9668 group.

Figure 4 *Post-hoc* analysis: ratio of change from randomisation to Day 28 in sputum biomarkers for AZD9668 versus placebo − data unadjusted versus adjusted for sputum weight. IL = interleukin; RANTES = Regulated on Activation, Normal T Expressed and Secreted; TNF = tumour necrosis factor; MCP-1 = monocyte chemoattractant protein-1; LTB-4 = leukotriene B4.

statistically significant) at the end of treatment. There was, however, a significant increase in lung function in the AZD9668 group (a difference of 100 mL in FEV_1 and a difference of 130 mL in SVC compared with placebo). There were also non-significant trends favouring AZD9668 for FVC, $FEF_{25-75\%}$, morning PEF, 24-h sputum weight, SGRQ-C (all scores) and for most inflammatory biomarkers, especially in the post-waking sputum samples. There were no differences between AZD9668 and placebo for BronkoTest® diary card symptom variables, reliever medication use and markers of tissue degradation.

The significant change in prebronchodilator FEV_1 in such a short study reflects a process independent of bronchodilation and is the kind of change seen after sputum clearance following antibiotic treatment[21] suggesting it reflects a different pathological process. However, in contrast, no significant changes in lung function tests were seen in

Table 3 Treatment emergent adverse events occurring in ≥5% of patients (safety analysis set).

MedDRA preferred term	n (%) patients	
	Placebo (n = 16)	AZD9668 60 mg bid (n = 22)
Headache	2 (13)	7 (32)
Diarrhoea	4 (25)	2 (9)
Nasopharyngitis	0 (0)	4 (18)
Peripheral oedema	2 (13)	1 (5)
Constipation	2 (13)	0 (0)
Oropharyngeal pain	2 (13)	0 (0)
Musculoskeletal chest pain	0 (0)	2 (9)
Contusion	0 (0)	2 (9)
Lethargy	1 (6)	1 (5)
Upper abdominal pain	1 (6)	1 (5)
Nausea	1 (6)	1 (5)
Vomiting	1 (6)	1 (5)
Back pain	1 (6)	1 (5)
Haemoptysis	1 (6)	1 (5)

MedDRA = Medical Dictionary for Drug Regulatory Affairs.

a 12-month study of nebulised gentamicin[22] even though sputum purulence, elastase and time to first exacerbation did improve. This may indicate that targeting certain aspects of the disease process, such as with an anti-inflammatory therapy, may offer different benefits from those offered by antimicrobials.

Two recently-published Phase II studies showed that AZD9668 did not improve lung function or respiratory signs and symptoms in patients with COPD.[23,24] The findings of the current study might have predicted the negative findings in COPD as NE activity is lower than in bronchiectasis so any benefit of AZD9668 would have been less marked and much slower to see. The potential masking effect of budesonide/formoterol in the second COPD study may also have contributed to the lack of effect.

While it is possible that higher concentrations of AZD9668 may be required to inactivate NE completely in the airways, in this study, AZD9668 was present in plasma and sputum supernatants at concentrations predicted to inhibit NE in the lung tissue. The IC_{50} for NE inhibition in *in vitro* assays is between 44 and 50 nM[15] and these levels were exceeded in both the plasma and sputum of most patients in this study. As such, the absence of any significant effect on NE activity or sputum neutrophil cell count is likely due to the large inter-patient variability in these data, especially as a non-significant trend for reduced NE activity was observed in AZD9668-treated patients. Furthermore, NE has been shown to play a role in mucus release[25]; therefore, it is possible that AZD9668 could have improved lung function via inhibition of NE and a resulting decrease in mucus secretion. This hypothesis would be partially supported by our observation of a non-significant trend for the reduction of 24-h sputum weight in the AZD9668 group versus an increase in the placebo group. In addition, inhibition of NE by AZD9668 may have improved lung function via an improvement in mucociliary clearance.[3]

While a direct effect of AZD9668 on NE activity in patients with bronchiectasis was not confirmed by our data, the observed trend for a decrease in NE activity is in line with that of another Phase IIa study assessing the efficacy of AZD9668 in cystic fibrosis,[26] which also showed a non-significant trend to reduced NE activity on active drug. One problem in the interpretation of sputum data in small studies is the large variability. Even though the assay validation was designed to minimise the analytical variation and any matrix interference, other technical factors such as sputum processing will have some effect. However, the major feature that contributes is large intra- and inter-patient variability seen even with sputum collection over longer periods than undertaken here, which will include variable dilution with nasopharyngeal secretions and airways hydration. Recent data have shown that with longer collection periods (over 4 h) and sequential daily sampling intrapatient variability can be reduced, optimised by using the average of at least 3 days' consecutive data.[27] This approach should be used in future studies to increase the power and/or reduce the number of patients needed.

Despite the variability, the trend for reductions in inflammatory markers in the AZD9668 group versus placebo was significant for post-waking sputum IL-6 and RANTES as well as plasma IL-8. Reductions in IL-8 and RANTES may be associated with the ability of NE to affect IL-8 and RANTES expression through regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB).[10,28-31] Similarly, *in vitro* studies using NE inhibitors support the link between NE and increased IL-6 production.[32,33] However, these data must be interpreted with caution, as the changes were generally small, with large variability within and between patients which was unaffected by adjusting for sputum weight or by pooling samples. Furthermore, statistical significance was not adjusted for multiple testing and the exploratory threshold p-value ($p < 0.1$) is associated with a substantially increased risk of chance findings, particularly given the number of sputum inflammatory marker assessments undertaken.

This study was limited by its small size, which probably resulted in a lack of precision in the mean estimates. In addition, the small numbers of patients recruited by some centres, together with randomisation in blocks of 4, may have contributed to some of the imbalance of the baseline characteristics. It is difficult to determine whether the baseline differences in lung function and the greater frequency of use of bronchodilator medications in the AZD9668 group could have contributed to the observed differences in lung function from placebo. Some of these baseline differences, however, were taken into account in the statistical analyses by including the baseline value as a covariate in the analysis of covariance, and others were considered unlikely to have had an impact on the outcome of the study.

In conclusion, despite the short duration of treatment, AZD9668 demonstrated some signals indicative of potential clinical efficacy in patients with bronchiectasis. AZD9668 was also well tolerated at a dose of 60 mg twice daily given for 28 days. However, given the significant inter- and intra-patient variability in inflammatory markers and neutrophil elastase levels, confirmation of these findings will require larger studies of longer duration.

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Conflict of interest statement

Robert Stockley has received honoraria, non-commercial grant funding and speaker fees from AstraZeneca, Boehringer Ingelheim, Grifols, GSK, Pfizer and Takeda, and has acted on advisory boards for Almirall, AstraZeneca, Baxter Biologicals, Boehringer Ingelheim, CSL Behring, Grifols, GSK, MSD, Novartis, Pfizer and Schering Plough. Anthony De Soyza has received speaker and consultancy fees from AstraZeneca, Bayer, Boehringer Ingelheim, Chiesi, Forest Labs, GSK, Novartis and Teva UK. Kulasiri Gunawardena, John Perrett, Kristina Forsman-Semb and Neil Entwistle are former AstraZeneca employees and shareholders. Noel Snell is a former AstraZeneca employee and shareholder and has received honoraria from Argenta, AstraZeneca, Chiesi, Napp and Pulmagen.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2012.12.009.

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