# SUPPLEMENTARY MATERIAL

## Methods

### *Additional exclusion criteria*

Significant liver disease; alanine aminotransferase (ALT)/aspartate aminotransferase (AST) level ≥1.5 x upper limit of normal (ULN) at enrolment; involvement in the planning and/or conduct of the study (applicable to both AstraZeneca staff/delegate staff and/or staff at the study site); previous randomisation of treatment in the present study; participation (defined as administration of at least one dose of investigational product) in another clinical study within 12 weeks of enrolment; an acute exacerbation (defined as an increase in respiratory symptoms requiring hospitalisation and/or a course of oral glucocorticosteroids and/or antibiotics, either prescribed or self administered), or acute respiratory infection (upper or lower) requiring oral steroids or antibiotics in the 6 weeks prior to randomisation; other acute infections requiring treatment in the 4 weeks prior to randomisation; lung transplant patients; any electrocardiogram abnormality (including a QTc >450 msec for males and >470 msec for females, or any arrhythmia) which, in the opinion of the investigator, may have put the patient at risk or interfered with the study assessments; the use of prohibited medications; the use of percutaneous intravenous catheters; infection with *Burkholderia cepacia* complex; any other clinical disease or disorder, which, in the opinion of the investigator, could have put the patient at risk, or influenced the results or the patient's ability to participate in the study; a history of excessive alcohol consumption or chronic alcohol-induced disease; a donation of >1350 mL of blood in the 12 months or >500 mL of blood in the 3 months before randomisation; a suspected or known risk of the patient transmitting

human immunodeficiency virus, hepatitis B or C; scheduled in-patient surgery or hospitalisation during the course of the study.

***Pre-study, concomitant and post-study treatments***

The use of concomitant inhaled corticosteroids, inhaled bronchodilators and maintenance or prophylactic antibiotics as part of the patient's standard CF therapy was permitted during the study, provided that the doses had been stable for at least 4 weeks before enrolment and remained stable during the study. All patients were required to continue their standard cystic fibrosis (CF) therapies throughout the study (including long-acting muscarinic antagonist [LAMA], long-acting β agonists [LABA], LABA and inhaled corticosteroid combinations).

Patients were required to withhold LAMA/LABA for 12 h, short-acting β agonists (SABA) for 6 h and short-acting muscarinic antagonists (SAMA) for 8 h before the days on which they had their pulmonary function tests. Normal doses of these medications were resumed after testing. Other respiratory medications could be continued unchanged.

Medications prohibited throughout the dosing period (randomisation to end of treatment) included: anti-diabetics/hypoglycaemics; anti epileptics/anticonvulsives; warfarin; fluvastatin; celecoxib; high dose, continuous non-steroidal anti-inflammatory drugs (documented prn use was allowed. Ibuprofen was permitted only at low to medium doses [<20 mg/kg/day]); torsemide; amitriptyline; fluoxetine.

Medications prohibited throughout the study included: oral corticosteroids in the 8 weeks prior to randomisation and throughout the study (NB use of inhaled corticosteroids was

allowed); immunomodulatory agents within 8 weeks prior to randomisation and throughout the study.

***Sputum sampling methodology***

Sputum was diluted with eight volumes of phosphate-buffered saline (PBS) and after mixing on ice, the sample was centrifuged and four volumes of supernatant were aliquoted for soluble biomarker analysis. Four volumes of PBS containing dithiothreitol (0.2%) were added to the remaining sputum mix and after further mixing and centrifugation, cytospin slides were prepared from the cell pellet material.

***Biomarker analysis***

Biomarkers were measured as follows: sputum NE activity using an initial linear-rate kinetic-read assay based on the cleavage of a fluorescent marker from a small peptide substrate; sputum IL-8 and serum amyloid A using commercially available immunoassay kits (R&D Systems and Cayman Chemical Company, respectively); sputum leukotriene $B_4$ ($LTB_4$) using a commercially available competitive enzyme immunoassay (EIA, Cayman Chemical Company); sputum tumour necrosis factor alpha (TNFα), interleukin (IL)-6, IL-1β, Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES) and monocyte chemoattractant protein 1 (MCP-1) using a custom made 5 plex sandwich immunoassay kit with electrochemiluminescent read-out (Meso Scale Discovery); normal range serum C-reactive protein (CRP; n-CRP) using an immunoturbidometric method run on an Olympus AU640 clinical chemistry analyser; plasma TNFα, IL-6, IL-8 and IL-1β using a 4-plex sandwich immunoassay with electrochemiluminescent read-out (Meso Scale Discovery); plasma total desmosine using liquid chromatography with mass spectrometry

(LC/MS-MS) following hydrolysis, solid-phase extraction and derivatisation; urinary creatinine, high performance liquid chromatography with ultraviolet absorbance (LC/UV) detection; urinary 'free' desmosine using solid-phase extraction followed by high performance LC/MS-MS detection in unhydrolysed samples; and urinary 'total' desmosine using solid-phase extraction followed by LC/MS-MS in hydrolysed samples.

***Statistical analyses – description of assessment timelines***

Samples collected on Visits 1a, 2, 3a and 4 were used for determination of induced sputum absolute and percentage neutrophil counts, neutrophil elastase (NE) activity and inflammatory biomarkers. The data were summarised by calculating baseline as the arithmetic mean of data from Visits 1a and 2 and end of treatment as the arithmetic mean of data from Visits 3a and 4.

For the analyses of urine and plasma desmosine levels and 24-h sputum weight, baseline was defined as data from Visit 1a and end of treatment as data from Visit 4. For analyses of lung function tests, revised CF quality of life questionnaire (CFQ-R) and inflammatory biomarkers in blood, baseline was defined as data from Visit 2 and end of treatment as data from Visit 4. The mean of the last 7 days prior to first dose was defined as baseline data for BronkoTest® diary card data analyses, while end of treatment was defined as the mean of the last 7 days on treatment.

## Results

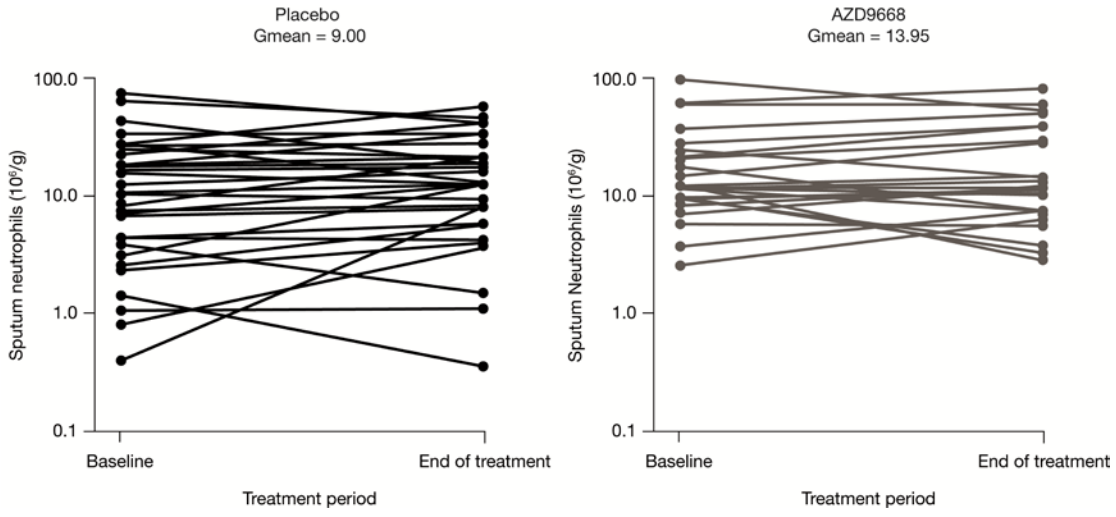
### *Concomitant medications after study entry*

The most frequently used medications received by patients during the study period were: Mucolytics-others (79% placebo, 77% AZD9668), Mucolytics-Pulmozyme (Dornase alfa) (59% placebo, 58% AZD9668), SABA (55% placebo, 46% AZD9668), inhaled corticosteroid (ICS)/LABA combination (34% placebo, 38% AZD9668), LAMA (14% placebo, 19% AZD9668), LABA (17% placebo, 15% AZD9668), SAMA (3% placebo, 15% AZD9668), ICS (10% placebo, 8% AZD9668), leukotriene receptor antagonists (7% placebo, 4% AZD9668) and SABA/SAMA (3% placebo, 0% AZD9668). The most frequently used antibiotics were macrolides (azithromycin), taken by more patients in the AZD9668 treatment group (15, 58%) than on placebo (8, 28%).

### *Absolute neutrophil counts*

**Figure E1**.

Absolute neutrophil counts for individual patients. Geometric means (Gmean) are for baseline values



***Spirometry***

**Table E1**.

Effect of AZD9668 versus placebo on signs and symptoms of cystic fibrosis at end of treatment (up to day 28) (efficacy analysis set)

| | **Mean at Baseline (SD)** | | **Change from Baseline, Mean (SD)** | | **Difference Between AZD9668 and Placebo (ANCOVA)** | | |
|---|---|---|---|---|---|---|---|
| | **Placebo (n=29)** | **AZD9668 60 mg bid (n=24)** | **Placebo (n=29)** | **AZD9668 60 mg bid (n=24)** | **LSM (SEM)** | **90% CI** | **p-value** |
| 24-h sputum weight (g)* | 13.86 (14.79) | 23.69 (29.01) | -4.34 (12.21) | -5.19 (11.92) | 2.83 (2.94) | -2.11, 7.78 | 0.341 |
| ***Lung function data*** | | | | | | | |
| $FEV_1$ (L) | 3.41 (1.16) | 2.63 (0.86) | -0.01 (0.21) | 0.00 (0.20) | -0.03 (0.06) | -0.13, 0.08 | 0.651 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SVC (L)[†] | 4.88 (1.09) | 4.37 (0.89) | -0.12 (0.32) | 0.04 (0.47) | 0.11 (0.13) | -0.10, 0.32 | 0.364 |
| FVC (L) | 4.87 (1.17) | 4.38 (0.91) | -0.01(0.27) | 0.00 (0.36) | -0.07 (0.09) | -0.21, 0.07 | 0.412 |
| $FEF_{25\%-75\%}$ (L/sec) | 2.64 (1.94) | 1.54 (1.02) | 0.08 (0.46) | -0.04 (0.26) | -0.14 (0.12) | -0.34, 0.05 | 0.231 |
| % predicted $FEV_1$ (%) | 78.40 (23.74) | 64.64 (19.66) | -0.15 (4.87) | -0.26 (5.09) | -1.10 (1.45) | -3.53, 1.33 | 0.451 |
| ***BronkoTest® diary card data*** | | | | | | | |
| PEF | | | | | | | |
| Morning (L/min) | 523.16 (109.95) | 467.96 (132.84) | 8.38 (34.19) | -4.83 (36.81) | -15.22 (10.21) | -32.37, -1.93 | 0.143 |
| Evening (L/min) | 538.25 (115.2) | 479.59 (125.74) | 3.15 (28.25) | -2.29 (33.59) | -5.72 (8.74) | -20.39, 8.95 | 0.516 |
| Night-time symptoms[‡] | 0.03 (0.08) | 0.16 (0.37) | 0.17 (0.67) | 0.11 (0.41) | -0.14 (0.15) | -0.40, 0.12 | 0.373 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Breathing difficulty[§] | 2.02 (0.17) | 2.08 (0.20) | 0.04 (0.23) | 0.11 (0.40) | 0.09 (0.09) | -0.06, 0.25 | 0.330 |
| Sputum colour[‡] | 3.59 (1.62) | 4.26 (1.31) | 0.00 (0.28) | -0.16 (1.01) | -0.11 (0.22) | -0.48, 0.26 | 0.612 |
| Sputum amount | 1.42 (0.87) | 2.04 (0.96) | 0.00 (0.33) | 0.05 (0.51) | 0.15 (0.13) | -0.06, 0.36 | 0.234 |
| Sputum type[‡] | 1.91 (0.59) | 2.19 (0.65) | 0.04 (0.37) | 0.06 (0.34) | 0.05 (0.10) | -0.11, 0.22 | 0.581 |
| How do you feel[§] | 2.04 (0.22) | 2.05 (0.16) | 0.02 (0.38) | 0.11 (0.42) | 0.09 (0.11) | -0.09, 0.27 | 0.413 |
| How often do you cough | 0.92 (0.58) | 1.06 (0.56) | 0.01 (0.38) | 0.11 (0.30) | 0.13 (0.10) | -0.04, 0.30 | 0.195 |
| Reliever medication taken today | 0.26 (0.85) | 0.00 (0.00) | -0.19 (0.78) | 0.04 (0.18) | -0.01 (0.08) | -0.15, 0.13 | 0.887 |
| ***CFQ-R scores*** | | | | | | | |
| Overall score (%)[†] | 946 (142) | 858 (164) | 1.0 (56.7) | -8.7 (86.8) | -24.4 (22.87) | -62.9, 14.1 | 0.293 |

Abbreviations: ANCOVA, analysis of covariance; BCSS, breathlessness, cough and sputum scale; bid, twice daily; CFQ-R, revised cystic fibrosis quality of life questionnaire; CI, confidence interval; $FEF_{25\%-75\%}$, forced expiratory flow between 25% to 75% of vital capacity; $FEV_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; LSM, least squares mean; PEF, peak expiratory flow; SD, standard deviation; SEM, standard error of the mean; SVC, slow vital capacity.

[*]Number of patients in the placebo group = 27.

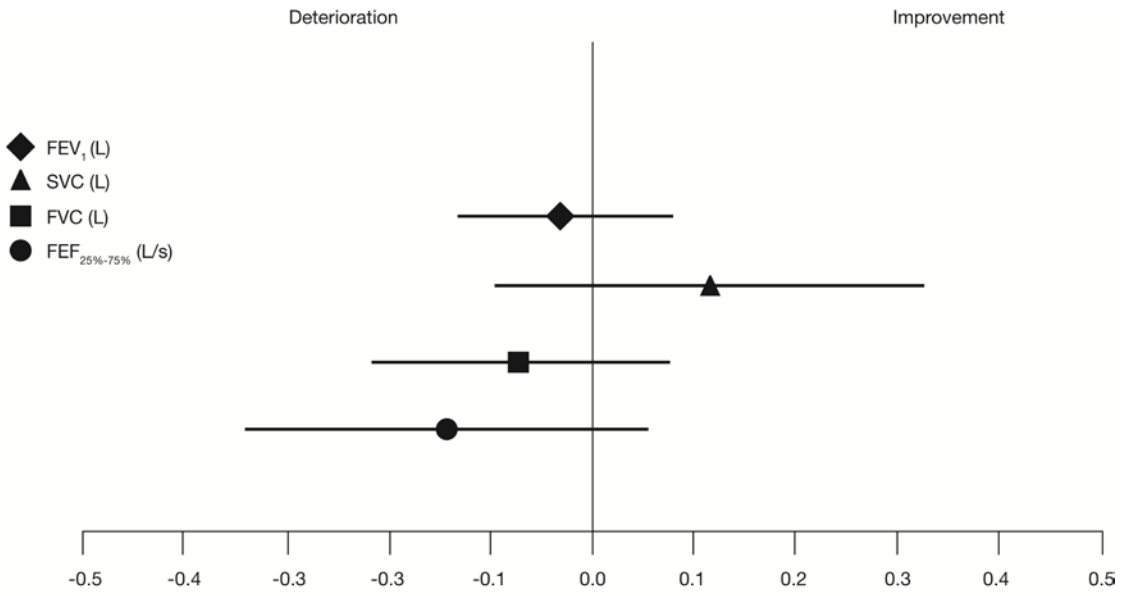
[†]Number of patients in the placebo group = 27 and in the AZD9668 group = 20.

[‡]Number of patients in the placebo group = 27 and in theAZD9668 group = 23.

[§]Number of patients in the placebo group = 28.

**Figure E2**.

Difference (and 90% confidence interval) between AZD9668 and placebo for the lung function tests (analysis of covariance)

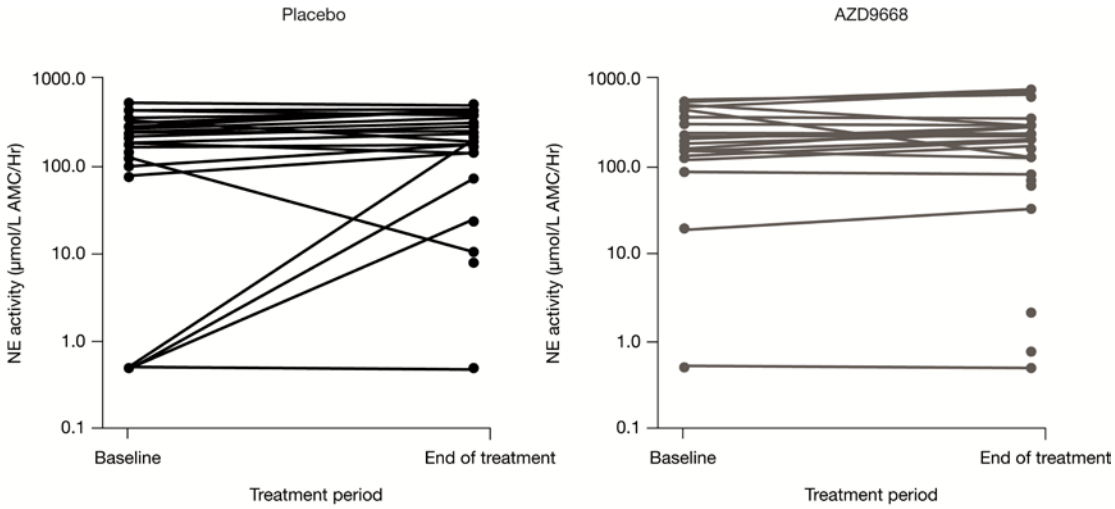



Abbreviations: $FEV_1$, forced expiratory volume in 1 second; SVC, slow vital capacity; FVC, forced vital capacity; $FEF_{25\%-75\%}$, forced expiratory flow between 25% to 75%

***NE activity in sputum***

**Figure E3**.

Individual patient neutrophil elastase activity data




Abbreviation: NE, neutrophil elastase

***Inflammatory biomarkers***

**Table E2**.

Sputum biomarker data

| **Biomarker** | **Treatment Groups** | **N** | **Baseline** | **End of Treatment** | **ANCOVA** | | |
|---|---|---|---|---|---|---|---|
| | | | **Gmean (CV, %)** | **Gmean (CV, %)** | **Ratio (AZD9668/Placebo)** | **90% CI** | **P-value** |
| TNF-α (pg/mL) | Placebo | 28 | 22.98 (181) | 30.75 (163) | | | |
| | AZD9668 | 24 | 28.86 (119) | 28.48 (130) | 0.73 | 0.51, 1.04 | 0.139 |
| IL-6 (pg/mL) | Placebo | 28 | 36.77 (159) | 36.70 (154) | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | AZD9668 | 24 | 30.95 (128) | 20.26 (119) | 0.59 | 0.44, 0.80 | 0.006 |
| IL-8 (pg/mL) | Placebo | 26 | 13693.91 (129) | 16215.69 (123) | | | |
| | AZD9668 | 24 | 15205.17 (57) | 14310.01 (59) | 0.83 | 0.64, 1.08 | 0.238 |
| IL-1β (pg/mL) | Placebo | 28 | 566.12 (269) | 732.62 (179) | | | |
| | AZD9668 | 24 | 1121.04 (85) | 1037.34 (96) | 0.87 | 0.63, 1.20 | 0.481 |
| $LTB_4$ (pg/mL) | Placebo | 25 | 996.13 (102) | 951.76 (94) | | | |
| | AZD9668 | 24 | 871.11 (95) | 808.64 (91) | 1.02 | 0.83, 1.24 | 0.901 |
| RANTES (pg/mL) | Placebo | 28 | 5.86 (74) | 6.26 (74) | | | |
| | AZD9668 | 24 | 4.89 (62) | 4.41 (70) | 0.77 | 0.59, 1.00 | 0.100 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MCP-1 (pg/mL) | Placebo | 28 | 163.05 (101) | 131.16 (99) | | | |
| | AZD9668 | 24 | 115.44 (82) | 88.50 (65) | 0.80 | 0.63, 1.02 | 0.129 |

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CV, coefficient of variation; Gmean, geometric mean; IL, interleukin; $LTB_4$, leukotriene $B_4$; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF-α, tumour necrosis factor α.

Summary statistics for baseline and change only contain patients included in the ANCOVA. A decrease is an improvement for all parameters except TNF-α where improvement/deterioration is not defined. ANCOVA includes treatment, country and baseline as covariates. The analysis was done on log-transformed data. The table presents the results back-transformed after the analysis on the original scale. If ratio CI contains 1, there is no evidence of a difference between AZD9668 and placebo.

***Safety***

**Table E3**.

Adverse event profile of AZD9668 (safety analysis set)

| | **Number (%) of Patients** | |
|---|---|---|
| | **Treatment Group** | |
| | **Placebo (n=29)** | **AZD9668 60 mg bid (n=26)** |
| **AE category**[*] | | |
| Any AE | 14 (48) | 12 (46) |
| Deaths | 0 | 0 |
| Any SAE | 2 (7) | 0 |
| Any AE leading to discontinuation | 2 (7) | 0 |
| Any other significant AE | 0 | 0 |
| **AEs by MedDRA preferred term**[†] | | |
| Headache | 5 (17) | 7 (27) |
| Nasopharyngitis | 3 (10) | 2 (8) |

| | | |
|---|---|---|
| Dyspnoea | 1 (3) | 2 (8) |
| Oropharyngeal pain | 1 (3) | 2 (8) |
| Cough | 1 (3) | 1 (4) |
| Fatigue | 1 (3) | 1 (4) |
| Back pain | 1 (3) | 1 (4) |
| Musculoskeletal chest pain | 1 (3) | 1 (4) |
| Blood creatinine phosphokinase increased | 1 (3) | 1 (4) |
| Non cardiac chest pain | 2 (7) | 0 |
| Pyrexia | 2 (7) | 0 |
| Constipation | 2 (7) | 0 |
| Diarrhoea | 2 (7) | 0 |
| Back pain | 1 (3) | 1 (4) |
| Musculoskeletal chest pain | 1 (3) | 1 (4) |
| Blood creatinine phosphokinase increased | 1 (3) | 1 (4) |

Abbreviations: AE, adverse event; bid, twice daily; MedDRA, Medical Dictionary for Regulatory Activities.

*Patients with multiple events in the same category were counted once in that category. Patients with events in more than one category were counted in each of those categories.

†Patients with multiple events were counted once for each preferred term

Note: MedDRA Version 13.0 used.