Supplementary Material

This supplementary material has been provided by the authors to give readers additional information about the work (last updated November 06, 2017).

Contents

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3. Expanded Methods Section

Pre-clinical

In-Situ Proximity Ligation Assay (isPLA)

isPLA on lung tissue: Lung tissue specimens were obtained from COPD patients who had resection for lung cancer, and healthy controls. All donors were between 46-65 years of age. The nine COPD donors were all (GOLD) stage IV patients and smokers with a male/female ratio of 5:4. The healthy control donors consisted of 3 males of which two were non-smokers and one ex-smoker with 7.5 pack years. One healthy control donor was a female ex-smoker with 2 pack years. Post-surgical formalin-fixed paraffin-embedded (FFPE) lung tissue from the COPD patients and the four control donor lungs was sectioned at 2 µm, dried O/N at 37°C, rehydrated by incubating for 2 x 5 min in xylene, 2 x 1 min in 99% ethanol, 1 min in 95% ethanol, 1 min in 70% ethanol, 1 min in 50% ethanol, and 5 min in double-distilled water. Antigen retrieval was performed by incubating the sections at 98°C for 47 min in a Tris-HCl buffer (pH 7.8) containing 1 mM EDTA. The sections were assayed using the p38α/MAPK14 antibody (#AF8691, R&D Systems, Minneapolis, MN) and the p-p38 (pT180/pY182) antibody (#4511, Cell Signaling Technology, Boston, MA). The isPLA assay was performed using the Duolink® In Situ PLA® Probe Anti-Mouse PLUS (#DUO92001, Sigma Aldrich, St Louis, MO), the Duolink® In Situ PLA® Probe Anti-Rabbit MINUS (#DUO92005, Sigma Aldrich, St Louis, MO) the Duolink® In Situ Detection Reagents Brightfield (#DUO80102, Sigma Aldrich, St Louis, MO) following the manufacturer's instructions. All assessments were performed blind to source of tissue and repeated on two separate occasions.

isPLA on lung alveolar macrophages: Alveolar macrophages were isolated from lung tissue of patients undergoing lung resection surgeries by flushing with PBS, incubated with 10 ng/mL LPS (#L4516 Sigma Aldrich, St Louis, MO) for 1 h, pelleted and fixed in formalin. The cell pellets were re-suspended in Histogel (#HG-4000-012, ThermoFisher Scientific, Waltham, MA), allowed to solidify and processed for dehydration using standard methodology. The FFPE cell pellets were sectioned at 2 µm, dried O/N at 37°C, rehydrated by incubating for 2 x 5 min in xylene, 2 x 1 min in 99% ethanol, 1 min in 95% ethanol, 1 min in 70% ethanol, 1 min in 50% ethanol, and 5 min in double-distilled water. Antigen retrieval was performed by incubating the sections at 98°C for 47 min in a Tris-HCl buffer (pH 7.8) containing 1 mM EDTA. The sections were assayed using the p38α/MAPK14 antibody (#AF8691, R&D Systems, Minneapolis, MN), p38β antibody (#MAB3274, R&D Systems, Minneapolis, MN), the

p38γ antibody (#2307, Cell Signaling Technology, Boston, MA), p38δ/MAPK13 antibody (#197202, R&D Systems, Minneapolis, MN), the p-p38 (pT180/pY182) antibody (#4511, Cell Signaling Technology, Boston, MA), and the p-p38 (pT180/pY182) antibody (#9216, Cell Signaling Technology, Boston, MA). The isPLA assay was performed using the Duolink® In Situ PLA® Probe Anti-Mouse PLUS (#DUO92001, Sigma Aldrich, St Louis, MO), the Duolink® In Situ PLA® Probe Anti-Rabbit MINUS (#DUO92005, Sigma Aldrich, St Louis, MO), the Duolink® In Situ PLA® Probe Anti-Rabbit PLUS (#DUO92002, Sigma Aldrich, St Louis, MO), the Duolink® In Situ PLA® Probe Anti-Mouse MINUS (#DUO92004, Sigma Aldrich, St Louis, MO), and the Duolink® In Situ Detection Reagents Brightfield (#DUO80102, Sigma Aldrich, St Louis, MO) following the manufacturer's instructions. For the analysis of the phosphorylated isoforms, an isoform-specific antibody and a phosphorylation-specific antibody (originating from different species) were used. For those analyses, Duolink® In Situ PLA® Probe Anti-Rabbit MINUS and Duolink® In Situ PLA® Probe Anti-Rabbit PLUS were employed. For the analysis of the total levels of each of the p38 isoforms, the isoform-specific antibodies were used in combination with both PLA probes recognizing the same species antibody (Duolink® In Situ PLA® Probe Anti-Mouse PLUS in combination with Duolink® In Situ PLA® Probe Anti-Mouse MINUS or Duolink® In Situ PLA® Probe Anti-Rabbit MINUS in combination with Duolink® In Situ PLA® Probe Anti-Rabbit PLUS). The analysis of the isPLA was performed using the Visiopharm software (Hoersholm, Denmark). Expression of p38 in isolated lung alveolar macrophages from COPD explanted lung specimens was measured by isPLA. Samples were prepared and assayed using antibodies against p38α, p38β, p38γ, p38δ, and phosphorylated p38 (p-p38). For the analysis of the phosphorylated isoforms, an isoform-specific antibody and a phosphorylation-specific antibody were used.

LPS-stimulated alveolar macrophages for cytokine release assay: Human alveolar macrophages were derived from lung resection tissue specimens. Cell count was estimated and cells seeded at a density of 200,000 cells per well in a 96 well cell culture plate (Costar). Cells were allowed to adhere for 1 hour at 37C in a 5% CO_2 humidified incubator in serum-free/phenol red free RPMI 1640 (Life Technology). After 1 hour, non-adherent cells were removed by washing with RPMI 1640. Adherent cells were rested overnight in the incubator in X-Vivo 10 (Lonza) media supplemented with 4mM L-Glutamine (Life Technology) and 1% penicillin-streptomycin (Life Technology). For compound treatment, media from overnight rested macrophages previously described were removed and fresh media added. Compounds diluted in media or DMSO alone (vehicle control, Sigma) were then added to the cells and incubated for 1h at 37°C. For stimulation, LPS from E. coli (Sigma) was used at a final concentration of

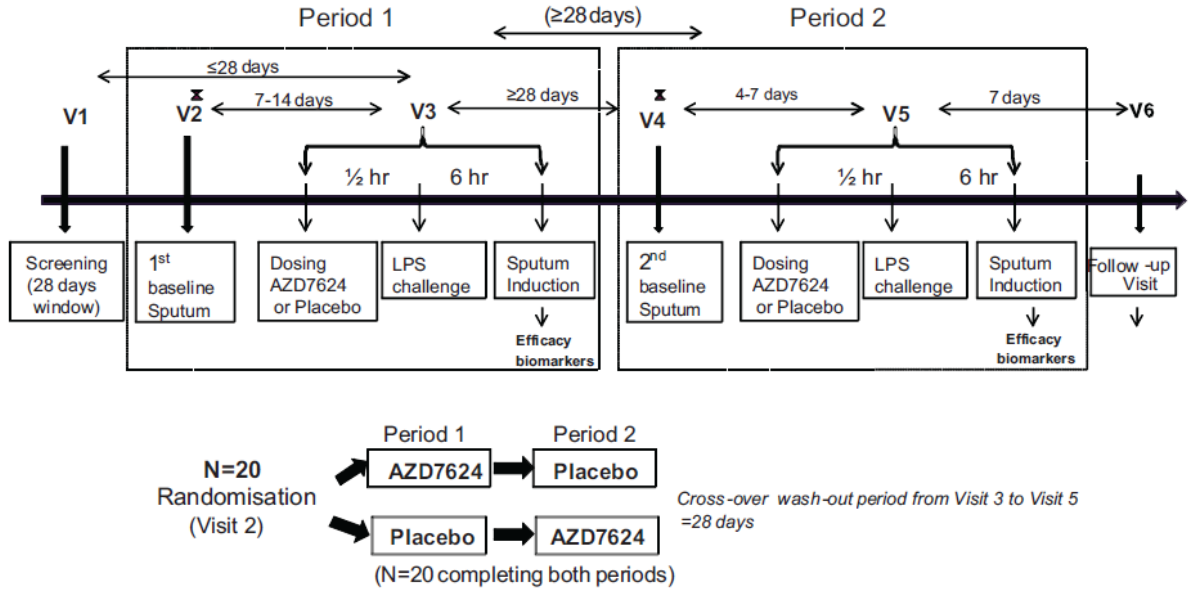
100 ng/mL and cells incubated for 18 h at 37°C. Unstimulated cells were included in all assays, as control. Following stimulation, the supernatants were collected and cytokine analyzed by multiplex immunoassay from MSD™ (Meso Scale Discovery). For calculating percentage inhibition of compounds on cytokine release the following formula was used (Max LPS response-LPS response in the presence of compound)/(max LPS response-unstimulated response)*100. To obtain concentration-responses, curve fitting was carried out using nonlinear regression, four-parameter equation with variable slope (GraphPad Prism 6). No constrains were placed on curve fitting. For deriving accurate pIC_{50} for inhibition of IL6, two data points from one donor was excluded from curve fitting. Curve fitted donor data are presented as mean± S.E.M.

Clinical

In the LPS Challenge proof of mechanism study, Male and female subjects of non-childbearing potential aged 18–55 years were screened within 28 days before the first administration of AZD7624, followed by a second pre-entry visit for sputum induction and methacholine challenge 7–14 days before dosing. A total of 30 volunteers were randomized to one of the two treatment sequences in a 1:1 ratio:

- Sequence 1, AZD7624 followed by placebo after a washout period of ≥28 days;
- Sequence 2, placebo followed by AZD7624 after a washout period of ≥28 days.

Subjects received a single inhaled lung-deposited dose of AZD7624 (1200 μg) or placebo 30 min prior to LPS challenge with sputum induction 6 h post challenge (6.5 h post-dose) for measurement of inflammatory biomarkers. Blood samples were collected 0.25, 6.5, 12, and 24 h post AZD7624 or placebo dosing for the analysis of biomarkers (see Figure S1)



LPS Lipopolysaccharide; V Visit
X Visit 2 and Visit 4 could have been repeated for additional 2 times to meet the acceptance criteria for quality of baseline sputum

Figure S1 Study flow chart of human LPS challenge study

The COPD proof of principle study was designed to examine an exacerbation based outcome in a relatively small and short duration study. This was done by incorporating several design elements designed to boost the event rate and potential treatment effect. Patients at high risk for future exacerbations were chosen based upon history of 2 or more exacerbations despite use of ICS/LABA[1]. Patients were enrolled preferentially during fall and winter months to capture patients at further increased risk[2]. Figure S2 outlines the study schema.

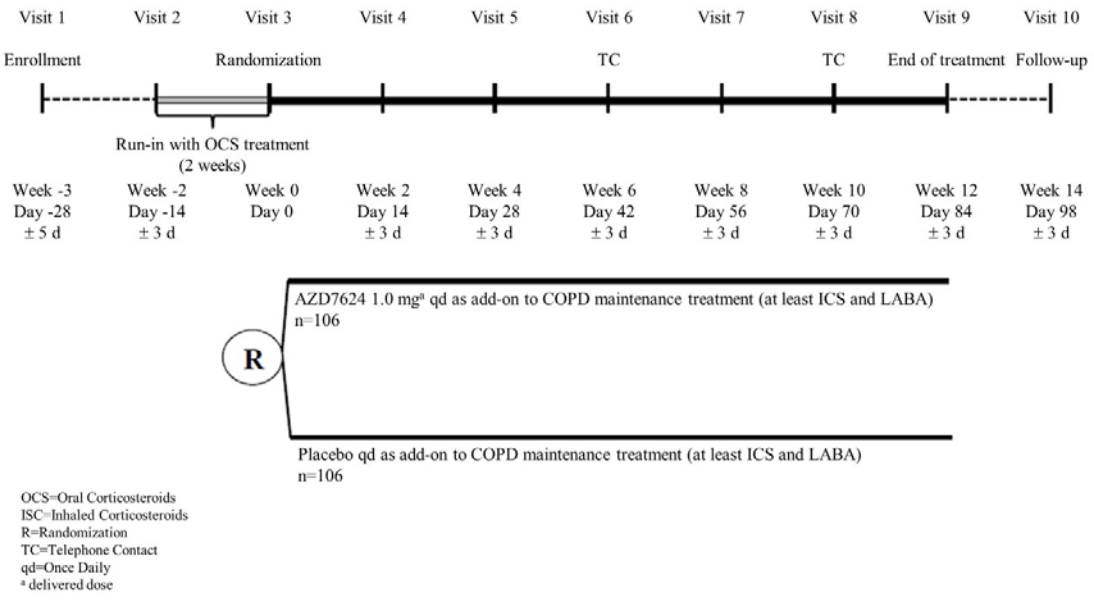


Figure S2 Study Schema – Proof of Principle COPD ExDo Study

For a list of complete inclusion and exclusion criteria, see below:

For inclusion in the study patients should fulfill the following criteria:

1. Provision of signed and dated written informed consent prior to any study specific procedures
2. Male and females aged 40–85 years
3. Females must have a negative pregnancy test, must not be lactating, and must be of non-childbearing potential, by fulfilling one of the following criteria:
    a. Postmenopausal defined as amenorrhea for at least 12 months or more following cessation of all possible exogenous hormonal treatments and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in the postmenopausal range
    b. Documentation of irreversible surgical sterilization by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation

4. Males must be surgically sterile or agree to use an acceptable method of contraception (defined as barrier methods in conjunction with spermicides) for the duration of the study (from the time they sign consent) and for 3 months after the last dose of investigational product to prevent pregnancy in a partner.
5. A weight of ≥50 kg (measured at Visit 1)
6. Clinical diagnosis of COPD for more than 1 year at Visit 1, according to the GOLD 2014 guidelines
7. Stable COPD maintenance treatment with at least ICS and LABA for at least 2 months prior to enrolment, to be continued unchanged during the study
8. A post-bronchodilator FEV1/FVC <0.70 and a post-bronchodilator FEV1 ≤70% of the predicted normal value
9. Documented history of 2 or more moderate or severe COPD exacerbations (requiring treatment with systemic corticosteroid or antibiotics, or both, or hospital admission) within 12 months of randomization, but not within the last 6 weeks before randomization. At least one of the exacerbations should be while on current COPD maintenance therapy (at least ICS and LABA)
10. Current or ex-smokers with a smoking history of at least 10 pack-years (1 pack year = 20 cigarettes smoked per day for one year)
11. Able to read and write and to comply with the study procedures
12. Ability to complete an eDiary correctly. Baseline diary data recorded for any 14 morning or evening diaries in the last 10 days of the run-in period to be eligible for randomization
13. Demonstrated ability to inhale with required inhalation flow using the Flow Indicator.
14. Demonstrated ability to inhale from the ADI device according to the provided instructions

Patients should not enter the study if any of the following exclusion criteria are fulfilled:

1. Involvement in the planning and conduct of the study (applies to both AstraZeneca staff and staff at third party vendors or at the investigational sites)
2. Previous randomization to treatment in the present study
3. Participation in another clinical study with any novel investigational medicinal product within 3 months before the first dose of investigational product in this study
4. Previously intake of any p38 inhibitor (same class as AZD7624)

5. Participation in, or scheduled for an intensive COPD rehabilitation program at any time during the study (N.B. patients are allowed to be in the maintenance phase of a rehabilitation program)
6. Planned in-patient surgery or hospitalization during the study

The primary efficacy outcome variable was a composite endpoint of moderate to severe exacerbations plus dropouts (ExDo). Rational for this is that based upon observation that subjects who drop-out early from COPD studies have a higher rate of exacerbations and risk factors for drop-out are similar to risk factors for exacerbations themselves[3]. This would suggest that a composite endpoint of exacerbation plus drop-outs might be able to serve as a surrogate for exacerbations, but with enhanced event rate. The oral corticosteroid run-in was used a stabilization approach to decrease intra- and interpatient variability before randomization. This has been proposed in asthma to allow better comparison of inhaled corticosteroid potency[4] and has been used in multiple COPD studies[5-7].

The primary efficacy outcome variable, Time to first event of moderate or severe COPD exacerbation or early drop-out related to worsening of COPD symptoms (ExDo), was calculated as the number of days from the date of randomization to the date of the first post-randomization event meeting the definition outlined below, i.e. Time to event = date of event – date of randomization + 1. The time to first COPD exacerbation for subjects who do not experience a COPD exacerbation during the treatment period was censored at the date of their last visit for the 12 week treatment period, or at the time point after which an exacerbation could not be assessed (for lost-to-follow-up subjects).

COPD exacerbation definition

COPD Exacerbations are classified using the following severity scale: **Mild exacerbations**: COPD symptom worsening that is self-managed by the patient, and not associated with use of systemic corticosteroids or antibiotics (N.B. mild exacerbations are not to be included in the 'ExDo' primary composite endpoint). **Moderate exacerbations**: COPD symptom worsening that requires treatment with systemic corticosteroids or antibiotics or both. Any course of steroid started within 7 days of finishing a previous course is considered treatment for a single exacerbation. Any course of antibiotics started within 7 days of finishing a previous course is considered treatment for a single exacerbation. Antibiotic treatment for upper or lower respiratory infections is not considered a COPD exacerbation unless the symptoms meet the COPD exacerbation definition outlined below. **Severe exacerbations**: COPD symptom worsening that requires hospital admission. Moderate and severe exacerbations as defined above will be included in the 'ExDo' composite endpoint.

The following analysis sets was used in this study:

All subjects analysis set

This analysis set comprises all subjects screened for the study and will be used for the reporting of disposition and screening failures.

Full analysis set

The full analysis set was used as the primary population for reporting efficacy data and to summarize baseline characteristics. This comprises all patients randomized into the study who receive at least 1 inhalation of study drug and will be analyzed according to randomized treatment (intention-to-treat principle). One patient was excluded from the full analysis set following review of protocol deviations due to lack of source data and GCP compliance.

Safety analysis set

The safety analysis set was used as the primary population for reporting safety data and to summarize baseline characteristics. This comprises all patients randomized into the study who receive at least 1 inhalation of study drug and will be analyzed according to the treatment they actually received irrespective of which treatment they were randomized to.

Analysis of the primary variable

All analyses of the primary variable were based on the full analysis set.

The primary efficacy variable was the time to first event of the composite endpoint referred to as “ExDo”. The primary analysis compared the primary efficacy variable for AZD7624 with placebo.

The following hypothesis was tested:

H_0: HR (AZD7624/placebo) equals 1 vs

H_1: HR does not equal 1.

The null hypothesis (H_0) was that the ExDo hazard rate during the 12-week double-blind treatment period on AZD7624 would be equal to the corresponding ExDo hazard rate on placebo. The alternative hypothesis (H_1) was that the ExDo hazard rate during the 12-week double-blind treatment period would be different on AZD7624 compared with the corresponding ExDo hazard rate on placebo. Of particular interest was the p-value corresponding to the value HR=0.55 which was the effect size that the study was powered for.

The analysis was performed using a Cox proportional hazard (PH) model, fitting treatment group, country, LAMA maintenance treatment, age (<50, ≥50 – <65, ≥65 years), baseline FEV_1, and sex (male, female) as covariates. Results were reported as a HR, 95% CI, and p-value.

Time to first ExDo event was displayed graphically for each treatment group using a Kaplan-Meier plot.

To validate the outcome of the PH model and to assess the effects of the inclusion of covariates, a log-rank test was also performed.

Analysis of secondary variables

All analyses of secondary efficacy variables were based on the full analysis set.

Analyses of exacerbation rates

The following secondary efficacy variables were analyzed using a Cox proportional hazard model:

- Time to first event of moderate or severe COPD exacerbations or early drop-out (including drop-outs due to any cause)
- Time to first moderate or severe exacerbation
- Time to first moderate or severe exacerbation (where worsening of COPD symptoms is defined as Anthonisens criteria fulfilled)
- Time to first symptom defined exacerbation (as defined by the EXACT daily diary)

For each variable, time to event was displayed graphically for each treatment group using a Kaplan-Meier plot.

Analyses of number of exacerbations

The following secondary efficacy variables (exacerbation rates) were compared for AZD7624 versus placebo.

- Number of moderate and severe COPD exacerbations and early drop-outs related to worsening of COPD symptoms (i.e. composite endpoint, ExDo)
- Number of moderate and severe COPD exacerbations and early drop-outs (including drop-outs due to any cause)
- Number of moderate and severe exacerbations
- Number of moderate and severe exacerbations (where worsening of COPD symptoms is defined as Anthonisens criteria fulfilled)
- Number of symptom defined exacerbations (as defined by the EXACT daily diary)

For each variable the following hypothesis was tested:

H_0: rate ratio (AZD7624/placebo) equals 1 vs

H_1: rate ratio does not equal 1.

The null hypothesis (H_0) was that the exacerbation rate during the 12-week double-blind treatment period on AZD7624 would be equal to the corresponding exacerbation rate on placebo. The alternative hypothesis (H_1) was that the exacerbation rate during the 12-week double-blind treatment period would be different on AZD7624 compared with the exacerbation rate during the 12-week double-blind treatment period on placebo.

The exacerbation rate in the AZD7624 group was compared to that observed in the placebo group using a negative binomial model. The response variable in the model was the number of exacerbations experienced by a subject over the 12-week double-blind treatment period. The model included covariates of treatment group, country, LAMA maintenance treatment, age (<50, ≥50 – <65, ≥65 years), baseline FEV_1, and sex (male, female). The logarithm of the subject's corresponding follow-up time was used as an offset variable in the model to adjust for subjects having different exposure times during which the events occurred.

The standard parameterization approach (NB2) of the Negative Binomial model was applied using PROC GENMOD (SAS procedure).

The estimated treatment effect (i.e., the rate ratio of AZD7624 vs pooled placebo), corresponding 95% confidence interval (CI), and two-sided p-value for the rate ratio were presented. In addition, the exacerbation rate and the corresponding 95% CI within each treatment group and the over-dispersion parameter were presented.

Time to any ExDo event

The time to any ExDo event was presented in a plot of cumulative number of events versus time, by treatment.

Duration of exacerbations

The total duration of moderate or severe exacerbations, moderate or severe exacerbations (where worsening of COPD symptoms is defined as Anthonisens criteria fulfilled), and of symptom defined exacerbations (as defined by the EXACT daily diary) was summarized by descriptive statistics including N, mean, standard deviation (SD), median, and range.

The total duration of symptom defined exacerbations (as defined by the EXACT daily diary) was analyzed using analysis of variance with treatment, country, and LAMA maintenance treatment as factors.

First clinically important deterioration

The time to first clinically important deterioration was analyzed using the same methodology as described for the analysis of exacerbation rates.

Night-time awakenings

The number and percentage of days where night-time awakenings were reported within each visit window and for the overall run-in and treatment periods were summarized by descriptive statistics including N, mean, standard deviation (SD), median, and range.

Changes from baseline in number of days were analyzed using a mixed effect repeated measures model (MMRM), with treatment, country, LAMA maintenance treatment, and visit as fixed effects, patient as a random effect, and baseline count as a continuous covariate. A term for visit window was included in the repeated statement (in SAS PROC MIXED or PROC GLM) and an unstructured covariance matrix was used, thus allowing adjustment for correlations between time points within patients. The denominator degrees of freedom were calculated using the Kenward-Roger method.

Use of reliever medication

The number of daytime inhalations, night-time inhalations, and total number of inhalations within each visit window and for the overall run-in and treatment periods were summarized by descriptive statistics including N, mean, standard deviation (SD), median, and range.

Changes from baseline in the total number of inhalations were analyzed using a MMRM, with treatment, country, LAMA maintenance treatment, and visit as fixed effects, patient as a random effect, and baseline count as a continuous covariate. A term for visit window was included in the repeated statement (in SAS PROC MIXED or PROC GLM) and an unstructured covariance matrix was used, thus allowing adjustment for correlations between time points within patients. The denominator degrees of freedom were calculated using the Kenward-Roger method.

Spirometry

Changes from baseline in trough FEV_1, FVC, and FEV_1/FVC were each analyzed using MMRM, with treatment, country, LAMA maintenance treatment, visit, sex (male, female), and smoking history (never, current, former) as fixed effects, patient as a random effect, and baseline assessment, age, BMI, and height as continuous covariates. A term for visit was included in the repeated statement (in SAS PROC MIXED or PROC GLM) and an unstructured covariance matrix was used, thus allowing adjustment for correlations between time points within patients. The denominator degrees of freedom were calculated using the Kenward-Roger method. Pre-bronchodilator spirometry

was only compared to other pre-bronchodilator values (baseline vs on-treatment) while post-bronchodilatory spirometry was only compared to other post-bronchodilator values.

From this analysis, the adjusted means for each treatment group, the difference between the adjusted means (AZD7624 vs placebo), 95% CI around the difference, and 2-sided p-value were calculated for each visit.

In patients with low lung function, the relative change from baseline in spirometry is considered to be a more relevant measure. To assess this, the analysis of FEV_1 and FVC described above were repeated on log-transformed data. The adjusted means, treatment differences, and 95% CIs were back-transformed and presented as geometric means and the ratio of geometric means, and 95% CI for the ratio.

The above analyses of spirometry data were repeated using Visit 2 as the baseline assessment.

Plots of mean trough FEV_1, FVC, and FEV_1/FVC versus time were produced.

Responder analyses were performed on the FEV_1 data, for each of the following definitions of responder:

- An increase of ≥5% compared to the Visit 2 baseline FEV_1
- An increase of ≥50 mL compared to the Visit 2 baseline FEV_1
- An increase of ≥100 mL compared to the Visit 2 baseline FEV_1

The response rates were summarized by visit, and compared between AZD7624 and placebo using logistic regression.

ER-S

Total symptom score and each of the three domains were summarized by visit and for the overall run-in and treatment periods.

Changes from baseline in the total score and each of the three domain scores were analyzed using MMRM, with treatment, country, LAMA maintenance treatment, and visit as fixed effects, patient as a random effect, and baseline assessment as a continuous covariate. A term for visit was included in the repeated statement (in SAS PROC MIXED or PROC GLM) and an unstructured covariance matrix was used, thus allowing adjustment for correlations between time points within patients. The denominator degrees of freedom were calculated using the Kenward-Roger method.

From this analysis, the adjusted means for each treatment group, the difference between the adjusted means (AZD7624 vs placebo), 95% CI around the difference, and 2-sided p-value were calculated for each visit.

SGRQ-C

Total score and each of the SGRQ-C domains were summarized by visit and for the overall run-in and treatment periods.

Changes from baseline in total score and each of the SGRQ-C domains were each analyzed using MMRM, with treatment, country, LAMA maintenance treatment, and visit as fixed effects, patient as a random effect, and baseline assessment as a continuous covariate. A term for visit was included in the repeated statement (in SAS PROC MIXED or PROC GLM) and an unstructured covariance matrix was used, thus allowing adjustment for correlations between time points within patients. The denominator degrees of freedom were calculated using the Kenward-Roger method.

From this analysis, the adjusted means for each treatment group, the difference between the adjusted means (AZD7624 vs placebo), 95% CI around the difference, and 2-sided p-value was calculated for each visit.

The number of patients with an improvement of more than 4 units was summarized by visit. The probability of achieving a ≥4 unit improvement after 12 weeks was compared between AZD7624 and placebo using logistic regression, fitting country, and LAMA maintenance treatment.

The MMRM analysis of SGRQ-C described above was repeated using visit 2 as the baseline assessment.

Plots of mean SGRQ-C total score and domains versus time were produced.

BDI / TDI

Total score and each of the three domain scores were summarized by visit and for the overall run-in and treatment periods.

TDI total score and each of the three domains were each analyzed using MMRM, with treatment, country, LAMA maintenance treatment, and visit as fixed effects, patient as a random effect, and corresponding BDI score as a continuous covariate. A term for visit was included in the repeated statement (in SAS PROC MIXED or PROC GLM) and an unstructured covariance matrix was used, thus allowing adjustment for correlations between time points within patients. The denominator degrees of freedom were calculated using the Kenward-Roger method.

BDI / TDI total scores were summarized by visit. The probability of achieving a ≥1 point improvement after 12 weeks was compared between AZD7624 and placebo using mixed model logistic regression, fitting treatment, country, and LAMA maintenance treatment as fixed effects, and modeling between visit correlations.

Plots of mean BDI/TDI total scores versus time were produced.

Safety and tolerability

All safety variables will be summarized using the safety analysis set and data presented according to treatment received.

COPD symptom worsening

The worsening of COPD symptoms can be either reported by the patient (patient driven) or related to a COPD daily eDiary alert (eDiary driven). Treating physicians together with patients then made decision on whether worsening symptoms required specific treatment.

Safety Evaluation

Safety outcomes in the ExDo proof of principle study included the frequency and type of adverse events and adverse drug reactions. On-treatment adverse events were summarized using the safety analysis set and data presented according to treatment received. Adverse events occurring during the screening and run-in period were listed, but not summarized. Serious adverse events (SAEs) were collected from informed consent throughout the study. We performed a complete physical examination at the time of screening and at follow-up and measured and recorded vital signs at baseline and at weeks 2, 4, 8, and 12 or at any unscheduled visit.

4. Expanded Preclinical Results Section

	COPD lung				Control lung			
Tissue	**p38α**	**p38β**	**p38γ**	**p38δ**	**p38α**	**p38β**	**p38γ**	**p38δ**
Epithelium (bronchi)	2	1	1	3	1	1	2	2
Epithelium (respiratory bronchioles)	1	1	1	3	1	1	2	3
Septa	2	1		2	1	1		2
Endothelial cells	1	1	2	2	1	1	3	2
Alveolar macrophages	2	1	2	3	1	1	3	3
Lymphocytes	1	1	1	2	1	1	2	2
Neutrophils	1	1	1	3	1	1	2	3
Broncho alveolar lavage	1	1	1	3	1	1	2	3
Smooth muscle cells	1	1	1	2	1	1	2	2

Table S1. Phosphorylated p38 expression in lung tissue from COPD patients and controls. Key: 1 = low signal, 2 = moderate signal, 3 = strong signal.

P38 isoform	pIC50	% Inhibition at 1 µM	Fold selectivity for p38α
p38α	10.0 ± 0.19 (n=10)	–	–
p38β	8.8 ± 0.15 (n=5)	–	15
p38γ	<6 (n=5)	27 ± 10	>10000
p38δ	<6 (n=5)	27 ±12	>10000

Table S2. Selectivity of AZD7624 on human p38 isoforms**.** All assays were performed with substrate concentrations substantially below the K_m and thus pIC_{50} values are equivalent to the pKi.

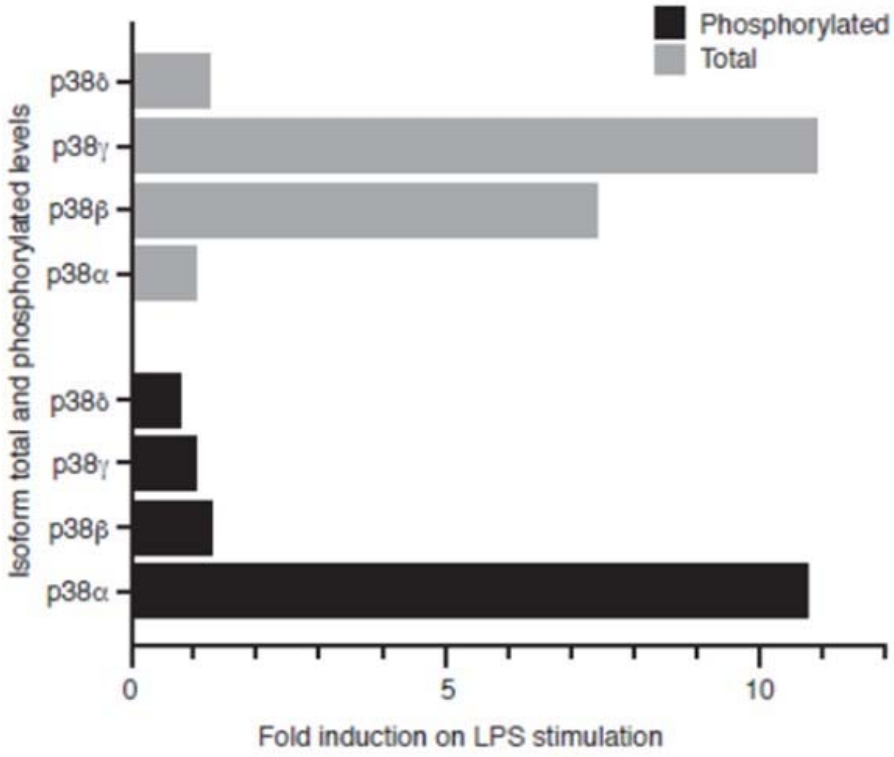


Figure S3. Phosphorylation of p38 isoforms and total level changes after LPS stimulation in human alveolar macrophages. Data are representative of 2 experiments.

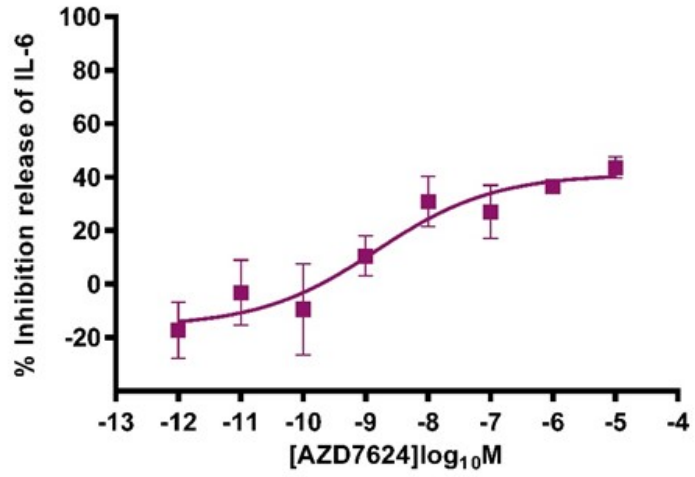


Figure S4. AZD7624 inhibition of LPS-stimulated pro-inflammatory cytokine IL-6 in human alveolar macrophages. Data are presented as mean ± SEM of n=4.

5. Expanded Clinical Results Section – LPS Proof of Mechanism Study

Characteristic	Study population (N=30)
Mean age, years (SD)	32 (9.2)
Male gender, N (%)	30 (100)
Race, N (%)	
White	24 (80.0)
Black or African American	3 (10.0)
Asian	2 (6.7)
Other	1 (3.3)
Ethnicity, N (%)	
Hispanic	2 (6.7)
Non-Hispanic	28 (93.3)
Mean height, cm (SD)	178.1 (4.9)
Mean weight, kg (SD)	77.9 (7.8)
Mean BMI, kg/m^2 (SD)	24.6 (2.3)

BMI, body mass index; SD, standard deviation

Table S3 Demographic and baseline characteristics of LPS-challenge study

	Change from baseline		Difference (placebo–AZD7624)	Relative change (%)	P-value
	AZD7624 (n=27)	Placebo (n=24)			
Sputum					
IL-6 (pg/mL)	16.52	70.33	53.81	76.5	0.062
IL-8 (pg/mL)	-1105	758	1863	245.8	0.024
MIP-1β (pg/mL)	267.00	874.58	607.58	69.5	0.0006
Blood					
Neutrophils, %	8.8	20.9	12.1	57.9	<0.0001
IL-6 (pg/mL)	0.59	13.77	13.18	95.7	0.0043
MIP-1β (pg/mL)	-7.07	20.92	27.99	133.8	<0.0001
CRP (mg/L) at 24 h	0.91	12.90	11.99	93.0	0.001

CRP, C-reactive protein; IL-6, interleukin 6; MIP-1β, macrophage inflammatory protein-1β

Table S4 Change from baseline for biomarkers in blood and sputum for AZD7624 and placebo after LPS challenge in human volunteers.

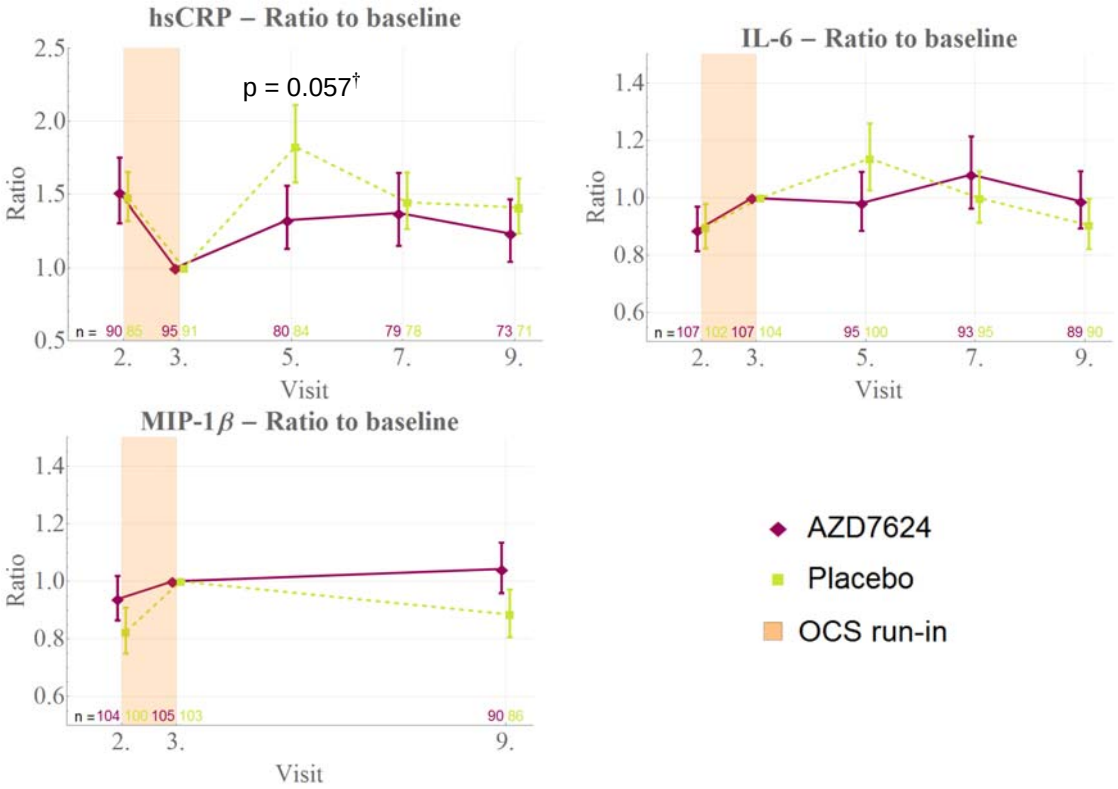
6. Expanded Clinical Results Section - COPD Proof of Principle Study

	AZD7624		Placebo		p-value†
Blood*	End of treatment	Change from baseline	End of treatment	Change from baseline	
hsCRP (mg/L)	0.91	0.01	0.66	0.08	0.44
MIP-1β (pg/mL)	171.12	44.42	128.67	-18.88	0.09
IL-6 (pg/mL)	9.60	-0.15	6.78	-2.02	0.20

* Data presented as means

† AZD7624 vs Placebo; mixed model repeated measures analysis, fitting treatment, country, LAMA maintenance treatment, visit, sex, smoking history, and treatment by visit interaction as fixed effects, patient as a random effect, and baseline assessment, age, BMI, and height as continuous covariates.

Table S5 Effect on blood biomarkers (CRP, IL-6 and MIP-1β) measured by change from pre-treatment baseline



* Figures account for all subjects with reported pre-treatment baseline value at visit 3 (n = number of subjects in each group at each visit). Data presented as geometric means. Error bars show standard errors.

† p-value derived from mixed model repeated measures analysis fitting treatment, country, LAMA maintenance treatment, visit, sex, smoking history and treatment by visit interaction as fixed effects, patient as a random effect, and baseline assessment, age, BMI, and height as continuous covariates.

Figure S5 Effect on blood biomarkers (CRP, IL-6 and MIP-1β) from before OCS run in to end of treatment

7. Data Monitoring Committee, Membership

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