A Single-Blind, Randomized, Placebo-Controlled 3-Part Study in Healthy Volunteers and Patients with Mild Asthma to Investigate the Safety, Tolerability and Pharmacokinetics of Inhaled AZD0449 Following Single and Multiple Ascending Doses and to Investigate the Anti-Inflammatory Effect of Inhaled AZD0449

ClinicalTrials.gov Identifier: NCT03766399

Protocol Amendment 10: Final, 04 February 2021

Clinical Study Protocol

A Single-blind, Randomized, Placebo-Controlled 3-Part Study in Healthy Volunteers and Patients with Mild Asthma to Investigate the Safety, Tolerability and Pharmacokinetics of Inhaled AZD0449 Following Single and Multiple Ascending Doses and to Investigate the Anti-Inflammatory Effect of Inhaled AZD0449

Parexel Study No.:	CCI [redacted]
Sponsor Study Code:	D5371C00001
EudraCT No.:	2018-003469-32
Test Product:	AZD0449
Therapeutic Indication:	Asthma
Pharmacological Class:	Inhaled JAK inhibitor
Development Phase:	Phase I
Sponsor:	AstraZeneca AB 151 85 Södertälje Sweden
Date of Protocol:	Final 1.0, 18 September 2018
Protocol Amendment No. 1	Final 1.0, 23 October 2018
Protocol Amendment No. 2	Final 1.0, 04 December 2018
Protocol Amendment No. 3	Final 1.0, 09 May 2019
Protocol Amendment No. 4	Final, 03 December 2019
Protocol Amendment No. 5	Final, 11 February 2020
Protocol Amendment No. 6	Final, 27 May 2020
Protocol Amendment No. 7	Final, 26 June 2020
Protocol Amendment No. 8	Final, 11 September 2020
Protocol Amendment No. 9	Final, 08 December 2020
Protocol Amendment No. 10	Final, 04 February 2021

This clinical study will be conducted according to the protocol and in compliance with Good Clinical Practice, with the Declaration of Helsinki (version 1996) and with other applicable regulatory requirements.

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A list and contact details of Investigators and other key study team members are provided in the Project Plan in the electronic Investigator's Site File. A list of participating Investigators will be provided in the Clinical Study Report (CSR).

PROTOCOL AMENDMENTS

Protocol Amendment No. 10, dated 04 February 2021

Protocol Amendment No. 9, dated 08 December 2020 was amended to update the timing to conduct the interim analysis of Part 3a. Sections that were updated were Section 3.1.4, Section 10.4.1 and Protocol Synopsis.

Protocol Amendment No. 9, dated 08 December 2020

Protocol Amendment No. 8, dated 11 September 2020 was amended to change the Chief Investigator from PPD to PPD

Protocol Amendment No. 8, dated 11 September 2020

The main changes made to the Protocol Amendment No. 7, dated 26 June 2020 are as follows:

1. **Investigators and study administrative structure**: Contact details for the Sponsor's lead physician and the Sponsor's biostatistician were updated to reflect changes in the study team.
2. **Treatments**: In response to a request from the German Federal Institute for Drugs and Medical Devices (BfArM), information about the dry-powder inhaler (DPI) to be used for the administration of study treatment during Part 3 was added in Section 3.5.4.1, Section 7.1, and Protocol Synopsis. In response to a request from an ethics committee, the actual dose administered in Part 3 was added in Section 4.2.5 and Section 7.6 for clarity.
3. **Determination of sample size:** In Section 10.4 and Protocol Synopsis, reference to the Statistical Analysis Plan (SAP) was removed. If the actual estimate of baseline FeNO standard deviation (SD) exceeds 0.55 during the interim analysis (IA), then the protocol amendment prepared to describe further adjustments in the sample size will include the criteria for sample size re-evaluation.
4. **Analysis of fraction exhaled nitric oxide (FeNO) in Part 2a and Part 3a**: Part 2a was removed from the treatment comparisons between DPI AZD0449 CCI and placebo. It was previously included in error as Part 2a does not include treatment with DPI AZD0449 CCI and placebo.
5. **Adverse Events Based on Examinations and Tests**: In response to a request from BfArM, Section 11.2.6 indicates that changes in Clinical Study Protocol (CSP)-mandated laboratory values, vital signs, electrocardiograms (ECGs), and other safety assessments will be reported as adverse events (AEs) if considered to be clinically relevant as judged by the investigator.

Protocol Amendment No. 7, dated 26 June 2020

The main changes made to the Protocol Amendment No. 6, dated 27 May 2020 are as follows:

1. **Extended Safety Monitoring Period**: The safety monitoring period for Study Part 2b, Part 3a, and Part 3b was extended to 15 days after last dose administration. This was in response to a Medicines and Healthcare Products Regulatory Agency (MHRA) request to extend safety monitoring and observation until the end of exposure, at least 5 half-lives or end of expected pharmacological activity, whichever is longer. First, 97% of the last dose is estimated to be eliminated after 11 days, which is equivalent to the 5 half-lives-condition. Second, the duration of the pharmacological effect was conservatively estimated to 15 days after the last dose. This estimate is based on pharmacokinetic (PK) and fractional exhaled nitric oxide (FeNO) data collected after repeated dosing of CCI in Part 2a in mild asthmatics. The longer of the 2 estimates was used to determine the safety monitoring period. This extended period of safety monitoring will continue until Day 27 (15 days after the last dose administration) and will apply to all patients and volunteers in Part 2b, 3a, and 3b. This procedure will be undertaken on a residential basis at the Clinical Unit until such time as the relevant Regulatory Authority is satisfied that outpatient based assessment is feasible and safe. Changes have been made to the following sections: Protocol Synopsis, Sections 3.1.3, 3.1.4, 3.1.6, 3.2.2, 3.2.4, and 5.1.2.1.1.

2. **Safety and PK assessments:** During this extended safety monitoring period, additional safety, PK, and PD assessments/sampling will be done. Changes have been made to the following section: Section 3.2.2.

3. **Blood volumes:** Minor updates to blood volumes for Part 1b, Parts 2a and 2b, and Parts 3a and 3b to reflect correct sampling during the study. Changes have been made to the following sections: Section 3.2.4, Table 3.2-12, Table 3.2-13, and Table 3.2-14.

4. **Exclusion criteria:** Minor corrections of typographical errors in Part 1a/b exclusion criterion number 7, patients with mild asthma Part 2a/3a exclusion criterion number 18, and healthy volunteers Part 2b/3b exclusion criteria number 7 and 8. Changes have been made to the following sections: Section 5.1.1.2, Section 5.1.2.2.1, and Section 5.1.2.2.2.

5. **Digital ECG schedule:** Minor correction of typographical errors in the footnotes for dECG schedule for Part 1b and the footnotes for dECG schedule for Part 2a. Changes have been made to the following section: Section 3.2.2, Table 3.2-9, and Table 3.2-10.

6. **Interim analysis:** Minor correction to remove duplication in text. The second paragraph in the section was deleted (deleted text in ~~strikethrough~~).

   "As mentioned above, an unblinded IA will be performed when 50% of the patients in have completed Part 3a. The collected data will be used to inform various decisions or actions

such as assessing the efficacy of AZD0449 in an unblinded manner and to perform an unblinded sample size re-estimation. Based on the results from the IA (PK, PD [FeNO] and safety), the PoM cohort size will be decided. Further details will be included in the SAP for Part 3. The latter will detail the nature and the exact criteria of the decisions as well as handling of the data and actual decision makers.

~~In Part 3a, an unblinded IA will be performed when 50% of the patients have completed the cohort. Based on the results from the IA (PK, PD [FeNO] and safety), the formulation cohort size will be decided.~~"

Changes have been made to the following section: Section 10.4.1.

7. **Protocol deviation:** Minor clarification in describing Data Review Meeting (deleted text in ~~strikethrough~~).

   "All protocol deviations will be discussed at the Data Review Meeting (DRM) before database hard lock in order to define the analysis sets for the study. The precise reasons for excluding patients from the study populations will be fully ~~defined~~ **agreed** on in the DRM report"

   Changes have been made to the following section: Section 10.5.

8. **Follow-up:** Minor clarifications throughout of "Follow-up Visit" to "Follow-up Visit/last day of study" or "last day of study" or "safety monitoring period" when describing Part 2b, 3a, and 3b. Changes have been made to the following sections: Protocol Synopsis, Sections 2.4, 5.2.2, 10.2, 10.6.4.9, 11.1.2, 11.2.1.

Protocol Amendment No. 6, dated 27 May 2020

The main changes made to the Protocol Amendment No. 5, dated 11 February 2020 are as follows:

1. **COVID-19 situation:** The first 2 multiple ascending dose (MAD) cohorts with the CCI [redacted] doses with the nebulized formulation have been completed in mild asthmatics. The primary (safety and tolerability) and secondary (PK and pharmacodynamics [PD]) objectives of Part 2 were evaluated. The remaining nebulized CCI cohort in mild asthmatics remains on hold due to the study sites suspension of recruitment of mild asthmatics related to the COVID-19 pandemic. As the intention is for the CCI nebulized PK and safety data to inform the same dose and formulation to support a potential COVID-19 study, the Sponsor has decided to replace the final mild asthmatic cohort in Part 2 of the study with a cohort of healthy volunteers. The secondary PD objective "to evaluate anti-inflammatory effect in patients with mild asthma" via FeNO has been removed from the third cohort in Part 2 and added to Part 3a to be performed at a time when

the recruitment of mild asthmatics becomes feasible. The Sponsor believes there is no change in the benefit: risk to either healthy volunteers or mild asthma subjects based on this amendment to the study. The primary objective and the secondary objective (PK) can be studied in healthy volunteers as well as mild asthmatics. Changes have been made to the following sections: Protocol Synopsis, Sections 1.3, 2.1, 2.2, 2.3, 2.4, 3.1, 3.2, 3.5, 3.6, 4.1, 4.2.5, 5.1, 6.2, 7.6, 7.10.1, 8.2.7, 8.5, 8.6, 10.4, 10.6.5.4, 10.6.6 and 10.6.7.

2. **Split of Part 2 to Part 2a and Part 2b:** For practical reasons Part 2 has been divided to Part 2a (Patients with mild asthma) and Part 2b (Healthy volunteers). Separate objectives [Table 2.3-1 and Table 2.3-2], Schedule of Assessments [Table 3.2-4 and Table 3.2-5], and inclusion and exclusion criteria (Section 5.1.2) are available in the amended protocol for Part 2a and Part 2b, respectively. Changes have been made to the following sections: Protocol Synopsis, Sections 1.3, 2.3, 3.1.3, 3.1.6, 3.2.1, 3.2.3, 3.2.4, 3.5.3, 3.6, 4.1.3, 4.2.5, 5.2.2, 5.2.3, 6.2, 8.5, 8.6, 10.4, 10.6.3.2, 10.6.5, 10.6.6 and 10.6.7.

3. **Split of Part 3 to Part 3a and Part 3b:** The primary and secondary objectives of Part 3 is identical to Part 2. In a situation (ie, COVID-19 pandemic) when the Sponsor cannot recruit mild asthmatics to the study the Sponsor will have the option to study the primary objective and one of the secondary objectives (PK) in an optional cohort in healthy volunteers. The decision to start this cohort would be made at a Safety Review Committee (SRC) meeting when Part 2a/b of the study has been completed.

   For practical reasons the Part 3 has been divided to Part 3a (Patients with mild asthma) and Part 3b (Healthy volunteers). Part 3b, if conducted, may be run in parallel or completed ahead of Part 3a. Separate objectives (Section 2.4), Schedule of Assessments (Table 3.2-6 and Table 3.2-7), and inclusion and exclusion criteria (Section 5.1.2) are available in the amended protocol for Part 3a and Part 3b, respectively. Changes have been made to the following sections: Protocol Synopsis, Sections 1.3, 2.3, 3.1.4, 3.1.6, 3.2.1, 3.2.3, 3.2.4, 3.5.4, 3.6, 4.1.4, 4.2.5, 4.3.1, 5.2.2, 5.2.3, 7.6.4, 7.10.1.4, 8.5, 8.6, 10.4, 10.6.4.3, 10.6.5, 10.6.6 and 10.6.7.

4. **Inclusion/exclusion criteria:** Inclusion/exclusion criteria have been updated to reflect the above proposed amendment for healthy volunteers to receive repeated dosing. The ongoing assessment of safety is provided and is aligned to that for the mild asthma subjects. Changes have been made to the Section 5.1.

   The exclusion of Women of Childbearing Potential (WOCBP) for Part 1b second IV cohort has been re-assessed taking into consideration the current predicted AUC and Cmax for intravenous (IV) AZD0449 CCI are predicted to be well below the safety exposure limits (6-fold lower for maximum observed plasma concentration [Cmax] and 9-fold lower for area under the concentration-time curve [AUC]) and this should minimize potential

reproductive risks. In view of the Clinical Trial Facilitation Group (CTFG) guidance, available data, and dose formulation of AZD0449 in this study, it was concluded that exclusion of WOCBP from Part 1b second IV cohort is not necessary, provided highly effective measures of contraception are implemented and that pregnancy testing is performed during the trial. Changes have been made to Section 5.2.3.

5. **Enlargement of sample size for the DPI cohort in mild asthmatics (Part 3):** The proof-of-mechanism (PoM) assessment has been moved from the nebulized cohort 3 (Part 2b) to the DPI cohort (Part 3a). As the nebulized cohort 3 will now be performed in healthy volunteers for PK and safety, the secondary objective of FeNO (PD) will be moved to Part 3a to be conducted in a powered cohort of mild asthmatics once recruitment of these subjects becomes feasible. The sample size of the Part 3a cohort is increased in order to power the cohort for statistical analysis of the PD, FeNO outcomes. Changes have been made to following sections: Protocol Synopsis, Sections 1.3, 2.3, 2.4, 3.1.4, 4.1.3, 4.1.4, 8.5.1, 10.4, and 10.6.6.1.

6. **Reduction of sample size for the third MAD cohort:** As indicated above, the CCI MAD cohort (Part 2b) will be conducted in healthy volunteers with the primary objective to study safety and tolerability and the secondary objective to study PK. Therefore, the number of subjects needed to study these objectives is the same as used in the Single Ascending Dose (SAD) cohorts (6 receiving AZD0449 and 2 placebo). Changes have been made to following sections: Protocol Synopsis, Sections 2.3, 3.1.3, 4.1.3, and 10.4.

7. **Twice daily option is removed:** Based on available data from Part 1 and Part 2a it has been decided to continue this study with once daily (QD) dosing. Therefore the twice daily (BID) option has been removed from the protocol. Changes have been made to following sections: Protocol Synopsis, Sections 3.1.3, 3.1.4, 3.1.6, 3.2.1, 3.2.2, and 10.6.5.4.

8. **Diagnostic testing for COVID-19:** Subjects will undergo serology (as available) and swab testing for COVID-19 at screening after signed informed consent and before randomization. If a study subject shows any symptom of a potential COVID-19 infection an ad hoc nasal and/or throat-swab specimen for the identification of a suspected respiratory infection including COVID-19 will be taken. Subjects who test positive for having active COVID-19 infection will be discontinued from the study and followed up until the final outcome of the AE. Changes have been made to Sections 3.2.2, 5.2, 6.2, and 8.2.7.

Protocol Amendment No. 5, dated 11 February 2020

The main changes made to the Protocol Amendment No. 4, dated 03 December 2019 are as follows:

1. Based on emerging data, an additional cohort with intravenous (IV) administration was added: the second IV dose cohort. The rationale behind adding an extra IV cohort was to improve the possibilities to characterize the elimination kinetics of AZD0449. Following administration of CCI the elimination kinetics of AZD0449 could not be sufficiently described. The concentrations in plasma declined rapidly with 2-compartment kinetics and the concentrations were quantifiable only 10 to 12 hours after administration. With the second IV dose cohort, it is predicted that a 4 times higher dose CCI will result in concentrations in plasma that are quantifiable up to 24 hours after dosing and thus improved possibilities to fully characterize the elimination kinetics of AZD0449 (Sections 2.2, 3.1.2 [including new section 3.1.2.2 and schedule of assessment table for the second IV cohort Table 3.2-3], 3.2, 3.5.2, 4.1.2, 5.1.1, 5.2.1, 7.6.2, 7.10.1.2).

2. Sections 3.5.2.1 and 4.1.2 were updated to include the dose strategy and rationale for the higher dose used in the second IV cohort in Part 1b CCI AZD0449). Results for the observed exposure at the lowest dose were added (as predicted, exposure was well below the maximum allowed exposure [1% of C_{max}, 0.3% of AUC]). The predicted exposure at the second dose was added (also expected to be well below the maximum allowed exposure [4.4% of C_{max}, 1.2% of AUC]).

3. The primary and secondary objectives for Part 1b (IV Cohorts) were updated to state "To characterize the blood plasma PK of AZD0449 following intravenous administration of ~~a~~ **2** single doses to healthy subjects" (new text in **bold**, deleted text in ~~strikethrough~~) in the Protocol Synopsis and Table 2.2-1.

4. Exploratory objectives were added to Part 2 (Cohort 3): CCI [redacted] and Part 3 CCI [redacted] (Protocol Synopsis and Table 2.4-1). Corresponding PK text was also updated where applicable (Protocol Synopsis, Sections 8.3.1, 8.3.2, 10.6.5.1, 10.6.5.2, 10.6.5.3).

5. Based on emerging data, an extra pharmacokinetic sample was added at 10 h for Cohort 3 onwards in Part 2 (multiple ascending dose [MAD]/proof-of-mechanism [PoM]) – once daily (QD) dosing [Table 3.2-4] and twice daily [BID] dosing [Table 3.2-5]) and Part 3 (Bridging – QD and BID dosing [Table 3.2-6 and Table 3.2-7].

6. In order to provide further data on the PK-PD relationship, the study design was updated to include an optional subset of up to 15 patients (Part 2 [MAD/PoM]) or up to 12 patients (Part 3 [Bridging]) who agree to remain in-house from Days 14 to 16, being discharged on

Day 17 (Day 18 for BID dosing in Part 3), to allow for additional PK and FeNO samples to be taken (Protocol Synopsis, Sections 3.1.3, 3.1.4, 3.1.6 and 5.1.2.1, inclusion criterion 3; schedule of assessment tables for Part 2 [Table 3.2-4 and Table 3.2-5] and Part 3 [Table 3.2-6 and Table 3.2-7]).

7. The blood volume tables were updated to address the above changes to assessments (Table 3.2-11, Table 3.2-12, Table 3.2-13, and Table 3.2-14).

8. A footnote was added to the blood volume tables for Part 2 (MAD/PoM; Table 3.2-13) and Part 3 (Bridging; Table 3.2-14), stating that for all cohorts, an additional 2.5 mL sample will be taken for imunoglobulins at Day -1 only. This was a correction which was omitted in the previous Amendment when the Clinical safety sampling was moved from Day -1 to Day -2.

9. Protocol Synopsis, Sections 3.2.3, 8.6.3, and Table 3.2-5 (Part 2 [MAD/PoM]) were revised to state that sputum samples were to be taken at participating sites only.

10. Assessment for collection of urine for exploratory analyses of metabolites of AZD0449 for Cohort 3 was added to the schedule of assessment table for Part 2 (MAD/PoM) – QD [Table 3.2-4] and BID [Table 3.2-5] dosing, and corresponding text was added in Section 8.5.2.

11. Section 3.1.4 and corresponding Protocol Synopsis text revised to state "Part 3 of the study will ~~only~~ be initiated after the completion of **Cohort 3 in** Part 2 (MAD/PoM) of the study ~~and after approval of the substantial amendment is received~~" (new text in **bold**, deleted text in ~~strikethrough~~).

12. Part 2 and Part 3 inclusion criterion 7 in Section 5.1.2.1 was clarified to state that lung function ≥70% predicted for Forced Expiratory Volume in 1 second (FEV_1) at the Screening Visit AND at the 12 h timepoint on Day -1 is in accordance with the American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria.

13. The pulmonary stopping criteria for in Section 6.2 were revised to include patients who received AZD0449 as well as subjects.

14. AZD0449 Dry-powder inhalation (DPI) and corresponding placebo were added to the Investigational Medicinal Product (IMP) table in the Protocol Synopsis and in Section 7.1 (Table 7.1-1). As a result, references to the Chemistry, Manufacturing, and Controls (CMC) documentation supporting the DPI formulation were removed.

15. CCI [redacted]

16. The interim analysis text in Section 10.4.1 was updated to include the percentage of patients required for sample size re-estimation for the Part 2 PoM and Part 3 inhalation cohorts. Reference to 'a special charter (interim analysis plan)' was removed.

17. Text referring to Protocol Amendment 5, i.e., "…substantial amendment of the protocol will be submitted before start of Part 3" was deleted throughout.

18. Text was added in Section 3.3.1.4 to describe periodic monitoring and regular oversight.

19. Cross references to schedule of assessment tables were corrected in the following sections: 3.2, 8.2.3.1, 8.2.3.2, and 8.5.7 (Table 3.2-5 link moved to Part 2, Table 3.2-6 and Table 3.2-7 added to Part 3); Section 5.2.3.1 (Table 3.2-5, Table 3.2-6, and Table 3.2-7 added); Section 8.5.4 (Table 3.2-4 and Table 3.2-5 added to Part 2 and link to Table 3.2-6 corrected); Sections 8.4.2, 8.5.5, and 8.5.6 (Table 3.2-5 added).

20. The Investigator Brochure Edition in the Reference List (Section 14) was updated.

21. Minor format and language changes were made throughout the document.

22. Protocol Synopsis text was updated per the above changes.

Protocol Amendment No. 4, dated 03 December 2019

The main changes made to the Protocol Amendment No. 3, dated 09 May 2019 are as follows:

1. The exclusion of Women of Childbearing Potential (WOCBP) in this study has been re-assessed taking into consideration recommendations related to contraception and pregnancy testing in clinical trials from the Clinical Trial Facilitation Group (CTFG) of the Heads of Medicines Agencies (HMA), dated 09 September 2015 [1]. Although there have been no reproductive studies conducted with AZD0449 so far, the current evidence based on pharmacological effects of oral Janus kinase (JAK) inhibitors suggests potential toxicity, as described in the AZD0449 Investigator's Brochure [2]. Systemic exposures of AZD0449 up to the CCI dose level, inhaled repeatedly, are predicted to be well below the safety exposure limits (6-fold lower for maximum observed plasma concentration [C_{max}] and 9-fold lower for area under the concentration-time curve [AUC]) and this should minimize potential reproductive risks. In view of the CTFG guidance, available data, and dose formulation of AZD0449 in this study, it was concluded that exclusion of WOCBP from study Part 2 (multiple ascending dose [MAD]) and Part 3 (proof-of-mechanism [PoM]) is not necessary, provided highly effective measures of contraception are implemented and that pregnancy testing is performed during the trial. Pregnancy testing will be done on women participants at screening, prior to randomization, and at the Follow-up Visit. Use of highly effective methods of contraception as per the above CTFG guidance will be mandatory for WOCBP participants, male participants, and WOCBP partners of male

participants. Text changes done to reflect inclusion of WOCBP in the study Part 2 and Part 3 are as follows:

1.1. Section 3.1.3, Part 2 (MAD/PoM) Study Design, Section 3.1.4, Part 3 (Bridging) Study Design, and Section 4.1.3, Rationale for Design of Part 2 (MAD/PoM): Text was updated to clarify that WOCBP will be recruited.

1.2. The following selection criteria were updated:

- Section 5.1.2.1, Inclusion Criteria, number 2: The requirement for female patients to be of non-childbearing potential has been removed.
- Section 5.1.2.1, Inclusion Criteria, number 4: Text has been amended to indicate female patients must not be pregnant.
- Section 5.1.2.2, Exclusion Criteria, number 22 was added to exclude female patients who are pregnant, breastfeeding, or planning a pregnancy during the study period or within 1 month after the last dose of investigational medicinal product (IMP).
- Section 5.1.2.2, Exclusion Criteria, number 23, was added to mention reproductive restrictions on WOCBP patients and WOCBP partners of male patients.

1.3. Text in Section 5.2.3, Reproductive Restrictions, was updated as follows:

- Section 5.2.3.1, Women of Childbearing Potential, was added to describe WOCBP and instructions on the use of highly effective methods of contraception.
- Section 5.2.3.3, Male Subjects, was updated for text on WOCBP partners of male patients. Previous text was summarized, and duplicate information was removed.

2. In the ongoing study Part 2 and Part 3, patients with mild asthma will be enrolled. Therefore, electrocardiogram (ECG) characteristics have been amended to be fit for a patient population rather than healthy volunteers. In the absence of any pre-clinical signal involving cardiac conduction system, the following modifications of the criteria were considered acceptable. A right-sided intraventricular conduction delay or right incomplete bundle branch block (IBBB) resulting in slightly wider QRS complex compared with healthy volunteers is not uncommon in asthma patients. The QRS duration >120 ms will be excluded since this could be related to an underlying, clinically relevant and significant cardiac pathology. First degree AV block (prolonged PR interval >200 ms and <240 ms) is acceptable in the absence of any clinical bradycardia with symptoms such as dizziness, presyncope/syncope, etc, as judged by the principal investigator (PI). Text changes made to reflect the changes in the ECG parameters were the following:

2.1. Section 5.1.2.2, Exclusion Criteria, number 10.5: PR (PQ) interval prolongation was changed from >220 ms to >240 ms.

2.2. Section 5.1.2.2, Exclusion Criteria, number 10.6, was modified to indicate that patients with intraventricular conduction delay (IVCD) and QRS >110 ms but <120 ms may be selected.

3. Table 3.210, ECG sampling for study Part 2: The once daily and twice daily dosing regimens were presented separately in 2 different panels in the table.

4. The 10 hour timepoint for ECG was eliminated because there is no PK sampling at this timepoint. Consequently the footnote "g" was deleted in Table 3.210, ECG sampling for study Part 2.

5. In the ongoing study Part 2 and Part 3, patients with mild asthma will be enrolled. On the basis of safety data from the Single Ascending Dose (SAD) part of this study (Part 1a) and considering the physiological variability in a patient population, the permissible levels for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) will now be up to 1.5 × upper limit of normal (ULN). Therefore, updates were made in Section 5.1.2.2, Exclusion Criteria, number 7 to make it appropriate for a patient population rather than healthy volunteers.

6. In the ongoing study Part 2 and Part 3, the requirement for the ratio of FEV_1 to forced vital capacity (FVC) to be >0.7 is now eliminated. Eliminating the specification of FEV_1/FVC ratio as an inclusion criterion would allow patients with high FVC in relation to FEV_1 (consistent with asthma patients) to be included. No changes were made to the schedule of measuring the FEV_1/FVC ratio; these assessments will be done as originally planned. Text has been amended in Section 5.1.2.1, Inclusion Criterion, number 7.

7. Forced Expiratory Volume in one second (FEV_1) and fractional exhaled nitric oxide (FeNO) assessed at the 12 hour timepoint on Day −1 will be considered for patient inclusion; previously, the timepoint had not been specified. Consequently, text has been amended in Section 5.1.2.1, Inclusion Criteria, numbers 7 and 8.

8. The follow-up period was extended from 6±1 days after the last dose to 10±1 days after the last dose. The systemic total concentrations of AZD0449 10 days after the last dose are predicted to be below the relevant systemic exposures (approximately 0.1 nM). This is based on modeling of intravenous and inhaled dose data from the SAD part of this study (Part 1a). Therefore, Table 3.2-4, Table 3.2-5, Table 3.2-6, Table 3.2-7, Schedules of Assessments were updated.

9. In the ongoing study Part 2, the starting dose level for Part 2 was chosen as CCI AZD0449 delivered dose. This dose level was chosen on the basis of Part 1a Cohort 6 data. Therefore, Section 3.5.3.1 and Figure 3-3 were updated.

10. Clinical chemistry tests done on Day −2 (not the tests on Day −1) will be considered for assessing patient selection. Updates were made in Section 5.1.2.2, Exclusion Criteria, numbers 7 and 3.10 for clarification.

11. Table 3.2-4, Table 3.2-5, Table 3.2-6, and Table 3.2-7, Schedules of Assessments: For the comment "Postmenopausal females only" against follicle stimulating hormone testing, a cross-reference was added to the definition of "postmenopausal" that is provided in Section 5.2.3.2, for clarification.

12. Table 3.2-4 and Table 3.2-5, Schedules of Assessments, were updated per Protocol Amendment 3, dated 09 May 2019 that CCI [redacted] The timepoints are now listed for these 2 cohorts, in the "Comments" column in these tables, for clarification.

13. In study Part 1, pharmacokinetic (PK) sampling timepoints had been added on the basis of emerging data. This is now indicated in the following sections:

13.1. Table 3.2-1: For Part 1a Cohort 5, 1 additional pharmacokinetic (PK) blood sample was taken at 72 hours post-dose on Study Day 4. This assessment was added as per decisions taken at the Safety Review Meeting held on 24 May 2019 to review PK data for Part 1a Cohort 4.

13.2. Table 3.2-1: For Part 1a Cohort 6, 3 additional PK blood samples were taken at 10 hours post-dose, 72 hours post-dose, and at the Follow-up Visit. These assessment were added as per decisions taken at the Safety Review Committee Meeting held on 20 June 2019 to review PK data for Part 1a Cohort 5.

13.3. Table 3.2-2: For Part 1b, 2 additional PK blood samples were taken at 14 hours and 16 hours post-infusion on the basis of emerging data at the request of the Sponsor.

14. For Part 1b, in Table 3.2-2, ECG sampling points on Day 3 are now specified in the table, for clarification.

15. Section 14 References was updated with addition of the first 2 new references. Consequently, the numbering of the previous references in the list and their citation in-text were also updated.

16. Section 15.3 Appendix, Actions Required in Cases Increases in Liver Biochemistry and Evaluation of Hy's Law, was updated with the current standard text used by the Sponsor.

17. Minor format and language changes (eg, replacing "subjects" with "patients" for Part 2 and Part 3, and replacing "study drug" with "IMP") were made throughout the document.

18. Protocol Synopsis: Text for study Part 2 was updated per the above changes.

Protocol Amendment No. 3, dated 09 May 2019

The following changes were made to the Protocol Amendment No. 2, dated 04 December 2018:

1. The starting dose has been updated to CCI
2. Part 1a of the study has been updated to reflect new timepoints for pulse oximetry, vital signs, pharmacokinetic (PK) sampling, and blood sample collection for exploratory assessments.
3. Part 1b of the study has been updated to reflect new timepoints for PK blood sampling.
4. Part 1b study design has been updated regarding subjects to be included in the cohort: healthy subjects who have participated in Part 1a and/or naïve subjects
5. Part 2 study design has been updated regarding treatment period and dose administration for twice daily dosing.
6. The schedule of assessments table for Part 2 and Part 3 has been duplicated to reflect timepoints for once daily dosing (Table 3.2-4 and Table 3.2-6) and twice daily dosing (Table 3.2-5 and Table 3.2-7).
7. The schedule of assessments table for Part 2 of the study has been updated to reflect discharge from the Clinical Unit on Day 14, new timepoints for spirometry, FeNO, CCI [redacted] and blood sample collection for PK and CCI [redacted]
8. The schedule of assessments table for Part 3 of the study has been updated to reflect, discharge from the Clinical Unit on Day 14, to remove CCI [redacted]
9. Part 2 and Part 3 of the study have been updated to state subjects will be discharged from the Clinical Unit on Day 14, Table 3.2-4 and Table 3.2-6 (Day 15 for twice daily dosing; Table 3.2-5 and Table 3.2-7).
10. Electrocardiogram (ECG) timepoints were updated in Part 1a, Part 1b and Part 2 of the study (Table 3.2-8, Table 3.2-9 and Table 3.2-10).
11. The blood volumes collected in Part 2 and Part 3 of the study have been updated, see Table 3.2-13 and Table 3.2-14.
12. All study flow charts have been updated.
13. Section 10.3.2 was updated for PK analysis set.

14. The determination of sample size was further detailed in Section 10.4.

15. Section 10.6.5 was revised for PK analysis.

16. Appendix 15.3 was updated in line with the new Hy's law template dated 10 January 2019.

17. Typographical errors were corrected throughout the document.

Protocol Amendment No. 2, dated 04 December 2018

The following changes was made to the Protocol Amendment No. 1, dated 23 October 2018.

1. The exploratory objective, CCI [redacted]
2. Part 1a of the study has been updated to state that blood samples for the assessment of CCI [redacted] will only be collected for Cohort 5 and onwards, see Table 3.2-1.
3. Part 1b of the study has been updated to move the 30 minutes post-dose spirometry assessment on Day 1 to 1 hour post-dose, see Table 3.2-2.
4. Part 1b of the study has been updated, to remove blood sample collection for the assessment CCI [redacted] see Table 3.2-2.
5. Part 2 of the study has been updated to state that blood samples for the assessment of CCI [redacted] will only be collected for Cohort 3 and onwards, see Table 3.2-4.
6. The time-points for 12-lead safety ECGs were updated to the start of each digital-ECG extraction window, see Table 3.2-8, Table 3.2-9 and Table 3.2-10.
7. The blood volumes collected in each part of the study have been updated, see Table 3.2-11, Table 3.2-12, Table 3.2-13 and Table 3.2-14.
8. Details on the presentation of CCI [redacted] results have been added, see Section 10.6.7.6.
9. Typographical errors were corrected throughout the document.

Protocol Amendment No. 1, dated 23 October 2018

The following changes was made to the original protocol, final 1.0 dated 18 September 2018 as requested by the MHRA and to correct inconsistencies.

1. The definition of true sexual abstinence was added to the reproductive restrictions as requested by the MHRA, see Section 5.2.3.3.

2. The number of blood samples for PK analyses in Part 2 and Part 3 were corrected and the corresponding blood volume updated, see Table 3.2-13 and Table 3.2-14.

3. Typographical errors were corrected throughout the document.

PROTOCOL SYNOPSIS

Title of the Study

A Single-blind, Randomized, Placebo-Controlled 3-Part Study in Healthy Volunteers and Patients with Mild Asthma to Investigate the Safety, Tolerability and Pharmacokinetics of Inhaled AZD0449 Following Single and Multiple Ascending Doses and to Investigate the Anti-Inflammatory Effect of Inhaled AZD0449

Chief Investigator

PPD

Study Centers

This study will be conducted in the United Kingdom and Germany at approximately 3 study centers.

Study Design, Study Rationale and Justification of Study Design

This will be a Phase I, first in human (FIH) study consisting of 3 study parts.

Part 1a (Single Ascending Dose [SAD])

AZD0449 has not been studied in man previously. This FIH clinical study will assess the safety and tolerability as well as the single dose pharmacokinetics (PK) of inhaled AZD0449 in healthy volunteers.

Part 1a of the study will be a randomized, single-blind, placebo-controlled, SAD, sequential group design study performed at a single study center. Six inhaled dose levels of AZD0449 nebulized suspension are planned to be investigated in 6 cohorts of 8 healthy volunteers, with 6 volunteers randomly assigned to inhaled AZD0449 and 2 volunteers randomly assigned to inhaled placebo in each cohort. Depending on emerging safety and PK data, up to 3 additional inhaled dose levels (cohorts) within the pre-specified dose range, may be added at the discretion of the Sponsor.

Healthy male volunteers and healthy female volunteers of non-childbearing potential will be included to minimize interference with other drugs or with disease processes.

Each cohort will be preceded by a sentinel cohort of 2 volunteers (1 will receive AZD0449 and 1 will receive placebo). The remaining volunteers of each cohort will only be dosed after the 24-hour safety data from the sentinel subjects has been reviewed. Escalation to the next dose cohort will only take place after safety and PK data from the previous cohort(s) have been reviewed.

This study part will comprise a Screening Visit within 28 days before dosing, a treatment period (Day -1 to Day 4) with a single inhaled dose of AZD0449 or corresponding placebo on Day 1 and a Follow-up Visit within 6±1 days after dosing. Assessments will be performed, and samples collected from before dosing until at least 36 hours post-dose. Healthy volunteers will be discharged after all samples have been collected and assessments have been performed on Day 4. Depending on the emerging data, the length of the stay at the Clinical Unit, timing and number of assessments and/or blood and urine samples may be adjusted and the collection period may be extended to 96 hours post-dose.

Part 1b (Intravenous [IV] Cohorts)

In Part 1b, AZD0449 will be administered as a single IV dose at 2 different dose levels to 2 cohorts of healthy volunteers in order to compare the PK between IV and inhaled administration, to determine absolute bioavailability and to characterize plasma clearance. This will make it possible to deduce the impact of lung absorption after inhalation. The first (and lowest) IV dose was chosen from the low end of the Part 1a (SAD) dose range. The second IV dose was selected after completion of Part 1a and the first IV cohort in Part 1b.

Healthy male volunteers and healthy female volunteers of either non-childbearing or childbearing (Part 1b second IV cohort only) potential will be included. Dosing in Part 1b of the study will be initiated after the completion of cohort 6 in Part 1a (SAD), or, if Part 1a is completed with fewer than 6 cohorts, after completion of the last cohort in Part 1a.

Part 1b will be open-label and consist of 2 dose cohorts CCI Six volunteers will be selected for the first IV dose cohort in Part 1b following a 2-stage design. If the first IV cohort of Part 1b cannot be completed with 6 volunteers from Part 1a or if some of the data are considered not evaluable, up to 6 additional naïve volunteers may be enrolled. In the first IV cohort, there will be a washout period of at least 2 weeks for the volunteers receiving both inhaled dosing in Part 1a and IV dosing in Part 1b. All 6 volunteers in the first IV cohort will receive a single IV dose CCI of AZD0449 solution. The first IV cohort will be preceded by a sentinel subject and the remaining volunteers will only be dosed after the 24-hour safety data from the sentinel volunteer have been reviewed. The second IV dose cohort will consist of 6 volunteers who have not previously participated

in the study. All 6 subjects will receive a single IV dose CCI of AZD0449 solution. Dosing will also start with 1 volunteer in a sentinel cohort.

For the IV treatment periods, volunteers will be admitted to the Clinical Unit. On Day 1, volunteers will receive a single IV dose of the AZD0449 solution. Assessments will be performed, and samples collected from before dosing until 48 hours post-dose. Volunteers will be discharged from the Clinical Unit after all samples have been collected and assessments have been performed on Day 3.

Volunteers will return to the Clinical Unit within 6±1 days after the last dose of AZD0449 for a Follow-up Visit.

Part 2a/b (Multiple Ascending Dose [MAD])

Part 2a/b of the study will be a randomized, single-blind, placebo-controlled, MAD, sequential group design study performed at approximately 3 study centers. Subjects aged 18 to 55 years will be included in this part of the study. Unlike in Part 1a and Part 1b first IV cohort, female subjects of childbearing potential may also be enrolled. This part of the study will recruit up to 56 subjects. A total of 26 evaluable subjects are needed for completion of Part 2a/b. Potentially, 30 extra subjects may be randomized to counteract drop-outs and allow for an extra cohort if needed.

Three dose levels of AZD0449 nebulized suspension are planned to be investigated in 3 cohorts. The cohorts could comprise either patients with mild asthma or healthy volunteers.

Part 2a will include cohorts 1 and 2, each comprising of 9 patients with mild asthma. Within each of these cohorts 6 patients will be randomized to receive AZD0449 nebulized suspension and 3 patients randomized to receive placebo. Additional cohorts with 9 patients could be added to study PK, PD and safety for doses lower than CCI Part 2b will consist of 1 cohort of 8 healthy volunteers; 6 volunteers will be randomized to receive AZD0449 nebulized suspension and 2 volunteers will be randomized to receive placebo.

Dosing for each ascending dose cohort will start with 2 subjects in a sentinel cohort, such that 1 subject will be randomized to receive AZD0449 nebulized suspension and 1 subject will be randomized to placebo.

Each subject will receive a single dose of AZD0449 nebulized suspension at the selected dose level or placebo on Day 1 followed by 10 daily doses from Day 3 to Day 12.

For the treatment period, subjects will be admitted to the Clinical Unit. The first dose will be administered on Day 1. Following this first dose, plasma PK samples will be collected up to 48 hours post-dose for the first dose (Day 1) to allow for characterization of the terminal elimination phase. Once daily dosing will start on Day 3, 48 hours post-Day 1 dose PK sampling and continue up to Day 12 with intervals of 24 hours between doses.

Each subject will receive inhaled doses of AZD0449 nebulized suspension at the selected dose level or placebo on the specified dosing days. Assessments will be performed, and samples collected from before dosing until up to 240 hours after last dose (Part 2a) and until up to 360 hours after last dose (Part 2b). Subjects will remain in house for the duration of the treatment period and will be discharged from the Clinical Unit on Day 14, 2 days after the last dose administration (Part 2a) and after an extended safety monitoring period on Day 27, 15 days after the last dose administration (Part 2b). If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the extended safety monitoring period may be conducted as non-residential visits.

Following review of emerging data, the Safety Review Committee (SRC) may decide to adjust the length of the stay at the Clinical Unit and the timing and number of assessments and/or blood and urine samples for subsequent cohorts. The time window between the single dose and start of repeated dosing may also be adjusted any time during the study.

Part 3a/b (DPI/PoM)

Part 3a/b of the study will be initiated after the completion of Part 2b.

Part 3a/b will be a randomized, single-blind, placebo-controlled, DPI/PoM study. Male and/or female patients with mild asthma (Part 3a) or healthy volunteers (Part 3b), aged 18 to 55 years will be included in this part of the study. This part of the study will be conducted in up to 44 subjects divided into 2 parts; 36 patients with mild asthma (Part 3a) and 8 healthy volunteers (Part 3b optional).

Part 3a will consist of 36 patients where 18 patients will be randomized to receive AZD0449 DPI and 18 patients randomized to receive placebo. An Interim Analysis will be conducted when approximately 50% of the patients have completed Part 3a. Should new data on the variability of the FeNO measurements emerge at the Interim Analysis (IA), the sample size of Part 3a could be changed.

If the optional Part 3b is conducted, it will be comprised of 8 healthy volunteers, 6 volunteers will be randomized to receive AZD0449 DPI and 2 volunteers randomized to receive placebo.

For the treatment period, subjects will be admitted to the Clinical Unit. The first dose will be administered on Day 1. Once daily dosing will start on Day 3, 48 hours post-Day 1 dose PK sampling and continue up to Day 12. Subjects will remain in house for the duration of the treatment period and undergo an extended safety monitoring period until being discharged from the Clinical Unit on Day 27, 15 days after the last dose

administration. If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the extended safety monitoring period may be conducted as non-residential visits.

Study Period

Estimated date of first subject enrolled: November 2018 (signing of informed consent)
Estimated date of last subject completed: Q3 2020

Study Duration

The expected duration of each volunteer in Part 1a of the study is up to 36 days and up to 53 days for volunteers participating in the first IV dose cohort in Part 1b plus 36 days for volunteers participating in the second IV dose cohort.

The expected duration of each subject (patients with mild asthma or healthy volunteers) in Part 2a is up to 52 days and in Part 2b and Part 3a/b is up to 55 days.

Target Population

Part 1

Healthy male and/or female volunteers, aged 18 to 55 years. Female volunteers of non-childbearing potential are eligible for inclusion in Part 1a/b; female volunteers of childbearing potential are eligible for inclusion in Part 1b second IV cohort.

Part 2a/b and Part 3a/b

Male and/or female subjects (patients with mild asthma or healthy volunteers), aged 18 to 55 years. Unlike in Part 1a and Part 1b (first IV cohort), female patients of childbearing potential may also be enrolled.

Number of Subjects/Patients Planned

Part 1 of the study will include up to 84 healthy volunteers (72 in Part 1a and 12 in Part 1b).

Part 2a/b of the study will include 18 patients with mild asthma and 8 healthy volunteers. A total of 26 evaluable subjects are needed for completion of Part 2a/b; 30 extra subjects may be randomized to counteract drop-outs and allow for an extra cohort if needed.

Part 3a/b of the study will include 36 patients with mild asthma and 8 healthy volunteers (optional cohort).

Study Objectives and Outcome Measures

Part 1a (SAD)

Primary Objective	**Outcome Measures**
To assess the safety and tolerability of AZD0449 following inhaled administration of single ascending doses to healthy volunteers.	Adverse events (AEs); vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG; 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Secondary Objective	**Outcome Measures**
To characterize the blood plasma PK of AZD0449 following inhaled administration of single ascending doses of AZD0449.	Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
Exploratory Objectives	**Outcome Measure**
CCI [redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

CCI	

Part 1b (IV Cohorts)

Primary Objective	**Outcome Measures**
To characterize the blood plasma PK of AZD0449 following intravenous administration of 2 single doses to healthy volunteers.	Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL, Vz, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
Secondary Objective	**Outcome Measures**
To assess the safety and tolerability of AZD0449 following intravenous administration of 2 single doses to healthy volunteers.	AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); ECG; dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Exploratory Objectives	**Outcome Measure**
CCI	

Part 2a in Patients with Mild Asthma

Primary Objective	**Outcome Measures**
To assess the safety and tolerability of AZD0449 following inhaled nebulized administration of multiple ascending doses.	AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG, 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2.
Secondary Objectives	**Outcome Measures**
To characterize the blood plasma PK of AZD0449 following inhaled nebulized administration of multiple ascending doses	Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$, AUC, CL/F, Vz/F, $AUC_{(0-t)}/D$, AUC/D, C_{max}/D, t_{last}
To evaluate anti-inflammatory effect.	Change from baseline in 2 hours post-dose FeNO and FeNO $AUC_{(0-12)}$ to Day 12 and Follow-up.
Exploratory Objectives	**Outcome Measure**
CCI	

Part 2b in Healthy Volunteers

Primary Objective	**Outcome Measures**
To assess the safety and tolerability of AZD0449 following inhaled nebulized administration of multiple ascending doses.	AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG, 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2

Secondary Objectives	Outcome Measures
To characterize the blood plasma PK of AZD0449 following inhaled nebulized administration of multiple ascending doses.	Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
Exploratory Objectives	**Outcome Measure**
CCI	

Part 3a in Patients with Mild Asthma

Primary Objective	Outcome Measures
To assess the safety and tolerability of AZD0449 following repeated inhaled administration using a DPI.	AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG; 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Secondary Objectives	**Outcome Measures**
To characterize the blood plasma PK of AZD0449 following repeated inhaled administration using a DPI.	Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
To evaluate anti-inflammatory effect.	Change from baseline in 2 hours post-dose FeNO and FeNO $AUC_{(0\text{-}12)}$ to Day 12 and during safety monitoring period

Exploratory Objectives	Outcome Measure
CCI	

Part 3b for Healthy Volunteers (optional cohort)

Primary Objective	Outcome Measures
To assess the safety and tolerability of AZD0449 following repeated inhaled administration using a DPI.	AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG; 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Secondary Objectives	**Outcome Measures**
To characterize the blood plasma PK of AZD0449 following repeated inhaled administration using a DPI.	Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
Exploratory Objectives	**Outcome Measure**
CCI	

CCI [redacted]	[redacted]

Investigational Medicinal Product (IMP)

All IMPs will be supplied by AstraZeneca AB, R & D Gothenburg. Dosing instructions and dispensing details will be provided by AstraZeneca.

AZD0449 for Inhalation	
Formulation 1:	AZD0449 nebulizer suspension CCI [redacted]
Formulation 2:	AZD0449 nebulizer suspension CCI [redacted]
Formulation 3:	AZD0449 nebulizer suspension CCI [redacted]
Dose:	Dose range CCI [redacted] delivered dose
Route of Administration:	Inhalation via Jet Nebulizer (Provo.X system)
Regimen:	Single and multiple ascending dose
Special Handling Requirements:	Sonication of suspension before use
Placebo for AZD0449 for Inhalation	
Formulation:	Placebo for AZD0449 nebulizer suspension
Strength/Concentrations/Dose:	N/A
Route of Administration:	Inhalation via Jet Nebulizer (Provo.X system)
Regimen:	Single and multiple ascending dose
Special Handling Requirements:	No

AZD0449 for Intravenous Administration	
Formulation:	AZD0449 solution for infusion CCI [redacted]
Strength/Concentrations:	CCI [redacted]
Dose:	Cohort 1: CCI [redacted] total dose; cohort 2: CCI [redacted] total dose
Route of Administration:	Intravenous via infusion pump set
Regimen:	Two single doses
Special Handling Requirements:	Sonication of solution before use

AZD0449 for Inhalation via dry-powder inhaler (DPI)	
Formulation:	AZD0449 inhalation powder: CCI [redacted]
Dose:	Dose range CCI [redacted] delivered dose
Route of Administration:	Inhalation via SD3FL version of Genuair
Regimen:	Repeated inhaled administration
Special Handling Requirements:	No
Placebo for Inhalation via DPI	
Formulation:	Placebo for AZD0449 inhalation powder
Strength/Concentrations/Dose:	N/A
Route of Administration:	Inhalation via SD3FL version of Genuair
Regimen:	Repeated inhaled administration
Special Handling Requirements:	No

Statistical Methods

Presentation and Analysis of Pharmacokinetic Data

Plasma concentrations for AZD0449 and CCI [redacted] will be summarized for the PK analysis set for each timepoint by treatment (dose level of AZD0449) Study Part and Study Day using protocol

scheduled times and appropriate descriptive statistics. Individual and combined individual plasma concentrations versus actual elapsed time after dose will be plotted on both the linear and semi-logarithmic scale for AZD0449.

Geometric mean (± gSD) plasma concentration versus nominal sampling time will be plotted on both the linear and semi-logarithmic scale for each Study Part and Study Day with all dose levels overlaid on the same plot for Part 1a Day 1 and Part 2a/b Day 1 and Day 12, with Day 1 and Day 12 overlaid on the same plot for each dose level for Part 2a/b and with Study Day 1 and Study Day 12 overlaid on the same plot for Part 3a/b. All plots will be based on the PK analysis set, with the exception of individual plots by subject, which will be based on the safety analysis set (SAF).

Dose-proportionality will be assessed following a single dose and following multiple dosing via least squares linear regression of the log-transformed PK parameters versus the log-transformed dose, ie, log (PK parameter)=α+βlog(Dose). An estimate of the slope and intercept of the regression line and the corresponding 2-sided 95% confidence interval (CI) for the slope will be obtained and tabulated for AUC and C_{max}.

The power model will be fitted by restricted maximum likelihood (REML) using SAS Proc Mixed. Both the intercept and slope will be fitted as fixed effects.

Scatter plots for each of the log-transformed PK parameters C_{max}, $AUC_{(0-t)}$ and AUC versus log-transformed dose will be presented with the regression line from the analysis overlaid on the same plot for Part 1a Day 1, and Part 2a/b Day 1, Day 12. In addition, figures of dose normalized C_{max}, $AUC_{(0-t)}$ and AUC versus dose, showing individual values and geometric mean will be presented separately for each PK parameter for Part 1a Day 1 and for Part 2a/b Day 1 and Day 12, to visually demonstrate dose-proportionality.

Presentation and Analysis of Safety and Tolerability Data

Subject disposition will be summarized. All safety data will be presented in the data listings. Use of concomitant medication will be reported.

AEs will be coded using the applicable dictionary and summarized by system organ class (SOC) and preferred term. Additional summaries by severity and causality will be presented.

Vital signs measurements and laboratory results will be listed and summarized including changes from baseline. Any out of range vital signs measurements and laboratory results will be flagged in the individual listings.

Results of the ECGs, including normal/abnormal and specific findings will be listed.

Spirometry values (Forced Expiratory Volume in 1 second [FEV_1] and forced vital capacity [FVC]) will be listed by treatment (dose level of AZD0449 and pooled placebo), subject and time-point including absolute values and percentage changes from baseline. Summary tabulations for absolute values and changes from baseline will be presented by treatment (each dose level of AZD0449, or pooled placebo, and pooled AZD0449) and time-point.

Peripheral capillary oxygen saturation will be listed and summarized including changes from baseline and percentages changes from baseline.

Analysis of Pharmacodynamic Data

Fractional Exhaled Nitric Oxide

The change from baseline in 2 hours post-dose FeNO level to 12 days (Part 2a and Part 3a) will be analysed based on a mixed effect model of repeated measures (MMRM) using all post-baseline 2 hours post-dose FeNO assessments. Analyses will be performed on the log-transformed FeNO data to normalize the skewed distribution of this endpoint.

The within-patient correlation will be modeled using the unstructured covariance matrix. The-Kenward-Roger approximation will be used to estimate denominator degrees of freedom. The analysis will be performed using only the observed cases (OC) without imputation of missing values. If the model does not converge, then the compound symmetry covariance structure will be used. REML method will be used for estimation.

Treatment effect and treatment differences will be estimated using contrasts of the Least-Square (LS) means on the correspondent treatment-by-day interaction, along with their SE and 2-sided 90% CI, and the p-value corresponding to the between-treatment group difference.

The same model will be used to evaluate treatment effect between the groups.

In addition, treatment effect of the change from baseline in 2 hours post-dose FeNO level at 12 days (Part 2a and Part 3a) will be analysed using analysis of covariance (ANCOVA) with treatment as a fixed effect, and baseline as a covariate. The last observation carried forward (LOCF) method will be used to deal with missing values. No multiplicity adjustments will be applied.

Fractional exhaled nitric oxide will be listed and summarized including changes from baseline. In addition, as sensitivity analysis, the change from baseline in FeNO area under the effect–time curve to Day 12 (AUC_{0-12}) will

be analysed using the same methods as the change from baseline in 2 hours post-dose FeNO level to 12 days (Part 2a and Part 3a).

CCI

Presentation and Analysis of Exploratory Data

CCI

Determination of Sample Size

Part 1 and the Non-PoM Cohorts

This part of the Phase I study is to investigate the safety and tolerability of a novel compound. The sample size was chosen to obtain reasonable evidence of safety and tolerability without exposing undue numbers of subjects to the compound at this phase of clinical development. No formal sample size calculation was done for Part 1 and the non-PoM cohorts in Part 2a/b and Part 3b.

Part 3a (PoM Cohort):

- Endpoint: change from baseline in exhaled FeNO (log scale) while receiving multiple inhaled doses of AZD0449, in active and placebo arms.
- Treatment difference (Treatment – Placebo) of change from baseline of mean Log FeNO, equivalent to a 25% reduction of the ratio of geometric mean.

For Part 3a, assuming a log mean baseline FeNO level of 4.24 with SD of 0.38 (geometric coefficient of variation [GCV]=39.4%) and a correlation between baseline and last day of study data of 0.7, as suggested by previous studies, the required sample size for a 25% absolute reduction in the ratio of geometric means (a 0.288 reduction of the mean log FeNO levels) is 18 evaluable patients per arm using a one-sided test at 5% significance level.

The current design presents an opportunity to perform a sample size calculation at an IA taking place when approximately half the data from Part 3a is available. If the actual estimate of baseline FeNO SD is higher than expected, reaching SD=0.55, the sample size of the PoM cohort in Part 3a will be potentially increased up to n=26 per arm.

Should the variation be even higher, requiring adjustments beyond this, an amendment will be submitted.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or special term	Explanation
AE	Adverse event (see definition in Section 11.1.1)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATD	Anticipated Therapeutic Dose (in this protocol used synonym for predicted (human) therapeutic dose)
ATP	Adenosine triphosphate
ATS	American Thoracic Society
AUC	Area under the concentration-time curve from time zero to infinity
$AUC_{(0-12)}$	Area under the plasma concentration-time curve from time zero to 12 hours post-dose
$AUC_{(0-24)}$	Area under the plasma concentration-time curve from time zero to 24 hours post-dose
AUC/D	Dose normalized AUC
%AUCextr	Percentage of AUC extrapolated
$AUC_{(0-t)}$	Area under the plasma concentration curve from time zero to the time of last quantifiable concentration
$AUC_{(0-t)}/D$	Dose normalized AUC(0-t)
AV	Atrioventricular
AZRand	AstraZeneca randomization system
CCI	[redacted]
BALF	Bronchoalveolar lavage fluid
BBB	Bundle branch block
BfArM	Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)
BID	Bis in die (twice daily)
BLQ	Below the limit of quantification
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
CCL17	Chemokine (C-C motif) ligand 17
CD28	Cluster of differentiation 28
cDC	Conventional dendritic cells
CI	Confidence interval

Abbreviation or special term	Explanation
CL	Total clearance of drug from plasma (IV administration)
CL/F	Apparent total clearance of drug from plasma (Extravascular administration)
C_{max}	Maximum observed plasma concentration
C_{max}/D	Dose normalized C_{max}
CMC	Chemistry, Manufacturing, and Controls
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CRF	Case report form
CRO	Contract research organization
CRP	C-reactive protein
CSP	Clinical Study Protocol
CSR	Clinical Study Report
DAE	Adverse event leading to the discontinuation of IMP
DC	Dendritic cells
DCF	Data clarification form
dECG	Digital electrocardiogram
DES	Data Entry Site – where serious adverse event reports from AstraZeneca Clinical studies are entered onto the AstraZeneca Patient Safety database by Tata Consultancy Services
DGR	Dangerous Goods Regulations
DILI	Drug-Induced Liver Injury
DMP	Data management plan
DNA	Deoxyribonucleic acid
DPI	Dry-powder inhaler
DVS	Data validation specification
ECG	Electrocardiogram
EClysis©	User-interactive, modular computer-based system for dECG data processing, analysis and measurement of ECG intervals and wave amplitudes, exports and reports, used by the AstraZeneca ECG Center
ECMO	Extracorporeal Membrane Oxygenation
ED	Effective dose
CCI	[redacted]
CCI	[redacted]
EMA	European Medicines Agency
EN	Enrolled analysis set
eNO	Exhaled nitric oxide

Abbreviation or special term	Explanation
EPCU	Early Phase Clinical Unit
EPO	Erythropoietin
ERS	European Respiratory Society
EU	European Union
FDA	Food and Drug Administration
FEV	Forced Expiratory Volume
FEV_1	Forced Expiratory Volume in one second
FeNO	Fractional exhaled nitric oxide
FIH	First in Human
CCI	[redacted]
FSH	Follicle-stimulating hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
gCV%	Geometric coefficient of variation
GGT	Gamma glutamyl transpeptidase (transferase)
GINA	Global Initiative for Asthma
GLP	Good Laboratory Practice
GM-CSF	Granulocyte macrophage-colony stimulating factor
GMP	Good Manufacturing Practice
gmean	Geometric mean
gSD	Geometric standard deviation
Hb	Hemoglobin
HbsAg	Hepatitis B surface antigen
HCT	Hematocrit
HED	Human equivalent dose
hERG	Human ether-a-go-go-related gene
HIV	Human immunodeficiency virus
HL	Hy's Law
HOP	Head out plethysmography
HR	Heart rate
i.t.	Intratracheal
IA	Interim Analysis
IATA	International Airline Transportation Association
IB	Investigator's Brochure
IBBB	Incomplete bundle branch block
IC_{50}	Half maximal inhibitory concentration

Abbreviation or special term	Explanation
ICAM-1	Intercellular Adhesion Molecule 1
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ICS	Inhaled corticosteroids
IEC	Independent Ethics Committee
CCI [redacted]	[redacted]
IMP	Investigational Medicinal Product
CCI [redacted]	[redacted]
IPF	Idiopathic pulmonary fibrosis
ISRB	Investigational Medicines Safety Review Board
IV	Intravenous
IVCD	Intraventricular conduction delay
JAK	Janus kinase
λz	Terminal elimination rate constant
λzN	Number of data points in the log-linear regression analysis
LDD	Lung-deposited dose
LLN	Lower limit of normal
LLOQ	Lower limit of quantification
LOCF	Last observation carried forward
LS	Least-Square
LSLV	Last Subject Last Visit
MABEL	Minimal anticipated biological effect level
max	Maximum
MAD	Multiple ascending dose
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
min	Minimum
MMRM	Mixed effect model of repeated measures
MRSD	Maximum Recommended Starting Dose
n	Total number of individual data values
N	Number of subjects
NA	Not applicable

Abbreviation or special term	Explanation
NC	Not calculated
NO(A)EL	No-observed-(adverse)-effect level
NQ	Not Quantifiable
NR	Not reportable
NS	No sample
OAE	Other significant adverse events
OTC	Over the counter
OVA	Ovalbumin
PAD	Pharmacologically active dose
PBMC	Peripheral blood mononuclear cells
PBPK	Physiology Based Pharmacokinetic
PD	Pharmacodynamics
PDF	Portable Document Format
PDS	Protocol deviation specification (document)
pECG	Paper printout ECG
PHL	Potential Hy's Law
PI	Principal Investigator
PK	Pharmacokinetics
PKS	Pharmacokinetic analysis set
PoM	Proof-of-mechanism
PP	Per Protocol analysis set
PV	Pharmacovigilance
QD	Quaque die (once daily)
QP	Qualified Person
QRS	ECG interval measured from the onset of the QRS complex to the J point
QT	ECG interval measured from the onset of the QRS complex to the end of the T wave
QTcF	QT interval corrected for heart rate using Fridericia's formula
R&D	Research and Development
RBC	Red blood cell
REML	Restricted maximum likelihood
RR	The time between corresponding points on 2 consecutive R waves on ECG
RS	Randomized Set
Rsq-adj	Regression coefficient adjusted for λz, N, Goodness-of-fit statistic for calculation of λz

Abbreviation or special term	Explanation
SABA	Short Acting Beta Agonist
SAD	Single ascending dose
SAE	Serious adverse event (see definition in Section 11.1.2).
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard deviation
SOC	System Organ Class
SOP	Standard operating procedure
SpO_2	Peripheral capillary oxygen saturation
SRC	Safety Review Committee
CCI	[redacted]
SUSAR	Suspected unexpected serious adverse reaction
$t_{1/2}\lambda z$	Terminal elimination half-life
t lower	Start of exponential fit of the terminal phase
t upper	End of exponential fit of terminal phase
CCI	[redacted]
TB	Tuberculosis
TBL	Total bilirubin
TCA	Tricyclic antidepressant
TCS	Tata Consultancy Services – an AstraZeneca partner who conduct data entry onto Sapphire
Th2	T-helper type 2
t_{last}	Time of last quantifiable concentration
t_{max}	Time of maximum observed concentration (first occurrence)
TSH	Thyroid-stimulating hormone
TSLP	Thymic stromal lymphopoietin
TYK2	Tyrosine kinase 2
UK	United Kingdom
ULN	Upper limit of normal
Vz	Apparent volume of distribution during the terminal phase (IV administration)
Vz/F	Volume of distribution during the terminal phase (extravascular administration)
WAD	Windows Allowance Document
WBC	White blood cell
WHO DD	World Health Organization drug dictionary
WOCBP	Women of Childbearing Potential

1. INTRODUCTION

1.1. Disease Background

Asthma affects an estimated 300 million individuals world-wide, and this number may grow by more than 100 million by 2025 [3]. Up to 10% of the population in developed countries may have asthma, and of these, up to 10% have severe disease, characterized by the requirement for high doses of inhaled corticosteroids (ICS) and an additional bronchodilator medication [4]. Around half of all asthma is driven by T-helper type 2 (Th2) cell inflammatory cytokines including interleukin (IL)-4, IL-5, and IL-13 [5]. This subgroup exists across the spectrum of asthma severity. Current clinical markers to identify this subtype include peripheral and sputum eosinophils, exhaled nitric oxide (eNO), as well as serum periostin. Additional asthma subtypes have been identified based on inflammatory processes including neutrophilic, mixed and paucigranulocytic [4].

Current asthma management is directed towards controlling inflammation, using oral and ICS, as well as providing short and long-term bronchodilation [6]. Although ICS address most of the pathways that drive asthma, not all patients respond to these medications. In the setting of severe exacerbations, patients require oral glucocorticoids for disease management, and many severe asthmatics require chronic oral glucocorticoid use. Inhaled glucocorticoids have potential side effects including thrush and have been shown to affect growth in children. Oral glucocorticoids have additional side effects including hyperglycemia, decreased bone density and increased infection risk [8]. More recently, biologic therapies directed towards the drivers of type-2 asthma have been developed. These medications can reduce oral corticosteroid use and improve lung function in severe asthmatics, but are often expensive, require specialist care and may need to be administered in office settings [9]. In this setting, there is a need for additional topical targeted therapies directed specifically towards Th2 asthma, but potentially applicable to all patients.

1.2. Investigational Medicinal Product Information

AZD0449 is an inhaled Janus kinase (JAK) 1 inhibitor derived from a cell-based platform that blocks phosphorylation of signal transducer and activator of transcription (STAT), including STAT6, STAT5, STAT3, and STAT1 induced by a range of cytokines. The JAK1/STAT6 pathway is a particularly attractive target for asthma management. Interleukin (IL)-4 and IL-13 activate JAK1, which in turn phosphorylates STAT6 leading to initiation of "T2" inflammatory processes including eosinophil recruitment and activation, antibody class switching to immunoglobulin E (IgE) production, goblet cell hyperplasia and mucus hypersecretion and airway hyperreactivity and remodeling.

1.2.1. Description of Mode of Action

AZD0449 is an adenosine triphosphate (ATP) competitive type I kinase inhibitor that binds to the active JAK1 conformation inhibiting its kinase activity. It is a potent and selective JAK1 inhibitor with a biochemical half maximal inhibitory concentration (IC_{50}) value of 0.003 µM for JAK1 in the presence of 5 mM ATP and no significant inhibition of JAK2, JAK3 or tyrosine kinase 2 (TYK2) at compound concentrations as high as 30 µM.

1.2.1.1. Primary Pharmacodynamics

Pharmacological characterization in a range of pre-clinical in vitro and in vivo models identified AZD0449 as a potent inhibitor of JAK1 dependent biological processes.

The effect of AZD0449 on IL-4/IL-13 induced signaling was studied in several cell models including human lung fibroblasts, small airway epithelial cells and peripheral blood mononuclear cells (PBMC). In these cells AZD0449 inhibited IL-4/IL-13 induced endpoints including STAT6 phosphorylation, eotaxin production, Intercellular Adhesion Molecule-1 (ICAM-1) expression and periostin secretion with IC_{50} values in the range of 0.011 to 0.057 µM. Ex vivo stimulation of sputum cells obtained from asthmatic donors with IL-4/IL-13 led to induction of STAT6 phosphorylation in the lymphocyte and monocyte populations that was inhibited by AZD0449 with IC_{50} values of 0.013 µM and 0.032 µM, respectively.

In human primary T cells, AZD0449 inhibited IL-2 induced STAT5 phosphorylation and proliferation, IL-21 induced STAT3 phosphorylation and interferon alpha (IFN-α) induced STAT1 phosphorylation with IC_{50} values in the range of 0.017 to 0.044 µM. AZD0449 was significantly less active on JAK2/TYK2-dependent STAT4 phosphorylation stimulated by IL-12 (IC_{50} of 9.03 µM), or JAK2-dependent erythropoietin (EPO)-induced cultured human erythroid precursor cell survival (IC_{50} of 1.35 µM), as expected from a JAK1 selective inhibitor. AZD0449 also inhibited the mixed lymphocyte reaction (IC_{50} value of 0.022 µM) without affecting the number of live cells in the reaction (IC_{50} >5 µM, n=2), indicating that AZD0449 can specifically block dendritic cell-driven T-cell proliferation, without being cytotoxic to the T-cells or the dendritic cells. In whole blood, AZD0449 could inhibit IL-2 induced STAT5 phosphorylation with an IC_{50} of 1.74 µM, which was about 40-fold less potency than in isolated T-cells. This is a 40-fold potency drop-off as compared to isolated T cells, but it can be explained by the strong binding of AZD0449 to plasma proteins. The JAK1 selectivity was maintained in whole blood configuration, as AZD0449 did not inhibit JAK2-dependent granulocyte-macrophage colony stimulating factor (GM-CSF) signaling even at the highest concentration tested (20 µM). Finally, R256/AZD0449 did not significantly inhibit phosphorylation events downstream of the T cell receptor as showed by minimal

inhibition of T cell receptor/cluster of differentiation 28 (CD28) induced IL-2 production (IC_{50} >4 µM).

Thymic stromal lymphopoietin (TSLP) is emerging as key cytokine in asthma as evidenced by the clinical efficacy of the anti-TSLP antibody tezepelumab in asthma patients [21]. Thymic stromal lymphopoietin induced signaling in most cell types, including dendritic cells (DC), is JAK1 dependent and involves phosphorylation of STAT5. For this reason, the impact of AZD0449 on TSLP induced DC responses was studied. AZD0449 blocked signaling downstream of the TSLP receptor in human conventional dendritic cells (cDCs) and monocytes as demonstrated by inhibition of TSLP-induced STAT5 phosphorylation in cDCs. Thymic stromal lymphopoietin-induced chemokine (C-C motif) ligand 17 (CCL17) production in monocytes was inhibited with an IC_{50} value of 0.066 µM. T2 skewing by DCs pre-treated with AZD0449 and subsequently stimulated with TSLP was blocked as indicated by a loss in the ability of these DCs to drive proliferation and production of Th2 cytokines in naïve and memory CD4+ T cells.

To inform further characterization of AZD0449 in in vivo pharmacokinetic (PK)/pharmacodynamic (PD) models and in secondary PD and safety pharmacology models (see Section 1.2.1.2) the cross-species activity of AZD0449 was determined in cell-based models. AZD0449 showed equipotent inhibition of IL-13 induced STAT6 phosphorylation in human, mouse and rat primary lung fibroblasts with IC_{50} values of 0.035 µM, 0.026 µM and 0.028 µM, respectively. AZD0449 also showed equipotent inhibition of IL-2 induced STAT5 phosphorylation in human and dog PBMCs with an IC_{50} value of 0.018 µM in both species.

To demonstrate suitability of AZD0449 for inhaled delivery and to guide dose to man prediction, AZD0449 was characterized in several in vivo models to demonstrate target engagement and effect on allergic airway inflammation when dosed by intratracheal (i.t.) instillation as well as by DPI. In these studies, clinically relevant formulations of AZD0449 (free base or fumarate salt) in the crystalline state were administered via the i.t. or inhaled (DPI) route. All stated doses refer to lung-deposited dose (LDD), which after i.t. dosing equals the delivered dose (dose deposited directly into the airways) and after DPI dosing is calculated from PK measurements (needed because not all of the nominal dose will reach the lung due to retention of substance in inhalation system and/or deposition in the upper respiratory tract).

Dosed i.t. AZD0449 (free base) demonstrated target engagement in vivo by inhibiting IL-13 induced STAT6 phosphorylation in mice. Significant inhibition (62%) was observed at a LDD of 5 µg/kg with calculated effective dose $(ED)_{50}$ and ED_{80} values of 1 and 4 µg/kg body weight, respectively.

The dose dependent effects on allergic airway inflammation of AZD0449 administered by i.t. instillation were investigated in ovalbumin (OVA) challenged Brown Norway rats. As read-out of efficacy, inhibition of OVA challenge induced eosinophilia in bronchoalveolar lavage fluid (BALF) was used. AZD0449 (fumarate salt) also demonstrated dose dependent effects in this model, with significant inhibition (38%) of airway eosinophilia observed at 3 µg/kg increasing to 62% at 300 µg/kg. The calculated ED80 value was 11 µg/kg body weight (corresponding to 9.1 µg/kg body weight of AZD0449 free base).

The same model was used to determine the effects of AZD0449 (fumarate salt) administered by DPI. Again, dose dependent inhibition of allergic airway inflammation was observed. The inhibitory effect of AZD0449 on lung eosinophil influx reached significance and a maximum (58% inhibition) at a LDD of 73 µg/kg body weight. The calculated ED80 value was 15 µg/kg for the AZD0449 fumarate salt (corresponding to 12.4 µg/kg body weight of AZD0449 free base). This value was used for scaling to a predicted efficacious LDD in man (70 kg) of approximatively 1.1 mg, corresponding to 0.91 mg AZD0449 free base. Assuming a lung-deposited fraction of 80%, this dose corresponds to 1.14 mg AZD0449 free base delivered dose.

1.2.1.2. Secondary Pharmacodynamics and Safety Pharmacology

A diverse set of in vitro radioligand binding, enzyme and functional and electrophysiological assays including cardiac ion channels, did not identify any off-target activities likely to be relevant in or near the expected plasma exposure range in humans.

In a head-out plethysmography (HOP) study in the rat, inhalation administration of AZD0449 at a total inhaled dose level of 26.2 mg/kg resulted in decreases in tidal volume of up to 30 to 40%. These decreases coincided with the 30-minute dosing period and were present for up to 1 hour post-dose. The decreases in tidal volume were accompanied by increases in respiratory rate and an overall decrease in minute volume. There were no effects on functional respiratory parameters at total inhaled dose levels of 0.707 or 2.72 mg/kg. In the repeat dose toxicity studies, decreases in tidal volume were observed in the rat at total inhaled dose levels ≥18.1 mg/kg/day, but there were no accompanying changes in respiratory rate or minute volume, while no effects were observed in the dog (also see Section 1.2.2.1).

AZD0449 had no effect on cardiovascular parameters assessed in conscious telemetered dogs following single inhaled administration of a total inhaled dose of 2 mg/kg/day. Thirty minutes following supplementary (bolus) intravenous (IV) dosing (administered 2 hours post inhalation dosing), a transient decrease in diastolic pressure (16%) occurred after a dose of 0.2 mg/kg; higher doses (1 and 2 mg/kg) transiently reduced systolic, diastolic and mean arterial blood pressure, with reductions of 41%, 36% and 40%, respectively, at 2 mg/kg, while left ventricular

contractility parameters were also decreased (eg, dP/dt_{max} by 43% and 42% after 1 and 2 mg/kg IV, respectively), tracking the blood pressure changes. All the cardiovascular parameters had recovered to baseline within 1 hour of the IV dosing. The no observable effect level (NOEL) for the cardiovascular effects was considered to be 2 mg/kg (inhaled) combined with 0.2 mg/kg IV. Furthermore, there was no AZD0449 related inhibition of the human ether-à-go-go-related gene (hERG) encoded potassium channel.

In the conscious rat, administration of AZD0449 at a total inhaled dose of 18.7 mg/kg, produced reductions in pupil diameter which were observed up to 2 hours post-dose. There was no other consistent behavioral effect or any effects on body temperature or locomotor activity that were attributable to AZD0449. There were no effects at inhaled dose levels of 0.712 or 4.55 mg/kg.

The margins from the NOEL in each of the safety pharmacology studies to the predicted human C_{max} plasma exposure associated with a maximal allowed dose of 5 mg (32.9 nmol/L/0.033; total/free) are as follows: nervous system (>4-fold); hERG (>65,000-fold); cardiovascular system (>8-fold); respiratory system (>2-fold; with the NOEL dose >10-fold higher the maximum allowed human dose).

1.2.2. Toxicology Pre-clinical Data

AZD0449 inhalation toxicity studies in the rat range up to 6 months in duration, while studies up to 3 months have been completed in the dog. Further information on pre-clinical toxicology findings is available in the Investigator's Brochure, but a summary of the key findings is presented below [2].

1.2.2.1. Respiratory Tract Effects

Inhalation administration of AZD0449 to rats and dogs at dose levels ≥3.75 mg/kg/day was associated with pathological findings in the respiratory tract.

In the rat, changes in the lung included polypoid lesions in the bronchi/bronchioles, deposition of eosinophilic material/pigments in the alveoli/bronchioles/bronchi, hypertrophy/hyperplasia of the bronchiolar/bronchial epithelium and alveolar macrophage aggregates at the alveolobronchiolar junction. Changes in the nasal turbinates consisted of atrophy/inflammation of the respiratory/olfactory epithelium and eosinophilic material/pigments/inflammatory cells in the lumen. Following polarized light evaluation, special staining, immunohistochemistry and mass spectrometry imaging, the pigments, seen embedded in the eosinophilic material of the lungs and nasal turbinates, were considered to be AZD0449.

In the dog, changes in the lung consisted of foamy alveolar macrophages, which were seen mostly in the proximity of terminal bronchioles and bronchioloalveolar junctions. These

changes were frequently associated with a minimal level of alveolar neutrophilic inflammatory cell infiltrate. There were no other respiratory tract changes in the dog.

There were no AZD0449 related respiratory tract pathologic findings in the rat at total inhaled dose levels ≤1.88 mg/kg/day (tested over 6 months) or in the dog at total inhaled dose levels ≤2.01 mg/kg/day (tested over 3 months, with a supplementary 1 mg/kg/day IV dose).

Functional respiratory assessments were performed in a standalone respiratory safety pharmacology study in the rat (see Section 1.2.1.2) and within repeat dose toxicity studies of up to 3 months in duration. In the rat decreases in tidal volume (up to ca. 40%) were observed at total inhaled dose levels ≥18.1 mg/kg/day. The decreases coincided with the 30-minute dosing period and were present for up to 1-hour post-dose. In the safety pharmacology study, the decreases coincided with an increase in respiratory rate and an overall decrease in minute volume, however these changes were not observed in the repeat dose toxicity studies. There were no changes in the rat at total inhaled dose levels ≤3.75 mg/kg/day. There were no changes in the dog at the highest dose level tested and assessed for functional respiratory changes (total inhaled dose level of 2.01 mg/kg/day plus a 1 mg/kg/day IV dose).

The respiratory tract pathology findings are likely to be a consequence of administration of high inhaled doses of a low solubility compound [2], while the absence of the polypoid lesion in the lung of the dog indicates that this particular finding is possibly rat or rodent specific. The cause of the functional respiratory changes is not known. Nevertheless, there were no pathological or functional changes observed at the no-observed-adverse-effect levels (NOAEL). The maximum allowed dose has been calculated by applying a 10-fold safety factor on the NOAEL in the most sensitive species (rat); see Section 3.4.7.

1.2.2.2. Systemic Effects

Administration of AZD0449 to rats and dogs was well tolerated, with no AZD0449 related clinical signs or premature deaths. Key findings are summarized below, however none were considered to be adverse.

In rats, lower than control body weight gain was observed (ca. 0.8-fold control at total inhaled doses ≥1.88 mg/kg/day over 6 months). There were no effects on body weight in the dog.

In the rat, decreases in white blood cell counts were observed, with the most consistent decrease recorded in lymphocytes (up to ca. 0.7-fold control); these changes were not observed in the dog.

Reticulocytes were also decreased (predominately in the rat; up to ca. 0.7-fold control), but the effect was only observed in studies up to 1 month in duration and there was no effect on total red blood cell counts.

Decreased lymphoid tissue (spleen and thymus) weights were recorded (up to ca. 0.6-fold control in rat and up to ca. 0.3-fold control in the dog [males only]); however, these changes were without histopathologic correlates and not consistently observed across all toxicology studies.

There were no AZD0449 related pathologic changes, other than those in the respiratory tract (see Section 1.2.2.1).

1.2.2.3. Intravenous Administration

Following IV administration of the intended clinical formulation of AZD0449 to rabbits, there were no signs of local irritation or microscopic findings at the injection sites that were considered to be related to AZD0449.

Furthermore, a suspension of AZD0449 was well tolerated following once daily IV administration to dogs at a dose level of 1 mg/kg for 3 months.

An in vitro blood compatibility study was conducted in human blood using the intended clinical formulation of AZD0449. There was no effect upon hemolysis, erythrocyte clumping or plasma precipitation.

1.2.3. Clinical Studies

AZD0449 has not been previously evaluated in clinical studies. This is a first-in-human (FIH) clinical study.

1.3. Overall Rationale for Conducting this Study

AZD0449 has not been previously studied in humans. This study will have 3 parts:

Part 1 will investigate the safety, tolerability and pharmacokinetics (PK) of AZD0449 after inhaled single ascending doses (SAD) in healthy volunteers (Part 1a) and will investigate two IV-dose cohorts to determine absolute bioavailability (Part 1b).

Part 2a/b will be conducted in patients with mild asthma (Part 2a) and healthy volunteers (Part 2b), investigating the safety, tolerability and PK of AZD0449 after multiple ascending doses (MAD) and studying the effect of AZD0449 on airway inflammation. Part 2a/b will be done with nebulized formulation.

Part 3a/b will be conducted in patients with mild asthma (Part 3a) and with an optional cohort of healthy volunteers (Part 3b), investigating the safety, tolerability and PK of AZD0449 after repeated dose and studying the effect of AZD0449 on airway inflammation to show target engagement and proof-of-mechanism (PoM). Part 3a/b will be done with dry-powder inhalation (DPI).

Details on design and rationale for the different study parts are provided in Section 3 and Section 4, respectively.

2. STUDY OBJECTIVES

The study objectives are detailed in Section 2.1 (SAD in healthy volunteers [Part 1a]), Section 2.2 (bioavailability in IV dose cohorts [Part 1b]), Section 2.3 (MAD - patients with mild asthma [Part 2a] and healthy volunteers [Part 2b]), Section 2.4 (patients with mild asthma [Part 3a] and an optional cohort of healthy volunteers [Part 3b]).

2.1. Part 1a Objectives

Part 1a will be a SAD study of inhaled AZD0449 nebulized suspension. The primary and secondary objectives and the corresponding outcome measures are presented below in Table 2.1-1.

Table 2.1-1 Part 1a Objectives and Outcome Measures

Primary Objective	**Outcome Measures**
• To assess the safety and tolerability of AZD0449 following inhaled administration of single ascending doses to healthy volunteers.	• Adverse events (AEs); vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG; 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Secondary Objective	**Outcome Measures**
• To characterize the blood plasma PK of AZD0449 following inhaled administration of single ascending doses of AZD0449.	• Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
Exploratory Objectives	**Outcome Measure**
• CCI [redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

• CCI	

BP: Blood pressure; ECG: Electrocardiogram; (d)ECG: (digital) electrocardiogram; CCI PK: Pharmacokinetics; SpO_2: Peripheral capillary oxygen saturation; CCI

2.2. Part 1b Objectives

In Part 1b, healthy volunteers in 2 cohorts will receive a single IV dose of AZD0449 solution at 2 different dose levels. The primary and secondary objectives and the corresponding outcome measures are presented below in Table 2.2-1.

Table 2.2-1 Part 1b Objectives and Outcome Measures

Primary Objective	**Outcome Measures**
• To characterize the blood plasma PK of AZD0449 following intravenous administration of 2 single IV doses in healthy volunteers.	• Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$, AUC, CL, Vz, $AUC_{(0-t)}/D$, AUC/D, C_{max}/D, t_{last}
Secondary Objective	**Outcome Measures**
• To assess the safety and tolerability of AZD0449 following IV administration of 2 single doses to healthy volunteers.	• AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG; 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Exploratory Objectives	**Outcome Measure**
• CCI	

AE: Adverse Event; BP: Blood pressure; (d)ECG: (digital) Electrocardiogram; CCI [redacted]; dECG: Digital electrocardiogram; CCI [redacted]; IV: Intravenous; PK: Pharmacokinetics; SpO_2: Peripheral capillary oxygen saturation.

2.3. Part 2a/b Objectives

Part 2a/b will be a MAD study of inhaled AZD0449 nebulized suspension in patients with mild asthma (Part 2a) and healthy volunteers (Part 2b). The primary, secondary and exploratory objectives and the corresponding outcome measures are presented below for patients with mild asthma in Table 2.3-1 and for healthy volunteers in Table 2.3-2.

Table 2.3-1 Part 2a Objectives and Outcome Measures in Patients with Mild Asthma

Primary Objective	**Outcome Measures**
• To assess the safety and tolerability of AZD0449 following inhaled nebulized administration of multiple ascending doses	• AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG, 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Secondary Objectives	**Outcome Measures**
• To characterize the blood plasma PK of AZD0449 following inhaled nebulized administration of multiple ascending doses	• Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
• To evaluate anti-inflammatory effect	• Change from baseline in 2 hours post-dose FeNO and FeNO $AUC_{(0\text{-}12)}$ to Day 12 and Follow-up
Exploratory Objectives	**Outcome Measure**
• CCI [redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

CCI	

AE: Adverse event; BP: Blood pressure; (d)ECG: (digital) Electrocardiogram; CCI ; FeNO: Fractional exhaled nitric oxide; PK: Pharmacokinetics; SpO_2: Peripheral capillary oxygen saturation.

Table 2.3-2 Part 2b Objectives and Outcome Measures in Healthy Volunteers

Primary Objective	**Outcome Measures**
• To assess the safety and tolerability of AZD0449 following inhaled nebulized administration of multiple ascending doses	• AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG, 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Secondary Objectives	**Outcome Measures**
• To characterize the blood plasma PK of AZD0449 following inhaled nebulized administration of multiple ascending doses	• Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
Exploratory Objectives	**Outcome Measure**
• CCI	

• CCI [redacted]	[redacted]

AE: Adverse event; BP: Blood pressure; (d)ECG: (digital) Electrocardiogram; CCI [redacted] PK: Pharmacokinetics; SpO_2: Peripheral capillary oxygen saturation; CCI [redacted]

2.4. Part 3a/b Objectives

Part 3a/b will be a PK, safety, and DPI/PoM study. Patients with mild asthma (Part 3a) and healthy volunteers (Part 3b) will receive repeated inhaled doses of 1 dose level of AZD0449 using a DPI. Part 3b is optional and will be conducted prior to, or in parallel to Part 3a depending on the speed of recruitment of subjects to the different parts. The primary, secondary and exploratory objectives and the corresponding outcome measures are presented below for patients with mild asthma in Table 2.4-1 and for healthy volunteers in Table 2.4-2.

Table 2.4-1 Part 3a Objectives and Outcome Measures in Patients with Mild Asthma

Primary Objective	**Outcome Measures**
• To assess the safety and tolerability of AZD0449 following repeated inhaled administration using a DPI	• AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG; 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Secondary Objectives	**Outcome Measures**
• To characterize the blood plasma PK of AZD0449 following repeated inhaled administration using a DPI	• Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
• To evaluate anti-inflammatory effect	• Change from baseline in 2 hours post-dose FeNO and FeNO $AUC_{(0\text{-}12)}$ to Day 12 and during safety monitoring period
Exploratory Objectives	**Outcome Measure**
• CCI [redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

• CCI	

AE: Adverse event; BP: Blood pressure; (d)ECG: (digital) electrocardiogram; DPI: Dry-powder inhaler; CCI FeNO: Fractional exhaled nitric oxide; PK: Pharmacokinetics; SpO_2: Peripheral capillary oxygen saturation; CCI

Table 2.4-2 Part 3b Objectives and Outcome Measures in Healthy Volunteers (optional cohort)

Primary Objective	**Outcome Measures**
• To assess the safety and tolerability of AZD0449 following repeated inhaled administration using a DPI	• AEs; vital signs (supine BP, pulse, respiratory rate and body temperature); 12-lead ECG; 12-lead dECG; telemetry; physical examination; laboratory assessments (hematology, biochemistry and urinalysis); spirometry and SpO_2
Secondary Objectives	**Outcome Measures**
• To characterize the blood plasma PK of AZD0449 following repeated inhaled administration using a DPI	• Where possible the following PK parameters will be assessed: C_{max}, t_{max}, λz, $t_{1/2}\lambda z$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, AUC, CL/F, Vz/F, $AUC_{(0\text{-}t)}/D$, AUC/D, C_{max}/D, t_{last}
Exploratory Objectives	**Outcome Measure**
• CCI	

• CCI [redacted]	[redacted]

AE: Adverse event; BP: Blood pressure; (d)ECG: (digital) electrocardiogram; DPI: Dry-powder inhaler; CCI [redacted] PK: Pharmacokinetics; SpO_2: Peripheral capillary oxygen saturation.

3. STUDY DESIGN

3.1. Overall Study Design

This will be a Phase I, FIH study consisting of the following parts:

- Part 1a – Section 3.1.1
- Part 1b – Section 3.1.2
- Part 2a/b – Section 3.1.3
- Part 3a/b – Section 3.1.4

3.1.1. Part 1a Study Design

Part 1a of the study will be a randomized, single-blind, placebo-controlled, SAD, sequential group design study performed at a single study center. Male and/or female healthy volunteers, aged 18 to 55 years will be included in this part of the study. Female volunteers must be of non-childbearing potential. This part of the study will be conducted in up to 72 volunteers.

Six inhaled dose levels of AZD0449 nebulized suspension are planned to be investigated in 6 cohorts. Depending on emerging safety and PK data, up to 3 additional inhaled dose levels (cohorts), within the pre-specified dose range (see Section 3.5.1), may be added at the discretion of the Sponsor.

Within each cohort, 6 volunteers will be randomized to receive an inhaled dose of AZD0449 nebulized suspension and 2 volunteers will be randomized to receive inhaled placebo. Dosing for each ascending dose cohort will start with 2 volunteers in a sentinel cohort, such that 1 volunteer will be randomized to receive AZD0449 nebulized suspension and 1 volunteer will be randomized to receive placebo (see Section 3.5.1.3).

In each cohort, volunteers will attend a Screening Visit within 28 days before receiving the dose of AZD0449 nebulized suspension or corresponding placebo.

For the treatment period, volunteers will be admitted to the Clinical Unit on Day -1. On Day 1, volunteers will receive a single inhaled dose of AZD0449 nebulized suspension or corresponding placebo. Assessments will be performed, and samples collected from before dosing until at least 36 hours post-dose. Volunteers will be discharged from the Clinical Unit after all samples have been collected and assessments have been performed on Day 4. Depending on the emerging data in cohort 1, the collection period may be extended to 96 hours post-dose.

Volunteers will return to the Clinical Unit within 6±1 days after the dose of AZD0449 nebulized suspension or corresponding placebo for a Follow-up Visit.

Following review of the study data, the Safety Review Committee (SRC) may decide to adjust the length of the stay at the Clinical Unit and the timing and number of assessments and/or blood and urine samples for subsequent cohorts.

Dosing, sentinel cohorts, dose escalation and stopping criteria are described in Section 3.5.1.

3.1.2. Part 1b Study Design

Intravenous (IV) dosing in Part 1b of the study will be initiated after the completion of cohort 6 in Part 1a or (if Part 1a is completed with less than 6 cohorts) after completion of the last cohort in Part 1a. Part 1b, will be open-label and consist of 2 dose cohorts (IV CCI) and be conducted in 12 healthy volunteers.

3.1.2.1. First IV Dose Cohort

Six (6) volunteers will be selected for the first IV dose cohort in Part 1b following a 2-stage design. If Part 1b cannot be completed with 6 volunteers from Part 1a or if some of the data are considered not evaluable, up to 6 additional naïve volunteers may be enrolled (for rationale see Section 4.1.2). The inhaled dose for naïve volunteers will be decided based on emerging data. The inhaled dose cannot be higher than the highest inhaled dose considered safe in previous cohorts.

All 6 volunteers will receive a single IV dose CCI of AZD0449 solution. Dosing will start with 1 healthy volunteer in a sentinel cohort (see Section 3.5.2.2).

There will be a washout period of at least 2 weeks for the volunteers receiving both inhaled dosing in Part 1a and IV dosing in Part 1b. For the IV treatment period, volunteers will be admitted to the Clinical Unit on Day -1. On Day 1, volunteers will receive a single IV dose CCI of the AZD0449 solution. Assessments will be performed, and samples collected from before dosing until 48 hours post-dose. Volunteers will be discharged from the Clinical Unit after all samples have been collected and assessments have been performed on Day 3.

Volunteers will return to the Clinical Unit within 6±1 days after the last dose of AZD0449 for a Follow-up Visit.

Dosing, sentinel cohorts, dose escalation and stopping criteria are described in Section 3.5.2.

3.1.2.2. Second IV Dose Cohort

A second IV dose cohort in Part 1b will be initiated after the evaluation of the PK and safety results from all cohorts in Part 1a and completion of the first IV dose cohort in Part 1b.

The second IV dose cohort will consist of 6 healthy volunteers who have not previously participated in the study (naïve volunteers).

All 6 volunteers will receive a single IV dose CCI of AZD0449 solution. Dosing will start with 1 healthy volunteer in a sentinel cohort (see Section 3.5.2.2).

The volunteers will be admitted to the Clinical Unit. On Day 1, volunteers will receive a single IV dose CCI of the AZD0449 solution. Assessments will be performed, and samples collected from before dosing until 48 hours post-dose. Volunteers will be discharged from the Clinical Unit after all samples have been collected and assessments have been performed on Day 3.

Volunteers will return to the Clinical Unit within 6±1 days after the last dose of AZD0449 for a Follow-up Visit.

Dosing, sentinel cohorts, dose escalation and stopping criteria are described in Section 3.5.2.

3.1.3. Part 2a/b Study Design

Part 2a/b of the study will be a randomized, single-blind, placebo-controlled, MAD, sequential group design study performed at approximately 3 study centers. Subjects aged 18 to 55 years will be included in this part of the study. Unlike in Part 1a or Part 1b (First IV Dose Cohort), female subjects of childbearing potential may also be enrolled. This part of the study will recruit up to 56 subjects. A total of 26 evaluable subjects are needed for completion of Part 2a/b. Potentially, 30 extra subjects may be randomized to counteract drop-outs and allow for an extra cohort if needed.

Three dose levels of AZD0449 nebulized suspension are planned to be investigated in 3 cohorts. The cohorts could comprise either patients with mild asthma or healthy volunteers.

Part 2a will include cohorts 1 and 2, each comprising of 9 patients with mild asthma. Within each of these cohorts 6 patients will be randomized to receive AZD0449 nebulized suspension and 3 patients randomized to receive placebo. Additional cohorts with 9 patients could be added to study PK, PD and safety for doses lower than CCI

Part 2b will consist of 1 cohort of 8 healthy volunteers; 6 volunteers will be randomized to receive AZD0449 nebulized suspension and 2 volunteers will be randomized to receive placebo.

Dosing for each ascending dose cohort will start with 2 subjects in a sentinel cohort, such that 1 subject will be randomized to receive AZD0449 nebulized suspension and 1 subject will be randomized to placebo (see Section 3.5.3.2).

Each subject will receive a single dose of AZD0449 nebulized suspension at the selected dose level or placebo on Day 1 followed by 10 daily doses from Day 3 to Day 12.

For the treatment period, subjects will be admitted to the Clinical Unit. The first dose will be administered on Day 1. Following this first dose, plasma PK samples will be collected up to 48 hours post-dose for the first dose (Day 1) to allow for characterization of the terminal elimination phase. Once daily dosing will start on Day 3, 48 hours post-Day 1 dose PK sampling and continue up to Day 12 with intervals of 24 hours between doses.

Each subject will receive inhaled doses of AZD0449 nebulized suspension at the selected dose level or placebo on the specified dosing days. Assessments will be performed, and samples collected from before dosing until up to 240 hours after last dose (Part 2a) and until up to 360 hours after last dose (Part 2b). Subjects will remain in house for the duration of the treatment period and will be discharged from the Clinical Unit on Day 14, 2 days after the last dose administration (Part 2a) and after an extended safety monitoring period on Day 27, 15 days after the last dose administration (Part 2b). If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the extended safety monitoring period may be conducted as non-residential visits.

Following review of emerging data, the SRC may decide to adjust the length of the stay at the Clinical Unit and the timing and number of assessments and/or blood and urine samples for subsequent cohorts. The time window between the single dose and start of repeated dosing may also be adjusted any time during the study.

Dosing, sentinel cohorts, dose escalation and stopping criteria are described in Section 3.5.3.

3.1.4. Part 3a/b Study Design

Part 3a/b of the study will be initiated after the completion of Part 2b.

Part 3a/b will be a randomized, single-blind, placebo-controlled, DPI/PoM study. Part 3 will be conducted in up to 36 patients with mild asthma (Part 3a) and 8 healthy volunteers (Part 3b, optional).

Part 3a will consist of 36 patients, aged 18 to 55 years, where 18 patients will be randomized to AZD0449 DPI and 18 patients to placebo. An Interim Analysis (IA) will be conducted when approximately 50% of the patients have completed Part 3a . Should new data on the variability of the FeNO measurements emerge at the IA, the sample size in Part 3a could be changed.

If the optional Part 3b is conducted, it will comprise 8 healthy volunteers; 6 volunteers will be randomized to AZD0449 DPI and 2 volunteers to placebo.

For information on dosing in Part 3a/b, please refer to Section 3.5.4.

Dosing and assessment time-points are presented in Table 3.2-6 and Table 3.2-7.

Each subject will receive a single dose of AZD0449 DPI at the selected dose level or placebo on Day 1 followed by 10 daily doses from Day 3 to Day 12.

For the treatment period, subjects will be admitted to the Clinical Unit. The first dose will be administered on Day 1. Once daily dosing will start on Day 3, 48 hours post-Day 1 dose PK sampling and continue up to Day 12.

Each subject will receive inhaled doses of AZD0449 DPI at the selected dose level or placebo on the specified dosing days. Assessments will be performed, and samples collected from before dosing until up to 360 hours after last dose. Subjects will remain in house for the duration of the treatment period and undergo an extended safety monitoring period until being discharged from the Clinical Unit on Day 27, 15 days after the last dose administration. If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the extended safety monitoring period may be conducted as non-residential visits.

Following review of emerging data, the SRC may decide to adjust the length of the stay at the Clinical Unit and the timing and number of assessments and/or blood and urine samples for subsequent cohorts. The time window between the single dose and start of repeated dosing may also be adjusted any time during the study.

Dosing and stopping criteria are described in Section 3.5.4.

Details of the statistical analyses are presented in Section 10.

3.1.5. End of Study

Study completion is defined as the last visit of the last subject for any protocol related activity (LSLV – Last Subject Last Visit).

3.1.6. Expected Duration of the Study

	Screening	Treatment Period*	Safety Monitoring** Period	Follow-up	Total Duration
Part 1a	28 days before dose	1 day	3 days	6±1 days after dose	Up to 36 days
Part 1b (First IV Cohort)	28 days before dose	1 day	2 days	6±1 days after dose	Up to 53 days
Part 1b (Second IV Cohort)	28 days before dose	1 day	2 days	6±1 days after IV dose	Up to 36 days
Part 2a	28 days before first dose	12 days	2 days	10 ±1 days after last dose	Up to 52 days
Part 2b	28 days before first dose	12 days	15 days	Not applicable	Up to 55 days
Part 3a and Part 3b	28 days before first dose	12 days	15 days	Not applicable	Up to 55 days

* This is a 12 day period with dosing on Day 1 and Day 3 to 12

** Period of in-house monitoring from last dose until discharge from the unit. If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the safety monitoring period for Part 2b, 3a, and 3b may be conducted as non-residential visits.

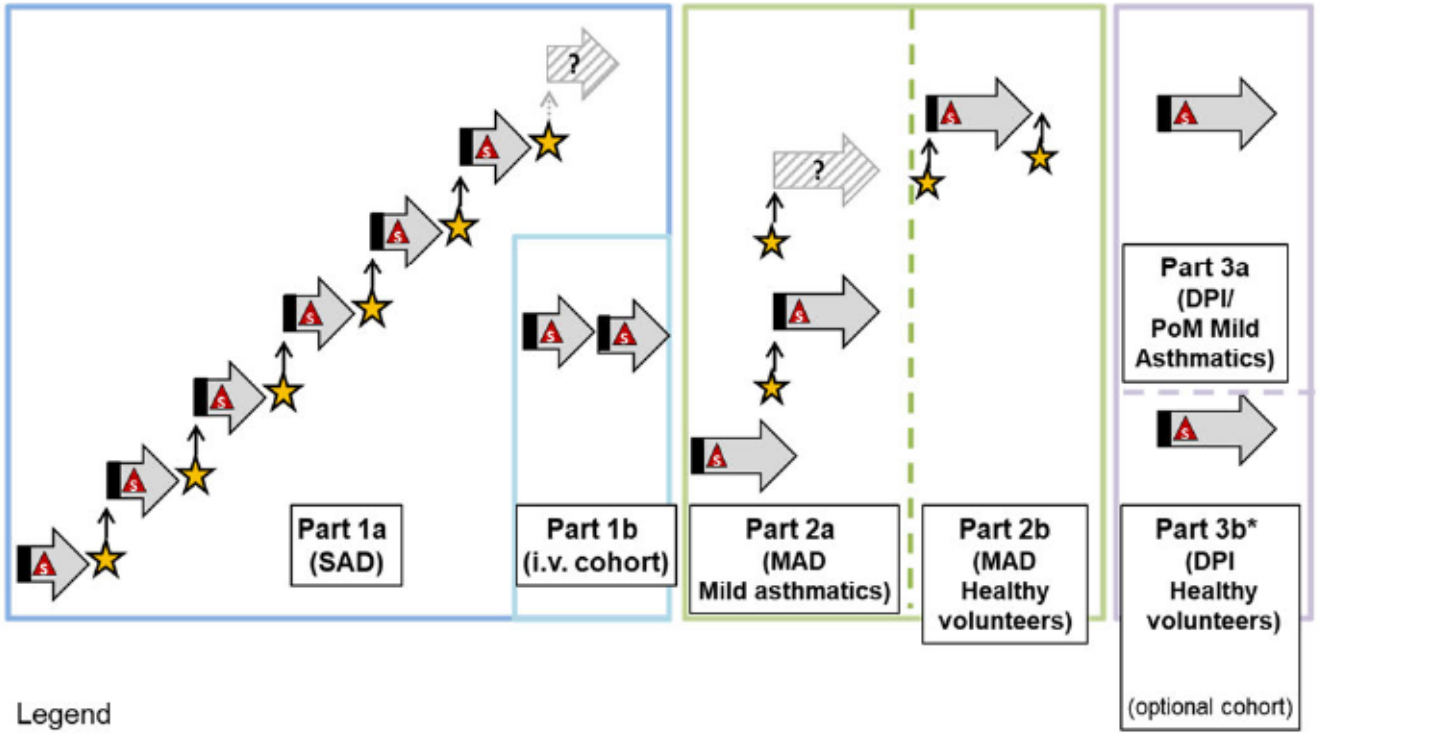
3.2. Study Flow Chart, Schedules of Assessments and Time Schedules for Digital Electrocardiography

A high-level overview of the study scheme is presented in Figure 3-1. Overviews and decision points of the individual study parts are presented in Figure 3-2 (Part 1a and Part 1b) and Figure 3-3 (Part 2a/b and Part 3a/b).

The Schedule of Assessments displaying assessments/tasks and time-points is presented in Table 3.2-1 (Part 1a), Table 3.2-2 and Table 3.2-3 (Part 1b), Table 3.2-4 and Table 3.2-5 (Part 2a/b), Table 3.2-6, and Table 3.2-7 (Part 3a/b). The time schedule for digital ECGs (dECGs) is presented in Table 3.2-8 (Part 1a), Table 3.2-9 (Part 1b) and Table 3.2-10 (Part 2a/b and Part 3a/b).

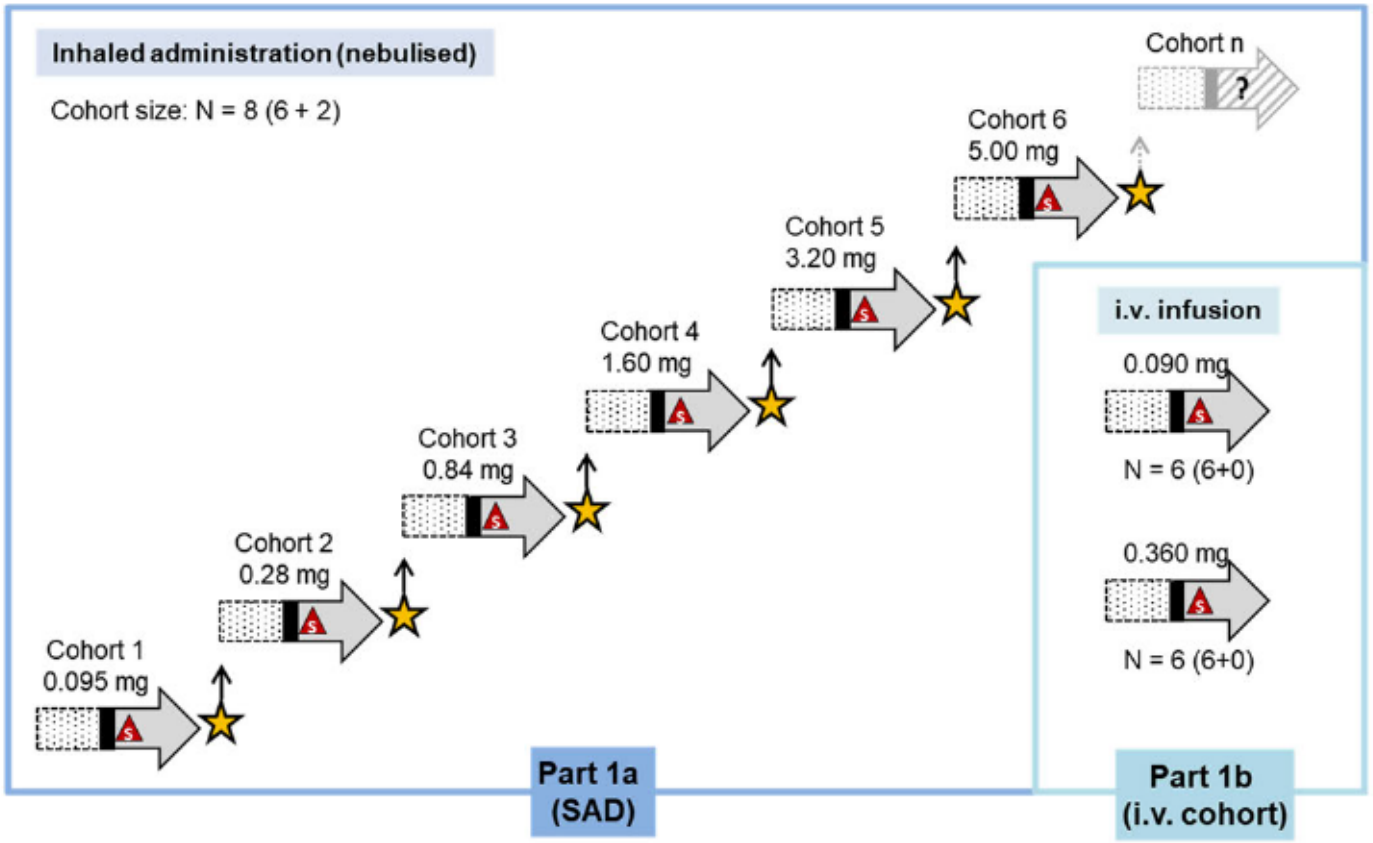
3.2.1. Study Flow Charts

Figure 3-1 High -Level Overview of the Study Scheme



For doses, subject numbers and other details please refer to the following detail charts

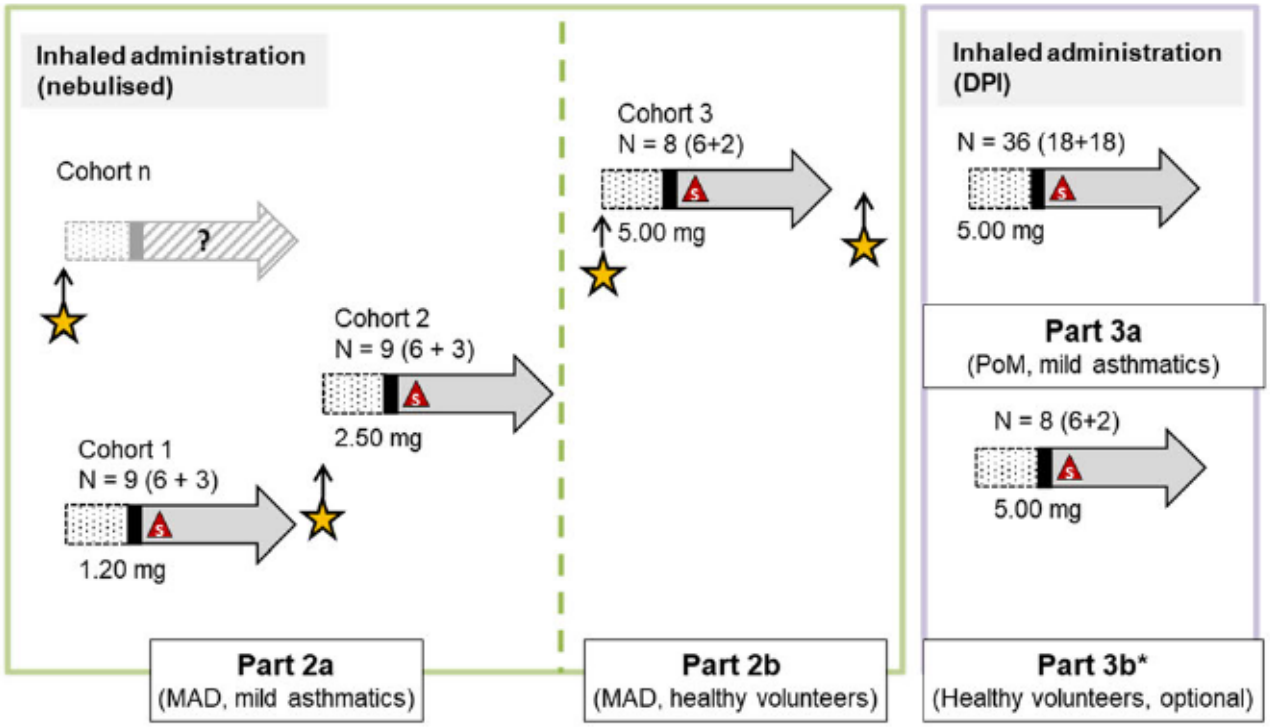
Figure 3-2 Dose Escalation and Decision Points in Part 1a/b



Legend

Study cohort treatment period		?	Additional cohorts may be added to test higher or lower dose levels at the discretion of the Sponsor and based on emerging data
Day 1 start of Sentinel Cohort dosing (2 subjects)			
Safety review of Sentinel Cohort data			Screening
Dose escalation based on SRC decision		N = x (y+z)	Number of subjects: x = total, y = AZD0449, z = placebo

Figure 3-3 Dose Escalation and Decision Points in Part 2a/b and Part 3a/b



Legend

- Study cohort treatment period
- Day 1 start of Sentinel Cohort dosing (2 subjects)
- Safety review of Sentinel Cohort data
- Dose escalation and initiation of optional cohort based on SRC decision
- ? Additional cohorts may be added to test higher or lower dose levels at the discretion of the Sponsor and based on emerging data
- Screening
- * Optional cohort
- N = x (y+z) Number of subjects: x = total, y = AZD0449, z = placebo

3.2.2. Schedules of Assessments

Table 3.2-1 Schedule of Assessments Part 1a (SAD)

Assessments	Screening	Treatment Period			Follow-up Visit	Comments
	Days -28 to--2	Day -1	Days 1 to 3 [a]	Day 4 [a]	(6±1 days post-dose)	
Informed consent	X					Includes optional genetic consent
Inclusion/exclusion criteria	X	X				
Demographic data	X					
Medical history	X					
Drug, alcohol and cotinine screen	X	X				
Serology	X					
QuantiFERON® TB	X					
Follicle-stimulating hormone testing	X					Post-menopausal females only (see Section 5.2.3.2 for definition)
Study Residency:						
Admission		X				
Discharge				X		At least 60 h post-dose, may be extended up to 96 h post-dose depending on emerging data
Non-residential visit	X				X	
Investigational Medicinal Product Administration:						
Inhaler Nebulizer Training		X				
Randomization			Day 1			
AZD0449/Placebo administration via nebulizer			Day 1 (0 h) [b]			
Safety and Tolerability:						
Adverse event questioning	Only SAEs	Only SAEs	X	X	X	

Table 3.2-1 Schedule of Assessments Part 1a (SAD)

Assessments	Screening	Treatment Period			Follow-up Visit	Comments
	Days -28 to–2	Day -1	Days 1 to 3 [a]	Day 4 [a]	(6±1 days post-dose)	
Spirometry	X	X	X	X	X	Day 1: Pre-dose and 30 min, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 4: 72 h post-dose
Pulse oximetry	X	X	X	X	X	**Cohorts 1-4:** Day 1: Pre-dose and 30 min, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 4: 72 h post-dose **Cohorts 5-6:** Day 1: Pre-dose, 1 h, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 4: 72 h post-dose
Blood pressure, pulse and respiratory rate (supine) and body temperature	X	X	X	X	X	**Cohorts 1-4:** Day 1: Pre-dose and 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h and 12 h post-dose Day 2: 24 h post-dose Day 3: 48 h post-dose Day 4: 72 h post-dose **Cohorts 5-6:** Day 1: Pre-dose and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h and 12 h post-dose Day 2: 24 h post-dose Day 3: 48 h post-dose Day 4: 72 h post-dose
12-lead dECG			X			See Table 3.2-8
12-lead safety ECG	X	X	X		X	12-lead safety ECG will be collected at the start of each dECG extraction window when time-points coincide, see Table 3.2-8

Table 3.2-1 Schedule of Assessments Part 1a (SAD)

Assessments	Screening	Treatment Period			Follow-up Visit	Comments
	Days -28 to–2	Day -1	Days 1 to 3 [a]	Day 4 [a]	(6±1 days post-dose)	
Telemetry		X	X			Day -1: at least 4 h Pre-dose (Day 1) to 24 h post-dose (Day 2)
Height, weight and BMI	X	X				
Clinical laboratory evaluations	X	X	X	X	X	Day 2: 24 h post-dose Day 3: 48 h post-dose Day 4: 72 h post-dose All samples will be collected after a 10 h fasting period. If the assessment period is extended, Day 4 assessments will be performed on the day the healthy volunteer are discharged
Pregnancy testing	X (serum)	X (urine)			X (serum)	Females only
Physical examination	X	X (brief)	X (brief)	X (brief)	X	Day 3: 48 h post-dose Day 4: 72 h post-dose
Pharmacokinetics:						

Pharmacokinetic blood sampling			X [d]	X [d]	X [e]	**Cohort 1:** Day 1: Pre-dose and 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h and 12 h after start of dosing Day 2: 24 h, 30 h and 36 h post-dose Day 3: 48 h and 60 h post-dose. **Cohorts 2-3:** Day 1: Pre-dose, as soon as possible after end of inhalation, 15 min after end of inhalation, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h and 12 h post-dose Day 2: 24 h, 30 h and 36 h post-dose **Cohorts 4:** Day 1: Pre-dose, as soon as possible after end of inhalation, 15 min after end of inhalation, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h and 12 h post-dose Day 2: 24 h, 30 h and 36 h post-dose Day 3: 48 h **Cohorts 5:** Day 1: Pre-dose, as soon as possible after end of inhalation, 15 min after end of inhalation, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h and 12 h post-dose Day 2: 24 h, 30 h and 36 h post-dose Day 3: 48 h post-dose Day 4: 72 h post-dose **Cohorts 6**: Day 1: Pre-dose, as soon as possible after end of inhalation, 15 min after end of inhalation, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h and 12 h post-dose Day 2: 24 h, 30 h and 36 h post-dose Day 3: 48 h post-dose Day 4: 72 h post-dose Follow-up Visit (single sample)

Table 3.2-1 Schedule of Assessments Part 1a (SAD)

Assessments	Screening	Treatment Period			Follow-up Visit	Comments
	Days -28 to -2	Day -1	Days 1 to 3 [a]	Day 4 [a]	(6±1 days post-dose)	
Exploratory Analyses:						
CCI			X			6 h and 24 h post-dose
CCI			X			Day 1: directly after dosing
CCI			X			Day 1: only if healthy volunteer agrees by signing a separate informed consent form
CCI		X [c, d]	X [c, d]			Day -1: single sample Day 1: 30 min, 1 h and 3 h post-dose
CCI		X [c, d]	X [c, d]			Day -1: single sample Day 1: 30 min, 1 h and 3 h post-dose
CCI		X			X	Day -1: single sample Follow-up Visit: single sample

BMI: Body mass index; dECG: Digital electrocardiogram; ECG: Electrocardiogram; CCI SAE: Serious adverse event; CCI TB: Tuberculosis

a) Healthy volunteers in cohort 1 will be discharged from the Clinical Unit after all samples have been collected and assessments have been performed on Day 4. Depending on the emerging data in cohort 1, the collection period may be extended to 96 hours post-dose, for cohort 2 onwards, in this case volunteers will only be discharged on Day 5.

b) The time-points for all procedures are relative to the start of the inhalation unless stated otherwise.

c) Only applicable to cohort 5 onwards.

d) Time-points may be updated based on emerging data.

e) For cohort 6 as indicated in the "Comments" column.

Table 3.2-2 Schedule of Assessments Part 1b (First IV Cohort)

Assessments	Screening [a]	Treatment Period			Follow-up Visit	Comments
	Days -28 to -2	Day -1	Days 1 to 2 [b]	Day 3 [b]	(6±1 days post-dose)	
Informed consent	X					Includes optional genetic consent
Inclusion/exclusion criteria	X	X				
Demographic data	X					
Medical history	X					
Drug, alcohol and cotinine screen	X	X				
Serology	X					
QuantiFERON® TB	X					
Follicle-stimulating hormone testing	X					Post-menopausal females only (see Section 5.2.3.2 for definition)
Study Residency:						
Admission		X				
Discharge				X		At least 60 h post-dose, may be extended up to 96 h post-dose depending on emerging data
Non-residential visit	X				X	
Investigational Medicinal Product Administration:						
AZD0449 IV administration [c]			Day 1 (0 hours)			60 min infusion duration
Safety and Tolerability:						
Adverse event questioning	Only SAEs	Only SAEs	X	X	X	
Spirometry	X	X	X	X	X	Day 1: Pre-dose and 1 h, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 3: 48 h post-dose
Pulse Oximetry	X	X	X	X	X	Day 1: Pre-dose and 30 min, 1 h, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 3: 48 h post-dose

Table 3.2-2 Schedule of Assessments Part 1b (First IV Cohort)

Assessments	Screening [a]	Treatment Period			Follow-up Visit	Comments
	Days -28 to -2	Day -1	Days 1 to 2 [b]	Day 3 [b]	(6±1 days post-dose)	
Blood pressure, pulse and respiratory rate (supine) and body temperature	X	X	X	X	X	Day 1: Pre-dose and 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h post-dose Day 2: 24 h post-dose Day 3: 48 h post-dose
12-lead dECG			X	X		See Table 3.2-9
12-lead safety ECG	X	X	X	X	X	12-lead safety ECG will be collected at the start of each dECG extraction window when time-points coincide, see Table 3.2-9
Telemetry		X	X			Day -1: at least 4 h Pre-dose (Day 1) to 24 h post-dose (Day 2)
Height, weight and BMI	X	X				
Clinical laboratory evaluations	X	X	X	X	X	Day 2: 24 h post-dose Day 3: 48 h post-dose All samples will be collected after a 10 h fasting period. If the assessment period is extended, Day 3 assessments will be performed on the day healthy volunteers are discharged
Pregnancy testing	X (serum)	X (urine)			X (serum)	Females only
Physical examination	X	X (brief)		X (brief)	X	Day 3: 48 h post-dose
Pharmacokinetics:						
Pharmacokinetic blood sampling			X	X		Day 1: Pre-dose and at 15, 30, 45 min after infusion start, **directly after the end of infusion (ca. 60 min),** 5, 10 and, 15 min after end of infusion, and 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 14 h, and 16 h after infusion start Day 2: 24 h, 36 h and 48 h after infusion start
Optional pharmacogenetic sample			X			Day 1: Only if healthy volunteer agrees by signing a separate informed consent form and has not participated in Part 1a of the study

Table 3.2-2 Schedule of Assessments Part 1b (First IV Cohort)

Assessments	Screening [a]	Treatment Period			Follow-up Visit	Comments
	Days -28 to -2	Day -1	Days 1 to 2 [b]	Day 3 [b]	(6±1 days post-dose)	
Exploratory Analyses:						
CCI [redacted]		X			X	Day -1: Single sample Follow-up Visit: Single sample

BMI: Body mass index; dECG: Digital electrocardiogram; ECG: Electrocardiogram; CCI [redacted] IV: Intravenous; SAE: Serious adverse event; CCI [redacted] TB: Tuberculosis

a) Screening only applicable if healthy volunteer did not participate in Part 1a of the study.
b) Healthy volunteers will be discharged from the Clinical Unit after all samples have been collected and assessments have been performed on Day 3.
c) Two separate catheters/lines must be used for sampling and IV infusion to avoid sample contamination. The time-points for all procedures are relative to the start of the infusion.

Table 3.2-3 Schedule of Assessments Part 1b (Second IV Cohort)

Assessments	Screening [a]	Treatment Period				Follow-up Visit	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Days 1 to 2 [b]	Day 3 [b]	(6±1 days post-dose)	
Informed consent	X						Includes optional genetic consent
Inclusion/exclusion criteria	X		X				
Demographic data	X						
Medical history	X						
Drug, alcohol and cotinine screen	X	X					
Serology	X						
COVID-19 serology testing [d)]	X					X	
COVID-19 PCR testing [d)]	X	X					
QuantiFERON® TB	X						
Follicle-stimulating hormone testing	X						Post-menopausal females only (see Section 5.2.3.2 for definition)
Study Residency:							
Admission		X					
Discharge					X		At least 60 h post-dose, may be extended up to 96 h post-dose depending on emerging data
Non-residential visit	X					X	
Investigational Medicinal Product Administration:							
AZD0449 IV administration [c]				Day 1 (0 hours)			48 min infusion duration
Safety and Tolerability:							
Adverse event questioning	Only SAEs	Only SAEs	Only SAEs	X	X	X	

Table 3.2-3 Schedule of Assessments Part 1b (Second IV Cohort)

Assessments	Screening [a]	Treatment Period				Follow-up Visit	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Days 1 to 2 [b]	Day 3 [b]	(6±1 days post-dose)	
Spirometry	X		X	X	X		Day 1: Pre-dose and 1 h, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 3: 48 h post-dose
Pulse Oximetry	X		X	X	X	X	Day 1: Pre-dose and 30 min, 1 h, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 3: 48 h post-dose
Blood pressure, pulse and respiratory rate (supine) and body temperature	X		X	X	X	X	Day 1: Pre-dose and 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h post-dose Day 2: 24 h post-dose Day 3: 48 h post-dose
Body temperature		X					
12-lead dECG				X	X		See Table 3.2-9
12-lead safety ECG	X		X	X	X	X	12-lead safety ECG will be collected at the start of each dECG extraction window when time-points coincide, see Table 3.2-9
Telemetry			X	X			Day -1: at least 4 h Pre-dose (Day 1) to 24 h post-dose (Day 2)
Height, weight and BMI	X		X			X	Height to be measured at screening only
Clinical laboratory evaluations	X	X		X	X	X	Day 2: 24 h post-dose Day 3: 48 h post-dose All samples will be collected after a 10 h fasting period. If the assessment period is extended, Day 3 assessments will be performed on the day volunteers are discharged

Table 3.2-3 Schedule of Assessments Part 1b (Second IV Cohort)

Assessments	Screening [a]	Treatment Period				Follow-up Visit	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Days 1 to 2 [b]	Day 3 [b]	(6±1 days post-dose)	
Pregnancy testing	X (serum)	X (urine)				X (serum)	Females only
Physical examination	X		X (brief)		X (brief)	X	Day 3: 48 h post-dose
Pharmacokinetics:							
Pharmacokinetic blood sampling				X	X		Day 1: Predose and at 12, 24, 36 min after infusion start, **directly after the end of infusion (ca. 48 min),** 5, 10 and, 15 min after end of infusion, and 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 14 h, and 16 h after infusion start Day 2: 24 h and 36 h after infusion start Day 3: 48 h after infusion start
Optional pharmacogenetic sample				X			Day 1: Only if healthy volunteer agrees by signing a separate informed consent form and has not participated in Part 1a of the study
Exploratory Analyses:							
CCI [redacted]			X			X	Day -1: Single sample Follow-up Visit: Single sample

BMI: Body mass index; dECG: Digital electrocardiogram; ECG: Electrocardiogram; CCI [redacted] IV: Intravenous; PCR: Polymerase Chain Reaction; SAE: Serious adverse event; TB: Tuberculosis

a) Screening only applicable if volunteer did not participate in Part 1a of the study.

b) Healthy volunteers will be discharged from the Clinical Unit after all samples have been collected and assessments have been performed on Day 3.

c) Two separate catheters/lines must be used for sampling and IV infusion to avoid sample contamination. The time-points for all procedures are relative to the start of the infusion.

d) A sample for COVID-19 serology testing will be collected at screening and at the Follow-up visit. A swab sample for PCR will be collected at screening only for volunteers who are IgM negative and IgG positive. PCR will be performed for all volunteers prior to admission to the Clinical Unit; further samples for PCR and/or serology testing will be collected at the discretion of the Investigator. In the event of reduced COVID-19 sample analysis capacity sites may initiate residency visits

from Day -3 if required. Ad hoc nasal and/or throat-swab specimen is to be collected for the identification of a suspected respiratory infection during any visit. Healthy volunteers who test positive for having active COVID-19 infection will be discontinued from the study and followed up until the final outcome of the AE.

Table 3.2-4 Schedule of Assessments Part 2a in patients with mild asthma

Assessments	Screening	Treatment Period					Follow-up	Comments
	Day -28 to Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing) [a] to Day 12 (last dosing day) [a]	Day 13 to 15	Day 22±1 (10±1 days post-last dose)	
Informed consent	X							Includes optional genetic consent
Inclusion/exclusion criteria	X		X	X				
Demographic data	X							
Medical history	X							
Drug, alcohol and cotinine screen	X	X						
Serology	X							
QuantiFERON® TB	X							
Follicle-stimulating hormone testing	X							Post-menopausal females only (see Section 5.2.3.2 for definition)
Study Residency:								
Admission		X						
Discharge						X		Discharge on Day 14
Non-residential visit	X					X	X	Only Day 15 is non-residential
Investigational Medicinal Product Administration:								
Inhaler Nebulizer Training			X					
Randomization				X				Day 1
AZD0449/Placebo administration via Nebulizer [a]				X [b]	X [b]			First dose on Day 1, second dose on Day 3 (48 hours post-dose), then once daily dosing from Day 4 to Day 12
Safety and Tolerability:								
Adverse event questioning	Only SAEs	Only SAEs	Only SAEs	X	X	X	X	

Table 3.2-4 Schedule of Assessments Part 2a in patients with mild asthma

Assessments	Screening	Treatment Period					Follow-up	Comments
	Day -28 to Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a]	Day 13 to 15	Day 22±1 (10±1 days post-last dose)	
Spirometry	X		X[c)]	X[c)]	X[c)]	X[c)]	X[c)]	Day -1: pre-dose, 6 h, 12 h (corresponding clock time) Day 1, Day 8 and Day 12: Pre-dose, 30 min, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 3 to 7 and Day 9 to 11: pre-dose and 30 min post-dose Day 13: 24 h post-dose, Day 14: 47 h post-dose, Day 15: 71 h post-dose, and Day 22: 239 h post-dose
Pulse oximetry	X		X	X	X	X	X	Day -1: pre-dose, 3 h and 12 h post-dose (corresponding clock time) Day 1 and Day 3 to Day 12: Pre-dose, 1 h, 1.5 h, 3 h, 6 h, 12 h -post-dose Day 2: 24 h post-dose Day 13: 24 h post-dose Day 14: 47 h post-dose, Day 15: 71 h post-dose, and Day 22: 239 h post-dose
Blood pressure, pulse and respiratory rate (supine) and body temperature	X		X	X	X	X	X	Day 1 (first dose) and Day 12 (last dose):Pre-dose and 1 h, 2 h and 6 h post-dose Day 2: 24 h post-dose Days 3 to 11: pre-dose Day 13: 24 h post-dose

Table 3.2-4 Schedule of Assessments Part 2a in patients with mild asthma

Assessments	Screening	Treatment Period					Follow-up	Comments
	Day -28 to Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a]	Day 13 to 15	Day 22±1 (10±1 days post-last dose)	
12-lead dECG				X	X	X		See Table 3.2-10
12-lead safety ECG	X		X	X	X	X	X	12-lead safety ECG will be collected at the start of each dECG extraction window when time-points coincide see Table 3.2-10
Telemetry			X	X	X			Day -1: at least 4 h Pre-dose (Day 1) to 24 h post-dose (Day 2) Pre-dose (Day 12) to 24 h post-dose (Day 13)
Height, weight and BMI	X		X					
Clinical laboratory evaluations	X	X		X	X	X	X	Day 2: 24 h post-dose Day 3: pre-dose Day 13: 24 h post-dose All samples will be collected after a 10 h fasting period
Pregnancy testing	X (serum)	X (urine)					X (serum)	Females only
Physical examination	X		X	X (brief)	X (brief)	X (brief)	X	Day 1: pre-dose Day 2: 24 h after the first dose Day 6: pre-dose Day 10: pre-dose Day 13: 24 h post-dose

Table 3.2-4 Schedule of Assessments Part 2a in patients with mild asthma

Assessments	Screening	Treatment Period					Follow-up	Comments
	Day -28 to Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing) [a] to Day 12 (last dosing day) [a]	Day 13 to 15	Day 22±1 (10±1 days post-last dose)	
Pharmacokinetics:								
Pharmacokinetic blood sampling				X [c)]	X [c)]	X [c)]	X [c)]	Day 1 and 12: pre-dose, as soon as possible after end of inhalation, 15 min after end of inhalation, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h [d)], and 12 h post-dose Day 2: 24 h, 30 h, and 36 h post-dose. Day 3 – Day 11: pre-dose Day 13: 24 h, 30 h, and 36 h post-last dose Day 14: 48 h post-last dose Day 15: 72 h post-last dose Day 22: 240 h post-last dose

Table 3.2-4 Schedule of Assessments Part 2a in patients with mild asthma

Assessments	Screening	Treatment Period					Follow-up	Comments
	Day -28 to Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing) [a] to Day 12 (last dosing day) [a]	Day 13 to 15	Day 22±1 (10±1 days post-last dose)	
Pharmacodynamics:								
Fractional exhaled nitric oxide	X		X [c)]	X [c)]	X [c)]	X [c)]	X [c)]	Single assessment at Screening Visit Day -1: (corresponding clock time) to pre-dose, 30 min and 2 h, 6 h and 12 h post-dose Days 1, 2, 3, 4, 6, 8, 10 and 12: Pre-dose (corresponding clock time), 30 min and 2 h, 6 h and 12 h post-dose Days 5, 7, 9 and 11: Pre-dose (corresponding clock time) and 2 h post-dose Day 13: 24 h and 26 h post-dose Day 14: 47 h and 50 h post-dose, Day 15: 71 h post-dose and Day 22: 239 h post-dose
Exploratory Analyses:								
CCI [redacted]	X		X		X	X		Single assessment at Screening Visit Day -1: pre-dose and 6 h post-dose (corresponding clock time) Days 8 and 12: pre-dose and 6 h post-dose Day 13: 24 h and 26 h post-dose
CCI [redacted]					X			Day 12: 2 h, 4 h, 8 h and 12 h post-last dose
CCI [redacted]			X			X	X	Day 13 and Day 22

Table 3.2-4 Schedule of Assessments Part 2a in patients with mild asthma

Assessments	Screening	Treatment Period					Follow-up	Comments
	Day -28 to Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing) [a] to Day 12 (last dosing day) [a]	Day 13 to 15	Day 22±1 (10±1 days post-last dose)	
CCI [redacted]				X	X		X	Day 1 (pre-dose), Day 12 (pre-dose) and Day 22 single sampling
CCI [redacted]				X				Day 1: only if patient agrees by signing a separate informed consent form
CCI [redacted]				X	X			Day 1: Pre-dose Day 12 (last dosing day): Pre-dose

BMI: Body mass index; dECG: Digital electrocardiogram; ECG: Electrocardiogram; CCI [redacted] SAE: Serious adverse event; TB: tuberculosis

a) The time-points for all procedures are relative to the start of the inhalation.
b) Single dose on Day 1 and once daily dosing on Day 3 – Day 12.
c) Time-points for assessments and sample collection may change based on emerging data. The Day 3 pre-dose blood sampling corresponds to 48 h post-Day 1 dose.
d) Only applicable to cohort 2 (Day 12 dosing only).

Table 3.2-5 Schedule of Assessments Part 2b in healthy volunteers

Assessments	Screening	Treatment Period					Safety Monitoring Period[e]	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a)]	Day 13 to 16	Day 17 to 27	
Informed consent	X							Includes optional genetic consent
Inclusion/exclusion criteria	X		X	X				
Demographic data	X							
Medical history	X							
Drug, alcohol and cotinine screen	X	X						
Serology	X							
COVID-19 serology testing[d)]	X						X	Day 27
COVID-19 PCR testing[d)]	X	X						
QuantiFERON® TB	X							
Follicle-stimulating hormone testing	X							Post-menopausal females only (see Section 5.2.3.2 for definition)
Study Residency:								
Admission		X						
Discharge							X	Discharge on Day 27
Non-residential visit	X							
Investigational Medicinal Product Administration:								
Inhaler Nebulizer Training			X					
Randomization				X				Day 1
AZD0449/Placebo administration via Nebulizer[a)]				X[b)]	X[b)]			First dose on Day 1, second dose on Day 3 (48 hours post-dose), then once daily dosing from Day 4 to Day 12

Table 3.2-5 Schedule of Assessments Part 2b in healthy volunteers

Assessments	Screening	Treatment Period					Safety Monitoring Period[e]	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a)]	Day 13 to 16	Day 17 to 27	
Safety and Tolerability:								
Adverse event questioning	Only SAEs	Only SAEs	Only SAEs	X	X	X	X	
Spirometry	X		X[c)]	X[c)]	X[c)]	X[c)]	X[c)]	Day -1: pre-dose, 6 h, 12 h (corresponding clock time) Day 1, Day 8 and Day 12: Pre-dose 30 min, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 3 to 7 and Day 9 to 11: pre-dose and 30 min post-dose Day 13: 24 h post-dose, Day 14: 47 h post-dose, Day 15: 71 h post-dose, Day 16: 95 h post-dose, Day 17: 120 h post-dose, Day 20: 192 h post-dose, Day 23: 264 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose

Table 3.2-5 Schedule of Assessments Part 2b in healthy volunteers

Assessments	Screening	Treatment Period					Safety Monitoring Period[e]	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a)]	Day 13 to 16	Day 17 to 27	
Pulse oximetry	X		X	X	X	X	X	Day 1 and Day 3 to Day 12: Pre-dose, 1 h, 1.5 h, 3 h, 6 h, 12 h -post-dose Day 2: 24 h post-dose Day 13: 24 h post-dose, Day 14: 48 h post-dose, Day 15: 72 h post-dose, Day 16: 96 h post-dose, Day 17: 120 h post-dose, Day 18: 144 h post-dose, Day 19: 168 h post-dose, Day 20: 192 h post-dose, Day 21: 216 h post-dose, Day 22: 240 h post-dose, Day 23: 264 h post-dose, Day 24: 288 h post-dose, Day 25: 312 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose

Table 3.2-5 Schedule of Assessments Part 2b in healthy volunteers

Assessments	Screening	Treatment Period					Safety Monitoring Period[e]	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a)]	Day 13 to 16	Day 17 to 27	
Blood pressure, pulse and respiratory rate (supine) and body temperature	X		X	X	X	X	X	Day 1 (first dose) and Day 12 (last dose): Pre-dose and 1 h, 2 h and 6 h post-dose Day 2: 24 h post-dose Days 3 to 11: Pre-dose Day 13: 24 h post-dose, Day 14: 48 h post-dose, Day 15: 72 h post-dose, Day 16: 96 h post-dose, Day 17: 120 h post-dose, Day 18: 144 h post-dose, Day 19: 168 h post-dose, Day 20: 192 h post-dose, Day 21: 216 h post-dose, Day 22: 240 h post-dose, Day 23: 264 h post-dose, Day 24: 288 h post-dose, Day 25: 312 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose
Body temperature		X						
12-lead dECG				X	X	X		See Table 3.2-10
12-lead safety ECG	X		X	X	X	X	X	Day 27 12-lead safety ECG will be collected at the start of each dECG extraction window when time-points coincide see Table 3.2-10

Table 3.2-5 Schedule of Assessments Part 2b in healthy volunteers

Assessments	Screening	Treatment Period					Safety Monitoring Period[e]	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a)]	Day 13 to 16	Day 17 to 27	
Telemetry			X	X	X	X		Day -1: at least 4 h Pre-dose (Day 1) to 24 h post-dose (Day 2) Pre-dose (Day 12) to 24 h post-dose (Day 13)
Height, weight and BMI	X		X				X	Day 27 Height to be measured at screening only
Clinical laboratory evaluations	X	X		X	X	X	X	Day 2: 24 h post-dose Day 3: pre-dose Day 13: 24 h post-dose Day 27: 360 h post-last dose All samples will be collected after a 10 h fasting period
Pregnancy testing	X (serum)	X (urine)					X (serum)	Day 27 Females only
Physical examination	X		X	X (brief)	X (brief)	X (brief)	X (brief)	Day 1: pre-dose Day 2: 24 h after the first dose Day 6: pre-dose Day 10: pre-dose Day 14: 48 h post-dose, Day 17: 120 h post-dose, Day 20: 192 h post-dose, Day 23: 264 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose (full examination)

Table 3.2-5 Schedule of Assessments Part 2b in healthy volunteers

Assessments	Screening	Treatment Period					Safety Monitoring Period[e]	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a)]	Day 13 to 16	Day 17 to 27	
Pharmacokinetics:								
Pharmacokinetic blood sampling				X	X	X	X	Day 1 and 12: Pre-dose, as soon as possible after end of inhalation, 15 min after end of inhalation, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, and 12 h post-dose Day 2: 24 h, 30 h, and 36 h post-dose. Day 3 to Day 11: Pre-dose Day 13: 24 h, 30 h, and 36 h post-last dose Day 14: 48 h, 54 h, and 60 h post-last dose Day 15: 72 h, 78 h, and 84 h post-last dose Day 16: 96 h, 102 h, and 108 h post-last dose Day 17: 120 h post-last dose, Day 20: 192 h post-last dose, Day 23: 264 h post-last dose, Day 26: 336 h post-last dose, and Day 27: 360 h post-last dose
Exploratory Analyses:								
CCI [redacted]					X			Day 12: 2 h, 4 h, 8 h and 12 h post-last dose
CCI [redacted]				X	X			Day 1: pre-dose, Day 12: 3-8 h post-last dose

Table 3.2-5 Schedule of Assessments Part 2b in healthy volunteers

Assessments	Screening	Treatment Period					Safety Monitoring Period[e]	Comments
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (start of once daily dosing)[a] to Day 12 (last dosing day)[a)]	Day 13 to 16	Day 17 to 27	
CCI [redacted]			X			X	X	Day 13 and Day 27
CCI [redacted]				X				Day 1: only if patient agrees by signing a separate informed consent form
CCI [redacted]				X		X		Day 1: Pre-dose Day 12 (last dosing day): Pre-dose

BMI: Body mass index; dECG: Digital electrocardiogram; ECG: Electrocardiogram; CCI [redacted] PCR: Polymerase Chain Reaction; SAE: Serious adverse event; TB: tuberculosis

a) The time-points for all procedures are relative to the start of the inhalation.

b) Single dose on Day 1 and once daily dosing on Day 3 – Day 12.

c) Time-points for assessments and sample collection may change based on emerging data. The Day 3 pre-dose blood sampling corresponds to 48 h post-Day 1 dose.

d) A sample for COVID-19 serology testing will be collected at screening and at the discharge visit. A swab sample for PCR will be collected at screening only for volunteers who are IgM negative and IgG positive. PCR will be performed for all volunteers prior to admission to the Clinical Unit; further samples for PCR and/or serology testing will be collected at the discretion of the Investigator. In the event of reduced COVID-19 sample analysis capacity sites may initiate residency visits from Day -3 if required. Ad hoc nasal and/or throat-swab specimen is to be collected for the identification of a suspected respiratory infection during any visit. Healthy volunteers who test positive for having active COVID-19 infection will be discontinued from the study and followed up until the final outcome of the AE.

e) Volunteers will remain in house for the duration of the safety monitoring period and will be discharged from the Clinical Unit on Day 27, 15 days after the last dose administration. If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the extended safety monitoring period may be conducted as non-residential visits.

Table 3.2-6 Schedule of Assessments Part 3a in patients with mild asthma

Assessments	Screening	Treatment Period						Safety Monitoring Period[e]	Comments
	Day -28 to Day -4	Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Informed consent	X								Includes optional genetic consent
Inclusion/exclusion criteria	X			X	X				
Demographic data	X								
Medical history	X								
Drug, alcohol and cotinine screen	X	X							
Serology	X								
COVID-19 serology testing [d]	X							X	Day 27
COVID-19 PCR testing [d]	X	X							
QuantiFERON® TB	X								
Follicle-stimulating hormone testing	X								Post-menopausal females only (see Section 5.2.3.2 for definition)
Study Residency:									
Admission		X							
Discharge								X	Discharge on Day 27
Non-residential visit	X							X	Day 17 to Day 27 (If permitted by local relevant regulatory authorities and considered feasible and safe)

Table 3.2-6 Schedule of Assessments Part 3a in patients with mild asthma

Assessments	Screening	Treatment Period						Safety Monitoring Period[e]	Comments
	Day -28 to Day -4	Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Investigational Medicinal Product									
DPI Training				X					
Randomization					X				Day 1
AZD0449/Placebo administration via DPI[a)]					X[b)]	X[b)]			First dose on Day 1, second dose on Day 3 (48 hours post-dose), then once daily dosing from Day 4 to Day 12
Safety and Tolerability:									
Adverse event questioning	Only SAEs	Only SAEs	Only SAEs	Only SAEs	X	X	X	X	
Spirometry	X			X[c)]	X[c)]	X[c)]	X[c)]	X[c)]	Day -1: pre-dose, 6 h, 12 h (corresponding clock time) Day 1, Day 8 and Day 12: Pre-dose, 30 min, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 3 to 7 and Day 9 to 11: pre-dose and 30 min post-dose Day 13: 24 h post-dose, Day 14: 47 h post-dose, Day 15: 71 h post-dose, Day 16: 95 h post-dose, Day 17: 120 h post-dose, Day 20: 192 h post-dose, Day 23: 264 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose

Table 3.2-6 Schedule of Assessments Part 3a in patients with mild asthma

Assessments	Screening	Treatment Period						Safety Monitoring Period[e]	Comments
	Day -28 to Day -4	Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Pulse oximetry	X			X	X	X	X	X	Day -1: pre-dose, 3 h and 12 h post-dose (corresponding clock time) Day 1 and Day 3 to Day 12: Pre-dose, 1 h, 1.5 h, 3 h, 6 h, 12 h -post-dose Day 2: 24 h post-dose Days 13: 24 h post-dose, Day 14: 48 h post-dose, Day 15: 72 h post-dose, Day 16: 96 h post-dose, Day 17: 120 h post-dose, Day 18: 144 h post-dose, Day 19: 168 h post-dose, Day 20: 192 h post-dose, Day 21: 216 h post-dose, Day 22: 240 h post-dose, Day 23: 264 h post-dose, Day 24: 288 h post-dose, Day 25: 312 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose

Table 3.2-6 Schedule of Assessments Part 3a in patients with mild asthma

Assessments	Screening	Treatment Period						Safety Monitoring Period[e]	Comments
	Day -28 to Day -4	Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Blood pressure, pulse and respiratory rate (supine)and body temperature	X			X	X	X	X	X	Day 1 (first dose) and Day 12 (last dose): Pre-dose and 1 h, 2 h and 6 h post-dose Day 2: 24 h post-dose Days 3 to 11: Pre-dose Day 13: 24 h post-dose, Day 14: 48 h post-dose, Day 15: 72 h post-dose, Day 16: 96 h post-dose, Day 17: 120 h post-dose, Day 18: 144 h post-dose, Day 19: 168 h post-dose, Day 20: 192 h post-dose, Day 21: 216 h post-dose, Day 22: 240 h post-dose, Day 23: 264 h post-dose, Day 24: 288 h post-dose, Day 25: 312 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose
Body temperature		X	X						
12-lead dECG					X	X	X		See Table 3.2-10
12-lead safety ECG	X			X	X	X	X	X	Day 27 12-lead safety ECG will be collected at the start of each dECG extraction window when time-points coincide, see Table 3.2-10

Table 3.2-6 Schedule of Assessments Part 3a in patients with mild asthma

Assessments	Screening	Treatment Period						Safety Monitoring Period[e]	Comments
	Day -28 to Day -4	Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Telemetry				X	X	X	X		Day -1: at least 4 h Pre-dose (Day 1) to 24 h post-dose (Day 2) Pre-dose (Day 12) to 24 h post-dose (Day 13)
Height, weight and BMI	X			X				X	Day 27 Height to be measured at screening only
Clinical laboratory evaluations	X	X			X	X	X	X	Day 2: 24 h post-dose Day 3: pre-dose Day 13: 24 h post-dose Day 27: 360 h post-last dose All samples will be collected after a 10 h fasting period
Pregnancy testing	X (serum)	X (urine)						X (serum)	Day 27 Females only
Physical examination	X			X	X (brief)	X (brief)	X (brief)	X (brief)	Day 1: pre-dose Day 2: 24 h after the first dose Day 6: pre-dose Day 10: pre-dose Day 14: 48 h post-dose, Day 17: 120 h post-dose, Day 20: 192 h post-dose, Day 23: 264 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose (full examination)

Table 3.2-6 Schedule of Assessments Part 3a in patients with mild asthma

Assessments	Screening	Treatment Period						Safety Monitoring Period[e]	Comments
	Day -28 to Day -4	Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Pharmacokinetics:									
Pharmacokinetic blood sampling					X[c]	X[c]	X[c]	X[c]	Days 1 and 12: pre-dose, as soon as possible after end of inhalation, 15 min after end of inhalation, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, and 12 h post-dose Day 2: 24 h, 30 h, and 36 h post-dose. Day 3 to Day 11: pre-dose Day 13: 24 h, 30 h, and 36 h post-last dose Day 14: 48 h, 54 h, and 60 h post-last dose Day 15: 72 h, 78 h, and 84 h post-last dose Day 16: 96 h, 102 h, and 108 h post-last dose Day 17: 120 h post-last dose, Day 20: 192 h post-last dose, Day 23: 264 h post-last dose, Day 26: 336 h post-last dose, and Day 27: 360 h post-last dose
Pharmacodynamics:									

Table 3.2-6 Schedule of Assessments Part 3a in patients with mild asthma

Assessments	Screening	Treatment Period						Safety Monitoring Period[e]	Comments
	Day -28 to Day -4	Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Fractional exhaled nitric oxide	X			X	X	X	X	X	Single assessment at Screening Visit Day -1: corresponding clock times to pre-dose, 30 min and 2 h, 6 h, and 12 h post-dose Days 1, 2, 3, 4, 6, 8, 10, and 12: Pre-dose (corresponding clock time), 30 min and 2 h, 6 h, and 12 h post-dose Days 5, 7, 9, and 11: Pre-dose (corresponding clock time) and 2 h post-dose Day 13: 23 h, 24.5 h, 26 h, 30 h, and 36 h post-last dose Day 14: 47 h, 48.5 h, 50 h, 54 h, and 60 h post-last dose Day 15: 71 h, 72.5 h, 74 h, 78 h, and 84 h post-last dose Day 16: 95 h, 96.5 h, 98 h, 102 h, and 108 h post-last dose Day 17, 20, 23, and 26: 5 measurements of FeNO (pre-dose, 30 min, 2 , 6 h, and 12 h post-hypothetical dose)[f] Day 27: 2 measurements of FeNO (pre-dose and 2 h post-hypothetical dose)
Exploratory Analyses:									

Table 3.2-6 Schedule of Assessments Part 3a in patients with mild asthma

Assessments	Screening	Treatment Period						Safety Monitoring Period[e]	Comments
	Day -28 to Day -4	Day -3	Day -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
CCI						X			Day 12: 2 h, 4 h, 8 h, and 12 h post-last dose
CCI				X			X	X	Day 13 and Day 27
CCI					X	X		X	Day 1 (pre-dose), Day 12 (pre-dose) and Day 27 single sampling
CCI				X		X			Day -1: single sample Day 12 (last dose): 30 min, 1 h and 3 h post-dose
CCI				X		X			Day -1 single sample Day 12 (last dose): 30 min, 1 h and 3 h post-dose
CCI					X				Directly after first dose
CCI					X				Day 1: only if patient agrees by signing a separate ICF
CCI					X	X			Day 1: pre-dose Day 12 (last dosing day): pre-dose

BMI: Body mass index; dECG: Digital electrocardiogram; DPI: Dry-powder inhaler; ECG: Electrocardiogram; CCI ICF: Informed consent form; CCI PCR: Polymerase Chain Reaction; SAE: Serious adverse event; CCI TB: tuberculosis

a) The time-points for all procedures are relative to the start of the inhalation.

b) Single dose on Day 1 and once daily dosing on Day 3 – Day 12.

c) Time-points for assessments and sample collection may change based on emerging data. The Day 3 pre-dose blood sampling corresponds to 48 h post-Day 1 dose.

d) A sample for COVID-19 serology testing will be collected at screening and at the discharge visit (or last non-residential visit). A swab sample for PCR will be collected at screening only for patients who are IgM negative and IgG positive. PCR will be performed for all patients at admission to the Clinical Unit; further samples for PCR and/or serology testing will be collected at the discretion of the Investigator. In the event of reduced COVID-19 sample analysis capacity sites may initiate residency visits from Day -3 if required. Ad hoc nasal and/or throat-swab specimen is to be collected for the identification of a suspected respiratory infection during any visit. Patients who test positive for having active COVID-19 infection will be discontinued from the study and followed up until the final outcome of the AE.

e) Patients will remain in house for the duration of the extended safety monitoring period and will be discharged from the Clinical Unit on Day 27, 15 days after the last dose administration. If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the extended safety monitoring period may be conducted as non-residential visits.

f) If it is permitted by local relevant regulatory authorities that the extended safety monitoring period may be conducted as non-residential visits, FeNO assessments will be performed at the following timepoints; Day 17: 120 h post-last dose, Day 20: 192 h post-last dose, Day 23: 264 h post-last dose, Day 26: 336 h post-last dose, and Day 27: 360 h post-last dose.

Table 3.2-7 Schedule of Assessments Part 3b in healthy volunteers

	Screening	Treatment Period					Safety Monitoring Period[e]	
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Informed consent	X							Includes optional genetic consent
Inclusion/exclusion criteria	X		X	X				
Demographic data	X							
Medical history	X							
Drug, alcohol and cotinine screen	X	X						
Serology	X							
COVID-19 serology testing [d)]	X						X	Day 27
COVID-19 PCR testing [d)]	X	X						
QuantiFERON® TB	X							
Follicle-stimulating hormone testing	X							Post-menopausal females only (see Section 5.2.3.2 for definition)
Study Residency:								
Admission		X						
Discharge							X	Discharge on Day 27
Non-residential visit	X						X	Day 17 to Day 27 (If permitted by local relevant regulatory authorities and considered feasible and safe)
Investigational Medicinal Product								

Table 3.2-7 Schedule of Assessments Part 3b in healthy volunteers

	Screening	Treatment Period					Safety Monitoring Period[e]	
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
DPI Training			X					
Randomization				X				
AZD0449/Placebo administration via DPI[a)]				X[b)]	X[b)]			First dose on Day 1, second dose on Day 3 (48 hours post-dose), then once daily dosing from Day 4 to Day 12
Safety and Tolerability:								
Adverse event questioning	Only SAEs	Only SAEs	Only SAEs	X	X	X	X	
Spirometry	X		X[c)]	X[c)]	X[c)]	X[c)]	X[c)]	Day -1: pre-dose, 6 h, 12 h (corresponding clock time) Day 1, Day 8 and Day 12: pre-dose, 30 min, 1.5 h, 3 h, 6 h and 12 h post-dose Day 2: 24 h post-dose Day 3 to 7 and Day 9 to 11: pre-dose and 30 min post-dose Day 13: 24 h post-dose, Day 14: 47 h post-dose, Day 15: 71 h post-dose, Day 16: 95 h post-dose, Day 17: 120 h post-dose, Day 20: 192 h post-dose, Day 23: 264 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose

Table 3.2-7 Schedule of Assessments Part 3b in healthy volunteers

	Screening	Treatment Period					Safety Monitoring Period[e]	
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Pulse oximetry	X		X	X	X	X	X	Day -1: pre-dose, 3 h and 12 h post-dose (corresponding clock time) Day 1 and Day 3 to Day 12: Pre-dose, 1 h, 1.5 h, 3 h, 6 h, 12 h -post-dose Day 2: 24 h post-dose Days 13: 24 h post-dose, Day 14: 48 h post-dose, Day 15: 72 h post-dose, Day 16: 96 h post-dose, Day 17: 120 h post-dose, Day 18: 144 h post-dose, Day 19: 168 h post-dose, Day 20: 192 h post-dose, Day 21: 216 h post-dose, Day 22: 240 h post-dose, Day 23: 264 h post-dose, Day 24: 288 h post-dose, Day 25: 312 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose

Table 3.2-7 Schedule of Assessments Part 3b in healthy volunteers

	Screening	Treatment Period					Safety Monitoring Period[e]	
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Blood pressure, pulse and respiratory rate (supine)and body temperature	X		X	X	X	X	X	Day 1 (first dose) and Day 12 (last dose): pre-dose and 1 h, 2 h and 6 h post-dose Day 2: 24 h post-dose Days 3 to 11: pre-Pre-dose Day 13: 24 h post-dose, Day 14: 48 h post-dose, Day 15: 72 h post-dose, Day 16: 96 h post-dose, Day 17: 120 h post-dose, Day 18: 144 h post-dose, Day 19: 168 h post-dose, Day 20: 192 h post-dose, Day 21: 216 h post-dose, Day 22: 240 h post-dose, Day 23: 264 h post-dose, Day 24: 288 h post-dose, Day 25: 312 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose
Body temperature		X	X					
12-lead dECG				X	X	X		See Table 3.2-10
12-lead safety ECG	X		X	X	X	X	X	Day 27 12-lead safety ECG will be collected at the start of each dECG extraction window when time-points coincide, see Table 3.2-10
Telemetry			X	X	X	X		Day -1: at least 4 h Pre-dose (Day 1) to 24 h post-dose (Day 2) Pre-dose (Day 12) to 24 h post-dose (Day 13)
Height, weight and BMI	X		X				X	Day 27 Height to be measured at screening only

Table 3.2-7 Schedule of Assessments Part 3b in healthy volunteers

	Screening	Treatment Period					Safety Monitoring Period[e]	
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Clinical laboratory evaluations	X	X		X	X	X	X	Day 2: 24 h post-dose Day 3: pre-dose Day 13: 24 h post-dose Day 27: 360 h post-last dose All samples will be collected after a 10 h fasting period
Pregnancy testing	X (serum)	X (urine)					X (serum)	Day 27 Females only
Physical examination	X		X	X (brief)	X (brief)	X (brief)	X (brief)	Day 1: pre-dose Day 2: 24 h after the first dose Day 6: pre-dose Day 10: pre-dose Day 14: 48 h post-dose, Day 17: 120 h post-dose, Day 20: 192 h post-dose, Day 23: 264 h post-dose, Day 26: 336 h post-dose, and Day 27: 360 h post-dose (full examination)

Table 3.2-7 Schedule of Assessments Part 3b in healthy volunteers

	Screening	Treatment Period					Safety Monitoring Period[e]	
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
Pharmacokinetics:								
Pharmacokinetic blood sampling				X [c]	X [c]	X [c]	X [c]	Days 1 and 12: pre-dose, as soon as possible after end of inhalation, 15 min after end of inhalation, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, and 12 h post-dose Day 2: 24 h, 30 h, and 36 h post-dose. Day 3 to Day 11: pre-dose Day 13: 24 h, 30 h, and 36 h post-last dose Day 14: 48 h, 54 h, and 60 h post-last dose Day 15: 72 h, 78 h, and 84 h post-last dose Day 16: 96 h, 102 h, and 108 h post-last dose Day 17: 120 h post-last dose, Day 20: 192 h post-last dose, Day 23: 264 h post-last dose, Day 26: 336 h post-last dose, and Day 27: 360 h post-last dose
Pharmacodynamics:								
Exploratory Analyses:								
CCI					X			Day 12: 2 h, 4 h, 8 h, and 12 h post-last dose
CCI			X			X	X	Day 13 and Day 27
CCI				X				Directly after first dose

Table 3.2-7 Schedule of Assessments Part 3b in healthy volunteers

	Screening	Treatment Period					Safety Monitoring Period[e]	
	Day -28 to Day -4 or -3	Day -3 to -2	Day -1	Day 1 and Day 2	Day 3 (second dosing day) to Day 12 (last dosing day)	Day 13 to 16	Day 17 to Day 27	
CCI [redacted]				X				Day 1: only if patient agrees by signing a separate ICF
CCI [redacted]				X	X			Day 1: Pre-dose Day 12 (last dosing day): Pre-dose

BMI: Body mass index; dECG: Digital electrocardiogram; ECG: Electrocardiogram; CCI [redacted] PCR: Polymerase Chain Reaction; SAE: Serious adverse event; TB: tuberculosis

a) The time-points for all procedures are relative to the start of the inhalation.

b) Single dose on Day 1 and once daily dosing on Day 3 – Day 12.

c) Time-points for assessments and sample collection may change based on emerging data. The Day 3 pre-dose blood sampling corresponds to 48 h post-Day 1 dose.

d) A sample for COVID-19 serology testing will be collected at screening and at the discharge visit (or last non-residential visit). A swab sample for PCR will be collected at screening only for volunteers who are IgM negative and IgG positive. PCR will be performed for all volunteers at admission to the Clinical Unit; further samples for PCR and/or serology testing will be collected at the discretion of the Investigator. In the event of reduced COVID-19 sample analysis capacity sites may initiate residency visits from Day -3 if required. Ad hoc nasal and/or throat-swab specimen is to be collected for the identification of a suspected respiratory infection during any visit. Healthy volunteers who test positive for having active COVID-19 infection will be discontinued from the study and followed up until the final outcome of the AE.

e) Volunteers will remain in house for the duration of the extended safety monitoring period and will be discharged from the Clinical Unit on Day 27, 15 days after the last dose administration. If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the extended safety monitoring period may be conducted as non-residential visits.

Table 3.2-8 Time Schedule for Digital Electrocardiogram Part 1a (SAD)

Study Days	ECG Number	Time-point	Start Time hour:min [a), b)]	Dose	Stop Time	dECG cont. [c), d), e)]	Other [f)]
1			-01:30		-01:00		Apply the electrodes [d)]
1			-00:40		-00:30		Rest in bed
1	1	Pre-dose	-00:30	Pre-dose	-00:20	10 minutes	
1			-00:20		-00:05		Toilet use recommended
1			00:00	Administration of AZD0449/placebo			
1	2	1 h	00:55		01:00	5 minutes [e)]	
1	3[g)]	2 h	01:55		02:00	5 minutes [e)]	
1	4	3 h	02:55		03:00	5 minutes [e)]	
1	5	4 h	03:55		04:00	5 minutes [e)]	
1	6 [h)]	5 h	04:55		05:00	5 minutes [e)]	
1	7	6 h	05:55		06:00	5 minutes [e)]	
1	8	8 h	07:55		08:00	5 minutes [e)]	
1	9 [h)]	10 h	09:55		10:00	5 minutes [e)]	
1	10	12 h	11:55		12:00	5 minutes [e)]	
2	11	24 h	23:55		24:00	5 minutes [e)]	
2	12	36 h	35:55		36:00	5 minutes [e)]	
3	13	48 h	47:55		48:00	5 minutes [e)]	
3	14 [h)]	60 h	59:55		60:00	5 minutes [e)]	

ECG: Electrocardiogram; dECG: Digital ECG; PK: Pharmacokinetics

a) Time-points for dECG may be adjusted according to emerging PK data.
b) Times are approximate as dECG and safety ECGs need to be completed before blood sampling.
c) The healthy volunteer must be in the same supine body position (max. 30 degrees flexion in the hip) at each time-point and at all visits. Healthy volunteer's feet should not contact the footboard of the bed.
d) Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied at least 30 minutes before first recording.
e) Healthy volunteer must rest in bed for at least 10 minutes before each ECG time-point.
f) Safety ECG will be collected at the start of each dECG extraction window.
g) For cohorts 1 to 4 only.
h) For cohorts 1 to 3 only.

Table 3.2-9 Time Schedule for Digital Electrocardiogram Part 1b (IV Cohorts)

Study Days	ECG Number	Time-point	Start Time hour:min[a) b), c)]	Dose	Stop Time	dECG cont. [c), d) e)]	Other [f)]
1			-01:30		-01:00		Apply the electrodes [d)]
1			-00:40		-00:30		Rest in bed
1	1	Pre-dose	-00:30	Pre-dose	-00:20	10 minutes	
1			-00:20		-00:05		Toilet use recommended
1			00:00	Infusion start			
1	2	5 min	00:05		00:10	5 minutes [e)]	After infusion start
1	3	30 min	00:25		00:30	5 minutes [e)]	
1	4	48 min [h)]	00:43		00:48	5 minutes [e)]	
1	5	1 h [g)]	00:55		01:00	5 minutes [e)]	
1	6	1.5 h	01:25		01:30	5 minutes [e)]	
1	7	2 h	01:55		02:00	5 minutes [e)]	
1	8	3 h	02:55		03:00	5 minutes [e)]	
1	9	4 h	03:55		04:00	5 minutes [e)]	
1	10	5 h	04:55		05:00	5 minutes [e)]	
1	11	6 h	05:55		06:00	5 minutes [e)]	
1	12	8 h	07:55		08:00	5 minutes [e)]	
1	13	10 h	09:55		10:00	5 minutes [e)]	
1	14	12 h	11:55		12:00	5 minutes [e)]	
2	15	24 h	23:55		24:00	5 minutes [e)]	
2	16	36 h	35:55		36:00	5 minutes [e)]	
3	17	48 h	47:55		48:00	5 minutes [e)]	

ECG: Electrocardiogram; dECG: Digital ECG; PK: Pharmacokinetics

a) Time-points for dECG may be adjusted according to emerging PK data.
b) Times are approximate as dECG and safety ECGs need to be completed before blood sampling.
c) The healthy volunteer must be in the same supine body position (max. 30 degrees flexion in the hip) at each timepoint and at all visits. Healthy volunteer's feet should not contact the footboard of the bed.
d) Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied at least 30 minutes before first recording.
e) Healthy volunteer must rest in bed for at least 10 minutes before each ECG timepoint.

f) Safety ECG will be collected at the start of each dECG extraction window.
g) For cohort 1 only.
h) For cohort 2 only.

Table 3.2-10 Time Schedule for Digital Electrocardiogram Part 2a/b, Part 3a/b

Study Days	ECG Number		Time-point	Start Time hour:min [a),b)]	Dose	Stop Time	dECG cont. [c),d),e)]	Other [f)]
1, 12				-01:30	Pre-dose	-01:00		Apply the electrodes [d)]
1, 12				-00:40		-00:30		Rest in bed
1, 12	1	12	Pre-dose	-00:30		-00:20	10 minutes	
1, 12				-00:20		-00:05		Toilet use recommended
1, 12				00:00	Administration of AZD0449/placebo			
1, 12	2	13	0.5 h [g)]	00:25		00:30	5 minutes [e)]	
1, 12	3	14	1 h	00:55		01:00	5 minutes [e)]	
1, 12	4	15	3 h	02:55		03:00	5 minutes [e)]	
1, 12	5	16	4 h	03:55		04:00	5 minutes [e)]	
1, 12	6	17	6 h	05:55		06:00	5 minutes [e)]	
1, 12	7	18	8 h	07:55		08:00	5 minutes [e)]	
1, 12	8	19	10 h [h)]	09:55		10:00	5 minutes [e)]	
1, 12	9	20	12 h	11:55		12:00	5 minutes [e)]	
2, 13	10	21	24 h	23:55		24:00	5 minutes [e)]	
2, 13	11	22	36 h	35:55		36:00	5 minutes [e)]	
14		23	48 h	47:55		48:00	5 minutes [e)]	

ECG: Electrocardiogram; dECG: Digital ECG; PK: Pharmacokinetics

a) Time-points for dECG may be adjusted according to emerging PK data.
b) Times are approximate as dECG and safety ECGs need to be completed before blood sampling.
c) The patient must be in the same supine body position (max. 30 degrees flexion in the hip) at each time-point and at all visits. Patient's feet should not contact the footboard of the bed.
d) Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied at least 30 minutes before first recording.
e) Patient must rest in bed for at least 10 minutes before each ECG time-point.
f) Safety ECG will be conducted at the start of each dECG extraction window.
g) Part 3a/b only.
h) Part 2 Cohort 1 only.

3.2.3. Order of Assessments

It is important that PK sampling occurs as close as possible to scheduled time. To achieve this, other assessments scheduled at the same time may be initiated before the time-point.

1. Electrocardiograms (ECGs)/dECGs preceded by 10 minutes supine rest, supine rest may be reduced accordingly for ECGs/dECGs within first hour of dosing.
2. Vital signs (systolic and diastolic BP, pulse, respiratory rate and body temperature) and pulse oximetry.
3. Pharmacokinetic blood sampling (will be drawn at the specified timepoint).
4. Exploratory blood samples CCI [redacted]
5. Fractional exhaled nitric oxide (only applicable to cohorts with patients with asthma in Part 2a and Part 3a).
6. CCI [redacted]
7. Spirometry.

Pre-dose assessments may be performed before dosing as specified in the separate window allowance document.

3.2.4. Total Blood Volume

The approximate total volume of blood that will be collected from each subject in this study, excluding repeat samples, is summarized in Table 3.2-11 (Part 1a [SAD]),Table 3.2-12 (Part 1b), Table 3.2-13 (Part 2a/b), and Table 3.2-14 (Part 3a/b).

Table 3.2-11 Total Blood Volume Part 1a

	Volume per Sample	Number of Samples	Total
Hematology	2 mL	6	12 mL
Clinical chemistry [a)]	5 mL	6	30 mL
QuantiFERON TB	4 mL	1	4 mL
Plasma glucose	2 mL	6	12 mL
PK AZD0449 (cohorts 1 – 4)	3 mL	15	45 mL
PK AZD0449 (cohort 5)	3 mL	16	48 mL
PK AZD0449 (cohort 6)	3 mL	18	54 mL
PK metabolites (exploratory)	2 mL	2	4 mL
CCI	4 mL	4	16 mL
CCI	2 mL	4	8 mL
CCI	0 mL	3	0 mL
CCI	10 mL	1	10 mL
Total (Cohorts 1 to 4)			**117 mL**
Total (Cohort 5)			**144 mL**
Total (Cohort 6)			**150 mL**

PK: Pharmacokinetics; TB: Tuberculosis

a) Serology tests at screening, pregnancy testing, follicle-stimulating hormone testing (female volunteers only) and Immunoglobulins will be performed on the clinical chemistry sample collected at the same timepoint.

Table 3.2-12 Total Blood Volume Part 1b

	Volume per Sample	Number of Samples	Total
Hematology	2 mL	5	10 mL
Clinical chemistry [a)]	5 mL	5	25 mL
QuantiFERON TB	4 mL	1	4 mL
Plasma glucose	2 mL	5	10 mL
PK AZD0449	3 mL	22	66 mL
CCI	0 mL	3	0 mL
CCI	10 mL	1	10 mL
COVID-19 serology (Part 1b Cohort 2)	1 mL	2	2 mL
Total (Cohort 1)			**125 mL**
Total (Cohort 2)			**127 mL**

COVID-19: Coronavirus disease 2019; PK: Pharmacokinetics; TB: Tuberculosis

a) Serology tests at screening, pregnancy testing, follicle-stimulating hormone testing (female volunteers only) and Immunoglobulins will be performed on the clinical chemistry sample collected at the same timepoint.

Table 3.2-13 Total Blood Volume Part 2a/b

	Volume per Sample	Number of Samples	Total
Hematology	2 mL	6	12 mL
Clinical chemistry [a)]	5 mL	6	30 mL
QuantiFERON TB	4 mL	1	4 mL
Plasma glucose	2 mL	6	12 mL
PK AZD0449 cohort 1 [b)]	3 mL	40	120 mL
PK AZD0449 cohort 2 [b)]	3 mL	41	123 mL
PK AZD0449 cohort 3 CCI [redacted]	4 mL	51	204 mL
CCI [redacted]	2 mL	4	8 mL
CCI [redacted]	2.5 mL	3	7.5 mL
CCI [redacted]	4 mL	3	12 mL
CCI [redacted]	2 mL	2	4 mL
CCI [redacted]	10 mL	1	10 mL
COVID-19 serology (Part 2b)	1 mL	2	2 mL
Total (Part 2a, Cohort 1)			**219.5 mL** [b)]
Total (Part 2a, Cohort 2)			**222.5 mL** [b)]
Total (Part 2b, Cohort 3)			**293.5 mL** [b)]

COVID-19: Coronavirus disease 2019; CCI [redacted]; PK: Pharmacokinetics; STAT: Signal transducer and activator of transcription; TB: Tuberculosis

a) Serology tests at screening, pregnancy testing, follicle-stimulating hormone testing (postmenopausal patients only) and Immunoglobulins will be performed on the clinical chemistry sample collected at the same timepoint.

b) If a cannula is used for PK blood samples, an additional 1 mL/sample will be drawn.

Table 3.2-14 Total Blood Volume Part 3a/b

	Volume per Sample	Number of Samples	Total
Hematology	2 mL 2.7 mL (Germany)	6	12 mL 16.2 mL (Germany)
Clinical chemistry [a]	5 mL 7.5 mL (Germany)	6	30 mL 45 mL (Germany)
QuantiFERON TB	4 mL	1	4 mL
Plasma glucose	2 mL	6	12 mL
PK AZD0449 and CCI	4 mL	51	204 mL
PK AZD0449 and CCI	4 mL	51	204 mL
CCI	2 mL	4	8 mL
CCI	4 mL	4	16 mL
CCI	2 mL	4	8 mL
CCI	2.5 mL 2.6 mL (Germany)	3	7.5 mL 7.8 mL (Germany)
CCI	4 mL	3	12 mL
CCI	2 mL	2	4 mL
CCI	10 mL	1	10 mL
COVID-19 serology (Part 3a)	1 mL	2	2 mL
COVID-19 serology (Part 3b)	1 mL	2	2 mL
Total (Part 3a)			**329.5 mL [b]** **349 mL [b] (Germany)**
Total (Part 3b)			**293.5 mL [b]** **313 mL [b] (Germany)**

COVID-19: Coronavirus disease 2019; CCI PK: Pharmacokinetics; TB: tuberculosis

a) Serology tests at screening, pregnancy testing, follicle-stimulating hormone testing (postmenopausal subjects only) and Immunoglobulins will be performed on the clinical chemistry sample collected at the same timepoint.

b) If a cannula is used for PK blood samples, an additional 1 mL/sample will be drawn.

Repeat blood samples may be collected for safety reasons and additional samples may be collected for PK analyses based on the emerging data. The maximum volume to be drawn from each patient must not exceed 500 mL.

3.3. Safety Review Committee

The SRC will consist of the following core members:

- Principal Investigator (PI) (Chair, voting member)
- AstraZeneca Lead Physician (voting member)
- AstraZeneca Clinical Pharmacokineticist (voting member)
- Parexel Project Manager (nonvoting member)

- Covance Pharmacokineticist (nonvoting member)

The SRC may also request to have attendance of or off-line support and input from the following functions as required:

- AstraZeneca Team Pharmacometrician
- Parexel and AstraZeneca Statisticians
- AstraZeneca and/or Parexel and/or external Medical Specialists (eg, Neurologist, ECG Center cardiologists)
- AstraZeneca Patient Safety Physician

Written statements and conclusions by the SRC will be in place before allowing study progression at the noted times as per Clinical Study Protocol (CSP). This includes documentation of appropriate quality control checks on the data reviewed.

3.3.1. Data Reviewed by Safety Review Committee for Dose Escalation Decision

3.3.1.1. Safety Review Committee Decision on Next Dose

The SRC will make the decision for the next dose level or whether to stop the study after reviewing all the pertinent safety and any other relevant data.

The PI(s) at the study site(s) or a medical delegate will be a core member of the SRC as a voting member. The Chief Investigator at Parexel Early Phase Clinical Unit (EPCU) will chair the meeting and will have the final decision should the SRC not be able to reach a final decision. In order to escalate to a next dose level a unanimous SRC decision is needed. Additional details, including the involvement of a Medical Monitor (MM) when adding additional site(s) for Part 2a/b of the study, are provided in the SRC Meeting Charter and the Medical Monitoring Plan. The Parexel MM will serve as a voting member of the SRC and is responsible for reviewing and assessing all the pertinent safety information before the meeting. The MM is responsible for the review of the medical/safety content of the SRC interim reports, safety data listings and supporting the CI and PI for making the final recommendations and conclusions on dose escalation or on the progress of the study based on reports and data provided. The MM will sign the dose escalation memo in agreement with the SRC voting members.

The decisions of the SRC on the next dose level will be documented and provided to all the appropriate parties involved with the study, including the Pharmacist to enable IMP preparation for the next scheduled dosing day.

- Where stopping criteria have not been met, the decision of the SRC may be to give the next higher dose according to the predefined dose increment, a smaller dose than previously given, a repeated dose or to stop dosing.

- A dose in which the safety stopping criteria have been met (please refer to Section 6, Study Stopping Rules) will not be repeated and further dose escalation **must not** occur.

  - In this case, the SRC will review the totality of data and restart of dosing is possible without a substantial amendment (eg, in the case of a laboratory error) if SRC review concludes that the relevant stopping criterion was not fulfilled.
  - A lower dose level expected to be tolerable and not to meet the stopping criteria, would be acceptable in this case.
  - If the safety stopping criteria are met and the SRC decides that there are reasons that the dose level should be repeated, or further dose escalation is warranted a summary of the data and justification (protocol amendment) will be submitted to Investigational Medicines Safety Review Board (ISRB), the Regulatory Authority and Independent Ethics Committee (IEC) for their approval before further dosing.
  - The SRC will decide the progress of the study from Part 2b to Part 3a (patients with mild asthma) or Part 3b (healthy volunteers) after conducting risk-benefit assessment and documenting the outcome of the assessment as part of the SRC decision. The assessment will consider the current situation of the COVID-19 pandemic, emerging data in the study and overall study objectives and endpoints.

3.3.1.2. Blinding at Safety Review Committee Meeting

The randomization code will be available at the SRC meeting and the data will be reviewed unblinded.

3.3.1.3. Assessments Adaptation

Following review of data from a cohort of subjects, the timing of assessments and/or blood samples may be adjusted for subsequent cohorts.

Additional assessment or sampling times may be added if indicated by the data; however, the maximum blood volume taken from each subject will not exceed 500 mL.

3.3.1.4. Safety Data Communication Between Study Sites Outside the Safety Review Committee Meetings

Per protocol, serious adverse events (SAEs) will be reported by Clinical Units to AstraZeneca within 24 hours. In addition to the AstraZeneca and Parexel standard safety and SAE processing and reporting processes that will be followed for this study, if an SAE occurs at one site, the PI or delegate will immediately inform other sites, and vice-versa. This will ensure prompt distribution and sharing of important safety information between sites. As per standard

AstraZeneca and Parexel reporting processes, Parexel Global Pharmacovigilance (PV) Reporting Group will also be responsible for distributing unblinded suspected unexpected serious adverse reaction (SUSAR) reports to ethics committees and study sites.

Communication between sites and Investigators should be done periodically according to the safety monitoring plan, in addition to SRC escalation meetings and expediting SAEs.

3.4. Overall Dose Strategy Based on Regulatory Guidance

3.4.1. Summary of Dose and Exposure Range

The dose range was defined by considering both the expected human therapeutic range and observations from non-clinical safety studies.

The starting dose for Part 1a (SAD) was chosen with consideration to both the Pharmacologically Active Dose (PAD), the Maximum Recommended Starting Dose (MRSD) [14], NOAEL and the Minimum Anticipated Effect Level (MABEL) dose [15].

The PAD was determined to be CCI AZD0449 delivered dose, as described in Section 3.4.4. The MRSD was calculated to be CCI AZD0449 delivered dose; detailed calculations are provided in Section 3.4.2. The MABEL dose was calculated to be CCI AZD0449 delivered dose; details are described in Section 3.4.3.

Conservatively, the MABEL dose was selected as the starting dose for Part 1a (SAD); more details on the dose rationale are provided in Section 3.5.1.2.

For scaling LDDs from rat to human, body weight-based scaling was chosen as the most appropriate approach, and has been applied to MABEL dose, PAD and predicted therapeutic dose. MRSD was calculated based on body surface area for reference only.

The human therapeutic dose is predicted to be CCI AZD0449 delivered dose; the assumptions and calculations are described in detail in Section 3.4.5.

The estimated human therapeutic dose range is CCI AZD0449 delivered dose, based on the considerations described in Section 3.4.6.

The NOAEL-based maximum allowed lung dose of CCI AZD0449 delivered dose was calculated as described in Section 3.4.7.

The maximum allowed exposure, C_{max}=188 nmol/L and AUC=1200 nmol*h/L, was determined as described in Section 3.4.8. Note, AUC for single doses or $AUC_{(0\text{-}24)}$ at steady state following repeated dosing.

Margins between starting dose, therapeutic dose and maximum allowed dose as well as predicted exposures are summarized in Section 3.6.

3.4.2. NOAEL-Based Maximum Recommended Starting Dose

The MRSD as defined by the Food and Drug Administration (FDA) [14] was calculated to be CCI AZD0449 delivered dose.

The value is based on the 6-month Good Laboratory Practice (GLP) toxicology data from the rat; since the rat had a lower human equivalent dose (HED) than the dog, it was selected as the most sensitive species.

The NOAEL was 0.188 mg/kg/day (lung-deposited dose) in the 6-month rat toxicology study and this dose has been used for calculation of a potential starting dose. The basis for the HED calculation has been the body burden doses in the toxicity studies defined by the fraction of inhaled drug delivered to lung.

The lung-deposited dose given in the rat toxicity study was 0.188 mg/kg/day. A corresponding HED of 0.030 mg/kg can be calculated by using the scaling factor (6.2) for rat. Applying a 10-fold safety factor and converting the dose to total human body dose (assuming a body weight of 60 kg) the starting dose results in CCI. When taking into account the predicted lung-deposited fraction of 0.8 in man (from the Provo.X system), the delivered MRSD is 0.22 mg.

A body weight of 60 kg was chosen as an especially conservative assumption for the starting dose, but for all therapeutic effect-related doses, 70 kg is assumed to be more realistic for a European population.

3.4.3. Minimum Anticipated Biological Effect Level Dose

In this protocol, the MABEL [15] dose is defined as the lowest dose for which an effect in humans can be anticipated. It was estimated to be CCI AZD0449 delivered dose.

The MABEL was determined based on data from a rat OVA model with intratracheal administration. In this model, a lung-deposited dose of 0.3 µg/kg AZD0449 showed no effect. The next higher investigated dose, 3 µg/kg, gave a 38% reduction of the eosinophil count in the bronchoalveolar lavage fluid (BALF). The minimum effect can be anticipated to lie between these 2 doses. All data of this experiment were fitted in a dose-response model. This model was then used to interpolate between the 0.3 and 3 µg/kg doses. An inhibition level of 20% was selected for read out, resulting in a dose of 1.3 µg/kg. This inhibition level is anticipated to be close to a minimum biological effect level.

The identified lung-deposited MABEL dose of 1.3 µg/kg was translated to an absolute human dose with the following calculation. A conservative assumption for human body weight of 60 kg was made. The lung-deposited fraction was predicted to be 80%. This results in an estimated MABEL dose of CCI AZD0449 delivered dose, which is 37% of PAD and 44% of MRSD.

As for the MRSD, a body weight of 60 kg was chosen as an especially conservative assumption for the starting dose, but for all therapeutic effect-related doses, 70 kg is assumed to be more realistic for a European population.

3.4.4. Pharmacologically Active Dose

The PAD is defined as the lowest dose tested in an animal species with the intended pharmacologic activity according to the FDA guidance [14].

The PAD was determined to be 3.0 µg/kg (expressed as free base of AZD0449) lung-deposited dose based on data from a rat OVA model. This dose caused a 38% reduction of the eosinophil count in the BALF. Assuming a human body weight of 70 kg and a lung-deposited fraction in human of 80%, the absolute human PAD is CCI AZD0449 delivered dose.

3.4.5. Predicted Human Therapeutic Dose/Anticipated Therapeutic Dose

In this protocol, predicted (human) therapeutic dose and Anticipated Therapeutic Dose (ATD) are used as synonyms.

The human therapeutic dose was predicted based on data from a rat OVA model with DPI. In this model, AZD0449 reduced the eosinophil count in the BALF by a maximum of approximately 60%. The dose resulting in 80% of this maximum inhibition (ED_{80}) was selected to be the expected human therapeutic dose. This selection was made considering that AZD0449 works as a competitive antagonist on JAK1. In contrast to agonists which may show clinical effects at low exposures, competitive antagonists like AZD0449 may generally require relatively high exposures to inhibit the target.

The selected dose, ED_{80}, was 13 µg/kg (expressed as free base of AZD0449) lung-deposited dose. Human body weight was assumed to be 70 kg. Lung-deposited fraction in human is assumed to be 80%. Applying these numbers, the predicted human therapeutic dose is CCI AZD0449 delivered dose.

3.4.6. Estimated Human Pharmacodynamic Dose Range

The lower end of the estimated human pharmacodynamic (PD) dose range can be assumed to be similar to the MABEL dose of CCI AZD0449 (see Section 3.4.3).

The upper end of the estimated human PD dose range is assumed to match the NOAEL-based maximum allowed dose of CCI AZD0449 (Section 3.4.7), as this dose corresponds to the dose giving 96% reduction of eosinophils in the rat OVA model (ED_{96}). This upper limit of CCI is approximately 4-times higher than the predicted human therapeutic dose (see Section 3.4.5).

3.4.7. NOAEL-Based Maximum Allowed Dose

The maximum allowed dose has been calculated by using an approach which converts the NOAEL from a dose per body weight (mg/kg) to a dose per g of lung weight (mg/g lung tissue), followed by a lung weight scaling to human and application of safety margins (10-fold for the rat and 6-fold for the dog [13]). Separate calculations for both species are given in the following paragraphs.

In the 6-month rat toxicity study, the NOAEL was observed at a total inhaled dose of 1.88 mg/kg/day, corresponding to 0.188 mg/kg/day lung dose, with 10% lung deposition [13]. This translates to 0.06 mg/animal (using the group mean body weight of 318 g) and 0.05 mg/g of lung tissue (using the group mean lung weight of 1.19 g). Assuming a lung weight of 1000 g in human, the corresponding human dose is 50 mg.

As the respiratory tract pathology findings are considered non-monitorable in the clinic, it was considered that the maximum clinical dose level should be limited to 10-fold lower than the dose level at which there were no respiratory tract pathology findings in the most sensitive species (rat). Applying a 10-fold safety margin results in a human delivered dose of CCI

In the 3-month dog toxicity study, the equivalent calculation was based on NOAEL at a total inhaled dose of 2.01 mg/kg/day, with 25% lung deposition [13] and using group mean values of 9.10 kg for body weight and 86.1 g for lung weight. This results in a human dose of 53 mg. Applying a 6-fold safety margin translates to a human delivered dose of **9 mg**.

Note, lung deposition in human is dependent upon inhalation device, but can here be expected to be approximately 80%; a total dose of eg, 5 mg therefore corresponds to a lung-deposited dose of 4 mg.

Conclusion

The maximum allowed dose was chosen to be CCI (delivered dose) and is based upon the data from the most sensitive species (rat).

A delivered dose of CCI is considered to be at the higher end of the estimated human therapeutic range and approximately 4 times higher than the predicted therapeutic dose (see Section 3.4.5).

The predicted human plasma exposure following the proposed maximum dose of CCI is 19.8 nmol/L (C_{max}) and 654 nmol*h/L (AUC) for a single inhaled dose.

3.4.8. Systemic Exposure Limits

The maximum allowed total human exposure is a C_{max} of 188 nmol/L and an AUC of 1200 nmol.h/L, corresponding to approximate free (non-plasma protein bound) exposures of 0.188 nmol/L and 1.20 nmol.h/L, respectively. Note, AUC for single doses or $AUC_{(0-24)}$ at steady state following repeated dosing.

Plasma protein binding in both the rat and dog are higher than human, however the exposure limit was set on the more conservative total concentration (% non-plasma protein bound [free]; rat: 0.24%; dog: 0.15%; human: 0.10%). Margins (between human plasma concentrations and systemic exposure limits) based on free concentrations are always ca. 2.5-fold (rat) and ca. 1.5-fold (dog) higher than margins based on total concentrations.

The adverse effects observed in both species were limited to the respiratory tract and were considered to be driven by administration of high inhaled dose levels which overloaded clearance mechanisms; there were no adverse systemic effects up to the highest dose levels and exposures in both the rat and dog.

Given that an adverse systemic exposure level was not identified, it is considered appropriate to limit systemic exposure to half of that achieved in species with the lower exposure (rat; using the exposure values from the 6-month study):

- $AUC_{(0-24)}$ achieved in rat in 6-month study=2400 nmol.h/L/2=1200 nmol.h/L
- C_{max} achieved in rat in 6-month study=376 nmol/L/2=188 nmol/L

For comparison the achieved exposure values in the dog with no adverse systemic findings were a C_{max} of 5440 nmol/L and an $AUC_{(0-24)}$ of 5940 nmol.h/L.

3.5. Dose Strategy in the Individual Study Parts

3.5.1. Dosing Part 1a

3.5.1.1. Targeted Delivered Doses

For inhaled dose administration, a jet nebulizer and a dosimeter will be used as per a separate handling instruction. The targeted delivered doses will be achieved by using the different strengths of the nebulizer suspension and varying the number of breaths. The number of breaths will always be selected to deliver a dose as close as possible to the target delivered dose while taking into account the pre-specified limits for dose escalation steps. For the targeted delivered starting dose CCI between 10-20 breaths of the lowest strength suspension will be

administered, and the actual dose, if it cannot be exactly CCI , will be slightly lower, but not higher.

3.5.1.2. Starting Dose Part 1a

For Part 1a, CCI AZD0449 delivered dose has been selected as the starting dose.

This starting dose was chosen with consideration to both the PAD, the MRSD [14], NOAEL and the MABEL dose [15].

Selecting the MABEL dose as starting dose represents the most conservative approach as the MABEL dose of CCI is 37% of PAD (0.26 mg) and 44% of MRSD (0.22 mg). The MRSD is based on the NOAEL dose but contains a 10-fold safety factor (see Section 3.4.2), so a starting dose of CCI represents 4.4% of the NOAEL dose (ie, the MRSD without the 10-fold safety factor). As the starting dose was thus chosen very conservatively, as the mode of action is well-understood and as there is rich clinical data from oral JAK inhibitors (see Section 4.2.1), a safety factor was not applied to the starting dose.

3.5.1.3. Sentinel Dosing Part 1a

Dosing for each ascending dose cohort will start with 2 volunteers in a sentinel cohort, such that 1 volunteer will be randomized to receive AZD0449 and 1 volunteer will be randomized to receive placebo. The safety data (AEs, vital signs, ECG, telemetry and clinical laboratory evaluations) from the sentinel volunteers up to 24 hours post-dose will be reviewed by the PI before the remaining volunteers in the cohort are dosed.

3.5.1.4. Dose Escalation Part 1a

The dose rationale and the selection of the starting dose are described in Section 3.5.1. Dose escalation will be guided by specific stopping rules (see Section 6).

In total, 6 dose levels are planned, with the option of adding 3 extra cohorts (within the pre-specified dose range). The starting dose is fixed at CCI , however the subsequent doses of AZD0449 may be adjusted based on available safety and PK data from the completed cohorts, ie, the dose escalation shown in the flow charts (Section 3.2.1) is preliminary. Escalation to the next dose level will only take place after the safety data (AEs, vital signs, ECG, telemetry and clinical laboratory evaluations) from the previous cohort has been reviewed (see Section 3.3). Data from a minimum of 5 volunteers on AZD0449 or 7 subjects in total per cohort must be reviewed and considered satisfactory before each dose escalation. Dose escalation below the predicted therapeutic dose will not exceed 3-fold. The estimated human pharmacodynamic dose range spans more than one order of magnitude (50-fold) (see Section 3.4.6). Therefore, a 3-fold step is not expected to cover the steep part of the

dose-response curve when escalating between 2 doses, ie, 3-fold dose escalation is considered cautious.

From the predicted therapeutic dose level onwards, the dose will increase no more than 2-fold. This is both due to general safety considerations and to ensure a thorough characterization of the PK in the dose range of predicted high pharmacological activity.

During dose escalation, the dose in the next cohort does not have to be higher than the previous one. Intermediate dose levels may be added. If the exposure limit has not been reached, any dose including the highest dose may be repeated or the size of any cohort may be increased. This may be required due to non-safety-related reasons, eg, subject dropout or loss of samples or unexpected or highly variable PK, which makes it necessary to add more data points around a certain dose level.

3.5.1.5. Stopping Criteria/End of Escalation Part 1a

Dosing will be stopped at any time based on the safety criteria described in Section 6.1.

In addition, dose escalation will be stopped if any of the PK criteria described in Section 6.2 have been met.

3.5.2. Dosing Part 1b

3.5.2.1. Dose Part 1b

The dose in the first cohort of Part 1b will be CCI AZD0449 administered as an IV infusion over a 60-minute period (0.1 mL/min). The dose in the second cohort of Part 1b will be CCI AZD0449 administered as an IV infusion over a 48-minute period (0.5 mL/min).

The first (and lowest) IV dose was selected based on the PK predictions that the dose is high enough to allow for quantification of plasma AZD0449 levels 24 hours after dosing. For general safety reasons, the selected dose level is at the low end of the estimated human PD dose range. The resulting observed exposure at the lowest dose was, as predicted, well below the maximum allowed exposure (1% of C_{max}, 0.3% of AUC). The rationale for the safety of the IV dose is discussed in Section 4.1.2.

The second IV dose was selected after completion of Part 1a and the first IV cohort in Part 1b. For the first IV dose, the concentrations in plasma were quantifiable 10 to 12 h after administration and that was not sufficient to fully characterize the PK of AZD0449 following IV administration. With the knowledge gained from the first IV dose and the Part 1a inhaled dose cohorts, it is predicted that with an IV dose of CCI, sufficient concentrations will be achieved to allow for quantification of plasma AZD0449 levels 24 hours after dosing. The

exposure at the second dose is also predicted to be well below the maximum allowed exposure (4.4% of C_{max}, 1.2% of AUC).

3.5.2.2. Sentinel Dosing Part 1b

Dosing of both IV cohorts will start with 1 volunteer in a sentinel cohort, and the safety data (AEs, vital signs, ECG, telemetry and clinical laboratory evaluations) from the sentinel volunteer up to 24 hours post-dose will be reviewed by the PI before the remaining volunteers in the cohort are dosed. The remaining 5 volunteers in the cohort will be dosed after the 24-hour safety data have been reviewed.

3.5.2.3. Stopping Criteria Part 1b

Dosing will be stopped at any time based on the safety criteria described in Section 6.1.

3.5.3. Dosing Part 2a/b

3.5.3.1. Starting Dose Part 2a/b

The starting dose for Part 2a will be a dose level that is around the mid dose range tested in Part 1a (SAD) and was predicted to be CCI AZD0449 delivered dose. A mid dose level avoids general safety concerns connected to starting with a high dose. It also makes it possible to escalate into the therapeutically relevant range with 3 dose levels. As per the discussions at the SRC meeting following completion of Part 1a cohort 6, the starting dose level selected was CCI AZD0449 delivered dose.

3.5.3.2. Sentinel Dosing Part 2a/b

Dosing for each ascending dose cohort will start with 2 subjects in a sentinel cohort, such that 1 subject will be randomized to receive AZD0449 and 1 subject will be randomized to receive placebo. The safety data (AEs, vital signs, ECG, telemetry and clinical laboratory evaluations) from the sentinel subjects up to 24 hours post-first dose will be reviewed by the PI before the remaining subjects in the cohort are dosed. The remaining subjects in each cohort will be dosed at least 48 hours after the sentinel cohort.

The rationale for the sentinel dosing is presented in Section 4.1.3.

3.5.3.3. Dose Escalation Part 2a/b

The dose rationale and the selection of the starting dose are described in Section 3.5.3.1. Dose escalation will be guided by specific stopping rules (see Section 6).

In total, 3 dose levels are planned, with the option of adding additional cohorts (within the pre-specified dose range). Escalation to the next dose cohort will only take place after the safety

data (AEs, vital signs, ECG, telemetry and clinical laboratory evaluations) from the previous cohort have been reviewed. Data from a minimum of 6 subjects on AZD0449 or 8 subjects in total (7 volunteers for cohort 3 in Part 2b) per cohort must be reviewed and considered satisfactory before each dose escalation.

Dose levels of AZD0449 will be adjusted based on review of available safety and PK data from Part 1a and previous cohorts in Part 2, ie, the dose escalation shown in the flow charts (Section 3.2.1) is preliminary.

When selecting the dose levels, the following criteria will be considered:

- Dose escalation below the predicted therapeutic dose will not exceed 3-fold. The estimated human PD dose range spans more than one order of magnitude (50-fold [see Section 3.4.6]). Therefore, a 3-fold step is not expected to cover the steep part of the dose-response curve when escalating between 2 doses, ie, 3-fold dose escalation is considered cautious.
- From the predicted therapeutic dose level onwards, the dose will increase no more than 2-fold. This is both due to general safety considerations and to ensure a thorough characterization of the PK in the dose range of predicted high pharmacological activity.
- During dose escalation, the dose in the next cohort does not have to be higher than the previous one. Intermediate dose levels may be added. If the exposure limit has not been reached, any dose including the highest dose may be repeated or the size of any cohort may be increased. This may be required due to non-safety-related reasons, eg, patient dropout or loss of samples, unexpected or highly variable PK.
- The highest dose investigated in Part 2a/b will not exceed the highest doses investigated in Part 1a.

Currently the dose levels are planned to be selected as follows:

- The starting dose is a dose from the middle of the dose range in Part 1a (SAD) (see Section 3.5.3.1 above).
- The highest dose in Part 2a/b (MAD) will be the highest dose tested in Part 1a (SAD), provided that no safety concerns related to C_{max} were raised during Part 1a and considering the specific stopping rules (see Section 6).

Including the highest dose from Part 1a (SAD study) into Part 2a/b (MAD study) is predicted to result in about a 50% increased C_{max} at the end of the MAD treatment compared with the SAD part while maintaining the same AUC (see Table 3.6-1). This increase in C_{max} is regarded

safe as the resulting predicted C_{max} value (32.9 nmol/L) still lies about 6-fold below the maximum allowed C_{max} of 188 nmol/L.

According to EMA guidance [15], the expected exposure after multiple dosing should have been covered in the SAD parts, but higher exposures in the MAD part can be considered provided this option is pre-specified and below the set maximum exposure. Both criteria are fulfilled in this protocol.

In addition, the following aspects support the safety of the chosen approach:

- In the dog, daily IV dosing of 1 mg/kg for a period of 3 months was well tolerated, with an achieved C_{max} of 5440 nmol/L. This value lies 29-fold higher than the C_{max} limit of 188 nmol/L and 165-fold higher than the predicted C_{max} after 14-day inhaled dosing of CCI AZD0449 in the highest MAD cohort.
- There were no adverse systemic findings in the pre-clinical toxicology studies (both species) related to either peak concentration (C_{max}) or prolonged exposure (AUC). The adverse respiratory tract pathology findings in the pre-clinical toxicology studies are considered to be driven by topical effects of the lung dose. Therefore, the maximum allowed human dose level is limited to 10-fold lower than the no effect level in the most sensitive species (rat).
- Extensive safety monitoring covering both local respiratory and systemic effects is performed throughout the study (see Sections 6 and Section 8.2), and the MAD part will be performed with a well-controlled clinical environment.

3.5.3.4. Stopping Criteria/End of Escalation Part 2a/b

- Dosing will be stopped at any time based on the safety criteria described in Section 6.1.
- In addition, dose escalation will be stopped if any of the PK criteria described in Section 6.2 have been met.

3.5.4. Dosing Part 3a/b

3.5.4.1. Dose Part 3a/b

The dose level for Part 3a/b will be CCI or equal to the highest MAD dose. Depending on emerging data from Part 2, the dose for Part 3a/b could be lower than CCI and then equal to the highest MAD dose that was safe and within the allowed exposure range. This dose level will allow for direct comparison of PK profiles. As the DPI formulation (Part 3) is expected to result in a lower lung-deposited fraction than the nebulized formulation (Part 2), it is expected that resulting exposure will remain below the exposure explored in Part 2. In Part 3a/b,

treatment will be administered with the SD3FL version of Genuair, a single integral, non-reusable DPI approved in EU.

The uncertainties associated with DPI dosing are discussed in Section 4.2.5.

3.5.4.2. Sentinel Dosing Part 3a/b

Dosing of the cohort in Part 3a as well as Part 3b will start with 2 subjects in a sentinel cohort, such that 1 subject will be randomized to receive AZD0449 and 1 subject will be randomized to receive placebo. The safety data (AEs, vital signs, ECG, telemetry and clinical laboratory evaluations) from the sentinel subjects up to 24 hours post-first dose will be reviewed by the PI before the remaining subjects in each cohort could be dosed. The remaining 34 patients (Part 3a) or 6 volunteers (Part 3b) in the respective cohort will be dosed at least 48 hours after the sentinel cohort.

The rationale for the sentinel dosing is presented in Section 4.1.4.

3.5.4.3. Stopping Criteria Part 3a/b

Dosing will be stopped at any time based on the safety and PK criteria described in Section 6.

3.6. Predictions of Exposures and Margins for Different Dose Levels

3.6.1. Methods for Modeling Doses and Estimated Exposure Levels

Plasma PK of AZD0449 were predicted using a Physiology Based Pharmacokinetic (PBPK) Model. In short, this model mechanistically describes lung deposition and PK in lung and plasma by combining information about the properties of compound, formulation, physiology and predicted human PK parameters. The PBPK model was fitted to describe experimental lung and plasma PK data from AZD0449 in the rat. Predictions for human PK were made considering the differences between rat and human physiology.

Model building and evaluation have been published by Boger et al. in 2016 [18].

Table 3.6-1 Predicted Exposures and Margins

	Description	Dose [a]	Predicted PK parameter [b]		Observed PK parameter [f]		Margin syst. exp. limit/parameter [b],[d]	
		[mg]	Cmax [nmol/L]	AUC [c] [h*nmol/L]	Cmax [nmol/L]	AUC [c] [h*nmol/L]	Cmax (-fold)	AUC (-fold)
Part 1a (SAD)	Start dose	CCI			0.436	1.71	431	702 [g]
	Max dose in SAD [e]	CCI			26.1	136	7	9 [g]
Part 1b (IV)	IV dose (60 min infusion)	CCI			2.05	3.67	92	327 [g]
		CCI	8.2	14			23	86
Part 2a/b (MAD)	Start dose	CCI			4.68	27.3	40	44 [g]
	Max dose in MAD [e]	CCI	36	131			5	9 [h]
Part 3a/b (DPI mild asthmatics)	Dose	CCI	36	131			5	9 [h],[i]

AUC: Area Under the Curve; C_{max}: maximum observed plasma concentration; i.v: intravenous; MAD: Multiple ascending dose; PK: Pharmacokinetics; SAD: Single ascending dose; Syst. exp. limit: systemic exposure limit

a) Target dose AZD0449 (expressed as free base). Part 1a and Part 2: inhaled delivered; Part 1b: intravenous.
b) PK parameters based on total blood plasma concentrations, see also comment below. For Part 2, parameters are given for the last treatment day, ie. steady state.
c) AUC is $AUC_{(0\text{-infinity})}$ for single dosing (Part 1a and Part 1b) and $AUC_{(steady\ state,\ 0\text{-}24\ h)}$ for multiple dosing (Part 2).
d) Systemic exposure limits from 6-month rat study (C_{max}=188 nmol/L, AUC=1200 h*nmol/L)
e) The maximum allowed dose represents both the upper end of the estimated human pharmacodynamic dose range (Section 3.4.6) and the NOAEL-based Maximum Allowed Dose (Section 3.4.7).
f) Observed PK parameter from Interim Analysis (IA).
g) Observed margin.
h) Prediction based on available data in SAD+IV; the prediction is that volunteers and patients with mild asthma will have similar PK.
i) High likelihood to get lower exposures with DPI and an assumed lower LDD.

Comments:

For improved readability, data is only shown for selected doses, including the lowest and highest possible dose; these form a range that includes the predicted exposure for all other doses mentioned in this protocol.

All parameters and margins shown are based on total plasma concentrations as this is the most conservative approach. The free (non-plasma protein bound) fractions in plasma are very low for both rat (0.24%) and human (0.10%). Margins based on free concentrations are always ca. 2.5-fold higher than margins based on total concentrations.

4. RATIONALE, UNCERTAINTY AND RISKS

4.1. Study Design Rationale and Justification

4.1.1. Rationale for Design of Part 1a

AZD0449 has not been studied in man previously. This FIH clinical study will assess the safety and tolerability as well as the single dose PK of inhaled AZD0449 in volunteers.

Study Population

The study will be performed in healthy male volunteers and healthy female volunteers of non-childbearing potential. Healthy volunteers will be selected to minimize interference with other drugs or with disease processes. The inclusion and exclusion criteria are defined such that the selected volunteers will be free of any significant illness when included in the study and that they do not receive medication expected to impact observations (see Section 4.4.1).

General Dosing Strategy

AZD0449 will be administered in a standard SAD sequential group design, ie, each volunteer in this study part will receive AZD0449 or placebo only once and dose will be increased step-wise between cohorts.

The rationale for dosing and dose escalation is described in detail in Section 3.5.1.

Each cohort will be preceded by a sentinel cohort (see Section 3.5.1.3). The remaining volunteers of each cohort will only be dosed after the 24-hour safety data from the sentinel volunteers have been reviewed. Likewise, escalation to the next dose cohort will only take place after safety and PK data from the previous cohort have been reviewed.

Placebo Control

The study includes placebo control in order identify effects that relate to drug administration rather than the study procedures or situation.

Randomization and Blinding

Treatments are randomized, and study volunteers will be blinded to treatment allocation (single-blind design) to minimize bias. The study is single rather than double-blind as specified Parexel personnel need to be unblinded for the dose escalation decisions made by the SRC. However, site personnel managing the study volunteers will be blinded to the extent possible while minimizing any impact on data collection.

4.1.2. Rationale for Design of Part 1b

In Part 1b, AZD0449 will be administered as a single IV dose at 2 different dose levels CCI to 2 cohorts of healthy volunteers in order to compare the PK between IV and inhaled administration. The results will be used to improve future study design and interpretation.

Study Population

The study will be performed in healthy male volunteers and healthy female volunteers. Female volunteers must be of non-childbearing potential for Part 1b first IV cohort. Healthy volunteers will be selected to minimize interference with other drugs or with disease processes. The inclusion and exclusion criteria are defined such that the selected volunteers will be free of any significant illness when included in the study and that they do not receive medication expected to impact observations (see Section 4.4.1).

Background - Inhalation PK

Contrary to plasma concentration-time (PK) profiles of oral drug administration, profiles of inhaled administration are influenced by absorption from the lung. Particularly for compounds with low solubility, lung absorption may be slow and may strongly affect the observed PK plasma profile.

If this is the case, the late phase of the plasma PK profile is driven by both processes – lung absorption and plasma clearance – at the same time. Estimating plasma clearance based on such an inhalation profile alone is misleading, as the clearance estimate will always contain the unknown component of lung absorption.

Background - Relevance and Application of IV PK Information

The individual contributions of plasma clearance and lung absorption can be separated by determining plasma clearance from an IV administration profile. With IV administration, lung absorption cannot influence the elimination phase, thus allowing for an unbiased characterization of plasma clearance. Once plasma clearance is known, the impact of lung absorption on the profile after inhalation can be deduced.

Characterizing the extent and time course of lung absorption will aid dose selection for future studies. Including experimental information on lung absorption into PK models will make predictions more informative and reliable; this in turn will make the models more valuable in supporting the design and interpretation of the ongoing and future studies.

Safety of IV Administration

The first (and lowest) IV dose in the proposed study was chosen to be at the low end of the investigated dose range (see Section 3.4.6). The resulting exposure from this dose was, as predicted, well below the maximum allowed exposure (1% of C_{max}, 0.3% of AUC). The exposure at the second dose is also predicted to be well below the maximum allowed exposure (4.4% of C_{max}, 1.2% of AUC). The resulting C_{max} is predicted to be 32% of the predicted C_{max} and 3% of the predicted AUC for the highest planned SAD dose, CCI (see Table 3.6-1).

Routes of metabolism and excretion of AZD0449 in plasma after IV administration will be the same as for the systemically available dose part after inhalation. As no lung absorption can take place with IV administration, plasma half-life is expected to be shorter than after inhalation.

As the studied IV dose is at the low end of the investigated dose range (see Section 3.4.6) and only a single dose is given, systemic effects are not expected based on pre-clinical data. In the dog, daily IV dosing of 1 mg/kg for a period of 3 months was well tolerated, with an achieved C_{max} of 5440 nmol/L and AUC of 5940 nmol.h/L, giving an 850-fold margin for C_{max} and a 283-fold margin for AUC compared to the predicted exposure of the planned human IV dose. Clinical data from oral JAK inhibitors including ruxolitinib or tofacitinib [17] suggest that a single dose is likely to be well tolerated (see also Section 4.2.1).

General Dosing Strategy

In Part 1b, AZD0449 will be administered initially (first IV cohort) as a single, fixed IV dose CCI to 6 volunteers who participated in and received a single inhaled dose of AZD0449 in Part 1a. If the volunteer is a naïve volunteer, then they will first receive the IV dose of AZD0449 followed by a single inhaled dose of AZD0449. For the second IV cohort, AZD0449 will be administered as a single, fixed IV dose CCI to 6 naïve volunteers. The rationale for the choice of dose is described in detail in Section 3.5.2.

Both IV cohorts will be preceded by a sentinel cohort (see Section 3.5.2.2). The remaining volunteers of each cohort will only be dosed after the 24-hour safety data from the sentinel volunteers have been reviewed.

2-stage Design

The 2-stage design makes it possible to study different treatments within the same individual, so that each volunteer serves as their own control. This reduces the impact of between-volunteer variability, thus making the study results clearer and more reliable. This in turn helps to reduce population size and makes the results more informative.

The volunteer receiving the first IV dose of AZD0449 will already have been exposed to a single inhaled dose of AZD0449 in Part 1a (SAD), or if the volunteer is a naïve volunteer (first IV cohort) then they will first receive the IV dose of AZD0449 followed by a single inhaled dose of AZD0449.

This will make this the first volunteer receiving multiple dosing of AZD0449. To prevent carry over, both dosing occasions will be separated by a washout period of at least 2 weeks.

4.1.3. Rationale for Design of Part 2a/b

Study Part 2a/b will assess the safety, tolerability, and multiple dose PK in healthy volunteers and patients with mild asthma. In addition, the effect of inhaled AZD0449 on FeNO in patients with mild asthma will be examined. The FeNO data from Part 2a will be analysed together with the data from Part 3a.

The combined data from Part 2-3 will guide the design of the future dose range finding study. The PK and safety data from Part 2a/b will be important to guide a potential treatment of COVID-19 associated inflammatory respiratory disease.

Study Population

Part 2 will be performed in patients with mild asthma (Part 2a) and healthy volunteers (Part 2b). Unlike in Part 1a and Part 1b (first IV cohort), female patients of childbearing potential who meet the relevant inclusion/exclusion criteria (eg negative pregnancy test, no breastfeeding, using a highly effective contraception method with an additional barrier method of either the female patient or the partner) may also be enrolled. The inclusion and exclusion criteria are defined such that the selected patients will be free of any significant illness other than mild asthma when included in the study. This mild asthma population will be otherwise healthy and stable, with no other comorbidities. Medication use will be limited by treatment history and occasional short acting beta agonists for rescue, this will ensure inclusion of a stable/robust population with a low risk for interferences caused by disease or medication.

Patients were chosen for the low dose levels in the MAD to identify target engagement, to have a disease marker, and to measure a response to treatment. Although not all patients with mild asthma have elevated FeNO, there is a subpopulation that will have elevated FeNO. Healthy volunteers were chosen for the highest dose level CCI in the MAD to not study target engagement in this cohort and instead perform that assessment in the Part 3a/b with DPI formulation. The DPI formulation is intended to be used in future clinical development studies for treatment of asthma.

Fractional exhaled nitric oxide will be used as a marker of target engagement since nitric oxide is down-stream from IL13/IL4 mediated activation of JAK1/STAT6 via iNOS. Fractional

exhaled nitric oxide levels are also associated with asthma disease activity including increased risk for exacerbations. In this study, the ability of AZD0449 to decrease FeNO both as a marker of target engagement will be tested.

Part 2a/b of this study will include both patients and healthy volunteers. The investigation of PK and safety can be done equally safe and informative in both populations. In Part 2a, target engagement can also be studied.

General Dosing Strategy

AZD0449 will be administered in a standard MAD sequential group design, ie, each patient in this study part will receive AZD0449 over 12 days and the dose will be increased step-wise between cohorts.

The rationale for dosing and dose escalation is described in detail in Section 3.5.3.

Each cohort will be preceded by a sentinel cohort (see Section 3.5.3.2) which will first receive a single dose of AZD0449. Dosing for the sentinel patients will be continued and the remaining patients of each cohort will only be dosed after the 24-hour safety data from the first dose in the sentinel patients have been reviewed. The remaining patients for each cohort will be dosed at least 48 hours after the sentinel cohort.

Observing the sentinel cohorts after the first dose over 24 hours is deemed sufficient as the pharmacology of JAK inhibition is well -understood. The risk of off -target effects is low as described in Section 4.2.3. Clinical data are available for oral JAK inhibitors, suggesting generally good safety and tolerability (more details in Section 4.2.1). Known adverse effects of JAK inhibition are unlikely to be observed in a sentinel cohort in this study, even over a time longer than 24 hours. As an example, the oral JAK inhibitor tofacitinib is approved in EU for the treatment of rheumatoid arthritis. For this medication an increased risk of infections including reactivation of viral infections and tuberculosis (TB) has been reported. Large overlap has been reported in the safety profiles of various JAK inhibitors [20]. With the low systemic exposure achieved after inhalation, and the limited duration of study treatment, the risk for infections is low. Sentinel dosing of any length does not seem suitable to address this low risk. The 24-hour sentinel dosing in this study was included as a general precaution in a first-inpatient- setting.

Placebo Control

The study includes placebo control in order identify effects that relate to drug administration rather than the study procedures or situation.

Randomization and Blinding

Treatments are randomized, and study patients will be blinded to treatment allocation (single-blind design) to minimize bias. The study is single rather than double-blind as specified Parexel personnel need to be unblinded for the dose escalation decisions made by the SRC. However, site personnel managing the study patients will be blinded to the extent possible while minimizing any impact on data collection.

4.1.4. Rationale for Design of Part 3a/b

In Part 3a and Part 3b , AZD0449 will be administered as a DPI in patients with mild asthma and healthy volunteers. For later clinical studies and commercial use a DPI formulation will be developed. A PK, PD, and safety DPI/PoM study will be performed in Part 3a to characterize the properties of AZD0449 when administered as a DPI formulation. Part 3a will have statistical power to study PoM based on FeNO. The results will be used to support future study design. The decision to start Part 3a would be made at an SRC meeting when Part 2a/b of the study has been completed. In a situation where mild asthmatics cannot be recruited to the study there will be an option to study safety and PK, but not PD, in an optional cohort in healthy volunteers (Part 3b).

Background DPI/PoM

The properties of nebulized formulations differ from those of DPI formulations, leading to differences in lung deposition patterns and PK profiles. It will be possible to compare plasma PK profiles for both formulations. The current study design will not allow for direct comparison between 2 identical cohorts when the only difference is formulation. Instead a combination of all available learnings from the different study parts will be used to characterize the possible PK and PD differences between DPI and nebulized formulation. Comparing the plasma PK profiles of both formulations will help to better understand the characteristics of the formulations.

The results will be used to support future study design and interpretation.

Study Population

This study part can be performed in patients with mild asthma with elevated FeNO with the same inclusion and exclusion criteria as for Part 2a or alternatively in healthy volunteers with the same inclusion and exclusion criteria as for Part 2b. This is to ensure that results are directly comparable.

General Dosing Strategy

AZD0449 will be administered at one dose level. The dose will be up to the highest dose that will be used in Part 2a/b (MAD).

The rationale for dosing is described in detail in Section 3.5.4.

The cohort will start with a sentinel pair (see Section 3.5.4.2) which will receive a single dose of AZD0449 first.

The details on this sentinel cohort and the rationale for it are the same as described for Part 2a/b (MAD) in the previous section (Section 4.1.3).

Placebo Control

The study includes placebo control in order identify effects that relate to drug administration rather than the study procedures or situation.

Randomization and Blinding

Treatments are randomized, and study patients will be blinded to treatment allocation (single-blind design) to minimize bias. The study is single rather than double-blind as specified Parexel personnel need to be unblinded for the dose escalation decisions made by the SRC. However, site personnel managing the study patients will be blinded to the extent possible while minimizing any impact on data collection.

4.2. Degree of Uncertainty

4.2.1. Uncertainty Associated with the Mode of Action

This will be the first time that AZD0449 is studied in humans, however, AZD0449 is not first in class, as there are JAK inhibitors both in clinical development and on the market.

GDC-0214 (RG-6151) and VR588 are examples of inhaled pan-JAK inhibitors that are in clinical development. GDC-0214 (RG-6151) is a JAK inhibitor developed by Genentech and is currently undergoing Phase I clinical trials. VR588 is an inhaled JAK inhibitor developed by Vertex that is currently in pre-clinical development.

Ruxolitinib and tofacitinib are examples of approved oral JAK inhibitors. Ruxolitinib is a potent and selective JAK1 and JAK2 inhibitor approved for the treatment of myelofibrosis. Tofacitinib is s a potent, selective JAK inhibitor that preferentially inhibits JAK 1 and JAK 3. The oral JAK inhibitor tofacitinib is approved in EU for the treatment of rheumatoid arthritis. This medication has been associated with an increased risk of reactivation of viral infections including herpes zoster, as well as potentially TB.

Risks Related to Immune Suppression

Janus kinase inhibitors have immunosuppressive properties and the major effect has been suggested to be on lymphocytes. The risk of serious infections of JAK inhibitors is similar to

tumor necrosis factor blockers and other biologics in rheumatoid arthritis patients. The 'herpes zoster signal' seems to be a "class effect" as most JAK inhibitors show an elevated risk [20]. It is considered that inhaled AZD0449 has a lower overall risk of causing infections compared with oral JAK inhibitors. However, inhaled administration of the drug necessitates special attention on pulmonary infections (see Section 4.4.2).

Dose-response Relationship

AZD0449 acts as an antagonist on JAK1 and is a competitive, reversible inhibitor of IL4-signaling, see Section 1.2.1 for more information on the mode of action. The dose-response of AZD0449 is well described in pre-clinical data over a wide dose range, spanning 4 orders of magnitude. The dose-exposure relationship was approximately linear; while the dose-effect relationship could be described in standard sigmoidal models.

In the human primary cell systems and animal in vivo models tested, there is no indication of steep dose-response curves. Similarly, there is no indication of a steep dose-response curve with regards to pre-clinical toxicity, while the proposed clinical dose range is ≥10-fold lower than NOAEL dose level in the most sensitive species.

4.2.2. Uncertainty Associated with Biomarkers

Fractional exhaled nitric oxide will be used as a biomarker to characterize the primary PD of AZD0449. Nitric oxide is produced by iNOS in lung epithelium which is a gene controlled by JAK1/STAT6.

Currently there is no biomarker available for safety in humans. As it is not possible to monitor for the lung pathology findings observed in the toxicology studies, a 10-fold safety factor has been applied to the NOAEL dose level in the most sensitive species to determine the maximum allowed inhaled dose (see Section 3.4.7).

4.2.3. Uncertainty Associated with the Nature of the Target

There is extensive knowledge available about the nature of the target (JAK1). Tissue distribution is broad and JAK1 is expressed in lung epithelium and immune cells. Janus kinase activation in response to cytokine receptors (eg, IL 4/IL 13 receptor, IL 6 receptor) is well described. The down-stream effects of JAK are mediated by STAT phosphorylation including STAT3, 5, 6. The systemic inhibition of JAK results in immune modulation.

There is a potential for off-target inhibition of targets that are functionally closely related to the target. Closely related kinases in the JAK family may be inhibited, however, good overall kinase selectivity has been demonstrated, with a diverse set of in vitro radioligand binding, enzyme and functional and electrophysiological assays including cardiac ion channels, not

identifying any off-target activities likely to be relevant in or near the expected plasma exposure range in humans.

Target interaction studies have been performed. In IL-13-challenged mice, the inhibition of STAT6 phosphorylation was demonstrated. Inhibition of allergic inflammation in OVA-challenged mice was also demonstrated.

4.2.3.1. Uncertainty Associated with the Available Animal Models

The studied animal models are directly relevant to humans.

Activation of IL4 receptor leads to down-stream activation of JAK1. The clinical efficacy of IL4/IL13 receptor antagonists (eg, dupilumab) have been demonstrated in asthma. Similar potency on the target has been observed between test species and human cell models. The translatability between animals and humans is good, as JAK1 is well conserved across species. Good translation between test species and clinical efficacy has been demonstrated for autoimmune diseases (eg, for tofacitinib which is approved for rheumatoid arthritis, as well as multiple other compounds in Phase II/III).

The pre-clinical data include repeated dose data in the rat OVA model. Systemic exposure in rat was described at the pharmacodynamically active dose levels. While systemic exposure is not directly relevant to local effects in the lung, the relationship between LDD and plasma levels was established pre-clinically and described in the PBPK model (see Section 3.6.1). Knowledge of this relationship will support interpretation of clinical data.

The MABEL, PAD and predicted human therapeutic dose are based on data from the rat OVA model. The direct translatability of effects in the OVA model to human asthma has not yet been shown.

The PD effects in mouse, rat and dog have been described (ie, there is no evidence of high species [human] specificity of the IMP). Similar potencies have been observed in animal and human cell-based systems, suggesting similar binding affinities and binding kinetics in animals and humans.

Pre-clinical PK were approximately dose linear. The exposure was not higher than dose proportional. Qualitatively, the metabolism between species used in the pre-clinical studies is similar to humans. The plasma half-life after inhalation is estimated to lie around 10 to 20 hours. The human PK predictions are driven by lung absorption, plasma clearance and distribution. Clearance is estimated based on well-established human hepatocyte assays. Absorption and distribution predictions are the outcome of a previously established PBPK model (see Section 3.6.1). Hence, human PK predictions are well-supported by pre-clinical data.

4.2.4. Uncertainty Associated with Non-clinical Safety Findings

AZD0449 was well tolerated in all animal toxicity studies and there were no AZD0449 related deaths or adverse clinical signs.

The target organ of toxicity is the lung.

Notable lung pathology (see Section 1.2.2.1) was observed in the rat but only at high doses (greater than 10-fold higher than the maximum proposed human inhaled dose) of AZD0449. The mechanism by which the lung pathology findings occur is uncertain, but they are considered to be primarily driven by the low solubility of AZD0449 and dose level (supported by a dose-response relationship and the identification of a clear no effect level), however a pharmacological contribution to the pathogenesis of the findings cannot be entirely excluded.

4.2.5. Uncertainties Associated with the Dosing in Part 3a/b

In Part 3a/b, the dose is planned to be the same as in the cohort from Part 2a/b (MAD) with the highest dose level CCI [redacted] There is a hypothetical risk that the DPI formulation used in Part 3a/b results in a higher systemic exposure than the same dose in the nebulized formulation in Part 2a/b. This is however unlikely as the DPI formulation is expected to result in lower lung deposition than the nebulized formulation. If no safety concerns arise in Part 2a/b and the updated predictions for Part 3a/b suggest that the systemic exposure limits will not be reached, the overall risk can be regarded as low.

4.3. Risks Associated with Study Design and Procedures

4.3.1. Risks Deriving from the Characteristics of the Study Population

It is not expected that polymorphisms in the study populations will pose a risk as systemic exposure will be low and no indication of a significant risk for drug-drug interactions was found in pre-clinical studies (see Section 4.3.2).

In general, a patient population is regarded more vulnerable than a healthy population. However, the mild asthmatic population in Part 2a and Part 3a will be otherwise healthy and stable, with no other comorbidities (see Section 4.1.3).

4.3.2. Risk for Drug-Drug Interactions

No risks of PK drug-drug interactions have been identified based on pre-clinical data. The major metabolic pathway for AZD0449 in human hepatocytes is direct glucuronidation. In vitro data as well as the low systemic exposure resulting from inhalation suggest a very low risk of AZD0449 to alter the PK of other compounds.

As AZD0449 is not associated with any drug-drug-interaction (DDI) risk via CYP3A4 induction, hormonal contraception will be considered as an acceptable highly contraceptive method in the early phase clinical studies with AZD0449. Any CYP3A4 inducing or inhibiting drug that may reduce the effect of hormonal contraceptives will not be allowed.

In summary, the risk for drug-drug interactions with inhaled AZD0449 is considered low.

4.3.3. Risks Deriving from Study Procedures

Procedures for drug administration, sampling and assessments used in this study are well-established and considered safe. No challenge agents will be administered during this study.

With IV administration, there is a general risk that maximum concentrations are higher than predicted, as this parameter is intrinsically difficult to predict, particularly in absence of human PK data. This risk will be mitigated by selecting a very low IV dose, see Section 3.5.2.1. Section 4.1.2 contains a dedicated sub-section discussing the rationale for safety of IV administration.

4.4. Risk-Benefit Assessment

4.4.1. Mitigation Plan for Rat Lung Safety Findings

The maximum allowable dose of CCI has been calculated by applying a 10-fold safety margin to the NOAEL (on a mg dose/g lung tissue basis) in the 6-month toxicity study in the rat. Rat was the most sensitive species for lung pathology findings (see Section 1.2.2.1).

While it is not possible to monitor for the lung pathology findings observed in the toxicology studies, lung function will be monitored with spirometry to evaluate any bronchoconstriction. Peripheral oxygen saturation will be monitored with pulse oximetry.

4.4.2. Mitigation Plan for Risks Related to Immune Suppression

Exclusion criteria have been defined in order to avoid including subjects with recent acute infections or risk of reactivation of latent infections such as TB and herpes zoster. Participating subjects will be monitored for infections and will have regular complete blood counts including differential white cell count during the study.

4.4.3. Benefit of Treatment with Inhaled AZD0449

Inhaled AZD0449 is expected to benefit patients with moderate to severe asthma by reducing overall inflammation, reducing respiratory exacerbations as well as improving lung function. It may also reduce the need for inhaled and oral corticosteroids, which can cause hoarseness and thrush for some patients, as well as prevent the need for biologics, which can be costly.

No benefit for individuals in this study is expected. There is however potential benefit for future patients receiving AZD0449.

4.4.4. Conclusion Risk-Benefit Assessment

Based on points described above, the risk-benefit ratio of AZD0449 is considered acceptable.

5. STUDY POPULATION AND RESTRICTIONS

5.1. Subject Selection Criteria

The Investigator should keep a subject screening log of all potential subjects who consented and were subjected to screening procedures.

Subjects who fail to meet the inclusion criteria or meet any exclusion criterion should not, under any circumstances, be randomized into the study. There can be no exceptions to this rule.

5.1.1. Part 1a/b

5.1.1.1. Inclusion Criteria

For inclusion in the study healthy volunteers should fulfill the following criteria:

1. Provision of signed and dated, written informed consent before any study specific procedures.
2. Healthy male volunteers and healthy female volunteers (for Part 1a and Part 1b first IV cohort, female volunteers must be of non-childbearing potential), aged 18 to 55 years with suitable veins for cannulation or repeated venipuncture.
3. Female patients must not be lactating and must have a negative pregnancy test at the Screening Visit and on admission to the Clinical Unit. Women of non-childbearing potential must fulfill one of the following criteria:

3.1. Postmenopausal defined as amenorrhea for at least 12 months or more following cessation of all exogenous hormonal treatments and follicle-stimulating hormone (FSH) levels in the postmenopausal range.

3.2. Documentation of irreversible surgical sterilization by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation.

4. Have a body mass index (BMI) between 18 and 30 kg/m^2 inclusive and weigh at least 60 kg.
5. Healthy volunteer has a Forced Expiratory Volume in one second (FEV_1) $\geq$80% of the predicted value regarding age, height, gender and ethnicity at the Screening Visit.
6. Male volunteers and their WOCBP partners should be willing to use highly effective contraception measures and should refrain from donating sperm or fathering a child from the first day of dosing until 3 months after the last dose of IMP.
7. Female volunteers in Part 1b second IV cohort should be willing to use highly effective contraception measures from the first day of dosing until 1 month after the last dose of IMP.

8. Provision of signed, written and dated informed consent for optional genetic research. If a volunteer declines to participate in the genetic component of the study, there will be no penalty or loss of benefit to the volunteer. The volunteer will not be excluded from other aspects of the study described in this protocol.

5.1.1.2. Exclusion Criteria

Healthy volunteers must not be randomized in the study if any of the following exclusion criteria are fulfilled:

1. History of any clinically important disease or disorder which, in the opinion of the Investigator, may either put the volunteer at risk because of participation in the study, or influence the results or the volunteer's ability to participate in the study.
2. History of any respiratory disorders such as asthma, chronic obstructive pulmonary disease (COPD) or idiopathic pulmonary fibrosis (IPF).
3. Healthy volunteer has an increased risk of infection:

3.1. History and/or presence of TB; positive result for interferon gamma release assay (IGRA) (ie, QuantiFERON TB-Gold), volunteers who have resided in regions where TB and mycosis are endemic during 90 days before screening, or who intend to visit such a region during the duration of the study ie, desert areas, Eastern Europe, Central and South America, Africa (except Egypt), Russia, Asia, Indonesia. The test may be repeated if the initial test result is indeterminate.

3.2. History of herpes zoster infection.

3.3. Any positive result for serum hepatitis B surface antigen, hepatitis C antibody, human immunodeficiency virus (HIV) and/or TB at the Screening Visit.

3.4. Is in high risk-group for HIV infection within the last 6 months (ie, men who have had unprotected sex with men, women who have had sex without a condom with men who have sex with men, people who have had sex without a condom with a person who has lived or travelled in Africa, people who inject drugs, people who have had sex without a condom with somebody who has injected drugs, people who have caught another sexually transmitted infection, people who have received a blood transfusion while in Africa, Eastern Europe, the countries of the former Soviet Union, Asia or Central and South America).

3.5. Other latent or chronic infections (eg, recurrent sinusitis, genital or ocular herpes, urinary tract infection) or at risk of infection (surgery, trauma, or significant infection) within 90 days of screening, or history of skin abscesses within 90 days of screening.

3.6. Clinically significant lower respiratory tract infection not resolved within 4 weeks prior to screening, as determined by the Investigator.

3.7. History and / or presence of COVID-19:

3.7.1. Healthy volunteer who has had a severe course of COVID-19 (ie, hospitalization, extracorporeal membrane oxygenation [ECMO], mechanically ventilated).

3.7.2. Healthy volunteer has clinical signs and symptoms consistent with COVID-19, eg, fever, dry cough, dyspnea, sore throat, fatigue or confirmed current infection by appropriate laboratory test within the last 4 weeks prior to screening or on admission to the Clinical Unit.

3.7.3. Healthy volunteer has confirmed COVID-19 infection by PCR test before randomization.

3.8. History of cancer within the last 10 years (20 years for breast cancer) except for basal and squamous cell carcinoma of the skin or in situ carcinoma of the cervix treated and considered cured. Any history of lymphoma is not allowed.

3.9. Disease history suggesting abnormal immune function.

3.10. Have received live or live-attenuated vaccine in the 4 weeks prior to dosing.

3.11. High-sensitivity C-reactive protein above upper limit of laboratory reference range at screening and on admission to the Clinical Unit.

3.12. Has a body temperature of >37.7°C on Day -1, or as judged by the Investigator.

3.13. Has an absolute neutrophil count <lower limit of normal (LLN) at the Screening Visit.

3.14. Has an absolute lymphocyte count <LLN at the Screening Visit.

4. History or presence of gastrointestinal, hepatic or renal disease or any other condition known to interfere with absorption, distribution, metabolism or excretion of drugs.

5. Any clinically important illness, medical/surgical procedure or trauma within 4 weeks of the first administration of IMP.

6. Any laboratory values with the following deviations at the Screening Visit and on admission to the Clinical Unit. Abnormal values may be repeated once at the discretion of the Investigator:

6.1. Alanine aminotransferase (ALT) >upper limit of normal (ULN).

6.2. Aspartate aminotransferase (AST) >ULN.

6.3. Creatinine >ULN.

6.4. White blood cell (WBC) count <LLN.

6.5. Hemoglobin <LLN.

7. Any clinically important abnormalities in clinical chemistry, hematology or urinalysis results, other than those described under exclusion criteria numbers 3 and 6, as judged by the Investigator.

8. Abnormal vital signs, after 10 minutes supine rest, as defined below. Abnormal values may be repeated once at the discretion of the Investigator.

8.1. Systolic BP <90 mmHg or >140 mmHg.

8.2. Diastolic BP <50 mmHg or >90 mmHg.

8.3. Pulse <45 or >85 beats per minute (bpm).

9. Any clinically important abnormalities in rhythm, conduction or morphology of the resting ECG and any clinically important abnormalities in the 12-lead ECG as considered by the Investigator that may interfere with the interpretation of QTc interval changes, including abnormal ST-T-wave morphology, particularly in the protocol defined primary lead or left ventricular hypertrophy.

9.1. Prolonged QTcF >450 ms.

9.2. Shortened QTcF <340 ms.

9.3. Family history of long QT syndrome.

9.4. PR (PQ) interval shortening <120 ms (PR >110 ms but <120 ms is acceptable if there is no evidence of ventricular pre-excitation).

9.5. PR (PQ) interval prolongation (>220 ms) intermittent second (Wenckebach block while asleep is not exclusive) or third-degree atrioventricular (AV) block, or AV dissociation.

9.6. Persistent or intermittent complete bundle branch block (BBB), IBBB, or intraventricular conduction delay (IVCD) with QRS >110 ms. Healthy volunteers with QRS >110 ms but <115 ms are acceptable if there is no evidence of eg, ventricular hypertrophy or pre-excitation.

10. Known or suspected history of drug abuse as judged by the Investigator.

11. Current smokers or those who have smoked or used nicotine products (including e-cigarettes) within the previous 6 months or has smoking history of >5 packyears.

12. History of alcohol abuse or excessive intake of alcohol as judged by the Investigator.

13. Positive screen for drugs of abuse, cotinine (nicotine) or alcohol at the Screening Visit or on admission to the Clinical Unit.

14. History of severe allergy/hypersensitivity or ongoing clinically important allergy/hypersensitivity, as judged by the Investigator or history of hypersensitivity to drugs with a similar chemical structure or class to AZD0449.

15. Excessive intake of caffeine-containing drinks or food (eg, coffee, tea, chocolate) as judged by the Investigator.
16. Use of drugs with enzyme inducing properties such as St John's Wort within 3 weeks before the first administration of IMP.
17. Use of any prescribed or nonprescribed medication including antacids, analgesics (other than paracetamol/acetaminophen), herbal remedies, megadose vitamins (intake of 20 to 600 times the recommended daily dose) and minerals during the 2 weeks before the first administration of IMP or longer if the medication has a long half-life.
18. Plasma donation within 1 month of the Screening Visit or any blood donation/blood loss >500 mL during the 3 months before the Screening Visit.
19. Non-sterilized male patients who are sexually active with a WOCBP and who do not agree to a highly effective method of contraception from Day 1 to 3 months after the last dose of the IMP. WOCBP who are sexually active with a fertile male partner and who do not agree to a highly effective method of contraception that is described in Section 5.2.3.1.
20. Has received another new chemical entity (defined as a compound which has not been approved for marketing) within 3 months of the first administration of IMP in this study. The period of exclusion begins 3 months after the final dose or 1 month after the last visit whichever is the longest.
    Note: Healthy volunteers consented and screened, but not randomized in this study or a previous Phase I study, are not excluded.
21. Healthy volunteers who have previously received AZD0449 (Part 1b second IV Cohort only).
22. Involvement of any AstraZeneca or Clinical Unit employee or their close relatives.
23. Judgment by the Investigator that the volunteer should not participate in the study if they have any ongoing or recent (ie, during the screening period) minor medical complaints that may interfere with the interpretation of study data or are considered unlikely to comply with study procedures, restrictions and requirements.
24. Healthy volunteers who cannot communicate reliably with the Investigator.
25. Vulnerable healthy volunteers, eg, kept in detention, protected adults under guardianship, trusteeship, or committed to an institution by governmental or juridical order.

In addition, any of the following is regarded as a criterion for exclusion from the genetic research:

26. Previous bone marrow transplant.

27. Non-leukocyte depleted whole blood transfusion within 120 days of the date of the genetic sample collection.

5.1.2. Part 2a/b and Part 3a/b

5.1.2.1. Inclusion Criteria

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

5.1.2.1.1. Patients with mild asthma (Part 2a and Part 3a)

1. Provision of signed and dated, written informed consent before any study specific procedures.
2. Male and female (including WOCBP) patients with mild asthma aged 18 to 55 years with suitable veins for cannulation or repeated venipuncture.
3. Patients must be willing to remain in house at the study center for 16 consecutive days (Part 2a) or for 30 consecutive days (Part 3a).
4. Female patients must not be lactating and must have a negative pregnancy test at the Screening Visit and on admission to the Clinical Unit. Women of non-childbearing potential must fulfill one of the following criteria:

4.1. Postmenopausal defined as amenorrhea for at least 12 months or more following cessation of all exogenous hormonal treatments and FSH levels in the postmenopausal range.

4.2. Documentation of irreversible surgical sterilization by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation.

5. Have a BMI between 18 and 35 kg/m^2 inclusive and weigh at least 50 kg.
6. Physician diagnosed (mild) asthma for at least 6 months prior to screening.
7. Lung function ≥70% predicted for Forced Expiratory Volume in 1 second (FEV_1) at the Screening Visit AND at the 12 h timepoint on Day -1, in accordance with the American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria.
8. Have a FeNO of ≥30 ppb at the Screening Visit and at the 12 h timepoint on Day -1.
9. Male patients and their WOCBP partners should be willing to use highly effective contraception measures and should refrain from donating sperm or fathering a child from the first day of dosing until 3 months after the last dose of IMP.
10. Female patients should be willing to use highly effective contraception measures from the first day of dosing until 1 month after the last dose of IMP.
11. Provision of signed, written and dated informed consent for optional genetic research. If a patient declines to participate in the genetic component of the study, there will be no penalty

or loss of benefit to the patient. The patient will not be excluded from other aspects of the study described in this protocol.

5.1.2.1.2. Healthy volunteers (Part 2b and Part 3b)

1. Provision of signed and dated, written informed consent before any study specific procedures.
2. Healthy male and female (including WOCBP) volunteers aged 18 to 55 years with suitable veins for cannulation or repeated venipuncture.
3. Females must not be lactating and must have a negative pregnancy test at the Screening Visit and on admission to the Clinical Unit. Women of non-childbearing potential must fulfill one of the following criteria:

3.1. Postmenopausal defined as amenorrhea for at least 12 months or more following cessation of all exogenous hormonal treatments and FSH levels in the postmenopausal range.

3.2. Documentation of irreversible surgical sterilization by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation.

4. Have a BMI between 18 and 30 kg/m^2 inclusive and weigh at least 60 kg.
5. Healthy volunteer has a Forced Expiratory Volume in one second (FEV_1) $\geq$80% of the predicted value regarding age, height, gender and ethnicity at the Screening Visit and at the 12 h timepoint on Day -1, in accordance with the ATS/ERS criteria.
6. Female volunteers should be willing to use highly effective contraception measures from the first day of dosing until 1 month after the last dose of IMP
7. Male volunteers and their WOCBP partners should be willing to use highly effective contraception measures and should refrain from donating sperm or fathering a child from the first day of dosing until 3 months after the last dose of IMP.
8. Provision of signed, written and dated informed consent for optional genetic research. If a healthy volunteer declines to participate in the genetic component of the study, there will be no penalty or loss of benefit to the healthy volunteer. The healthy volunteer will not be excluded from other aspects of the study described in this protocol.

5.1.2.2. Exclusion Criteria

Subjects must not be randomized in the study if any of the following exclusion criteria are fulfilled:

5.1.2.2.1. Patients with mild asthma (Part 2a and Part 3a)

1. History of any clinically important disease other than asthma, or disorder which, in the opinion of the Investigator, may either put the patient at risk because of participation in the study, or influence the results or the patient's ability to participate in the study.
2. History of any respiratory disorders such as, COPD or IPF.
3. Patient has an increased risk of infection:

3.1. History and/or presence of TB; positive result for IGRA (ie, QuantiFERON TB-Gold), patients who have resided in regions where TB and mycosis are endemic during 90 days before screening, or who intend to visit such a region during the duration of the study ie, desert areas, Eastern Europe, Central and South America, Africa (except Egypt), Russia, Asia, Indonesia. The test may be repeated if the initial test result is indeterminate.

3.2. History of herpes zoster infection.

3.3. Any positive result for serum hepatitis B surface antigen (or anti-HBc antibody in Germany only), hepatitis C antibody, HIV and/or TB at the Screening Visit.

3.4. Is in high risk-group for HIV infection within the last 6 months (ie, men who have had unprotected sex with men, women who have had sex without a condom with men who have sex with men, people who have had sex without a condom with a person who has lived or travelled in Africa, people who inject drugs, people who have had sex without a condom with somebody who has injected drugs, people who have caught another sexually transmitted infection, people who have received a blood transfusion while in Africa, Eastern Europe, the countries of the former Soviet Union, Asia or Central and South America).

3.5. Other latent or chronic infections (eg, recurrent sinusitis, genital or ocular herpes, urinary tract infection) or at risk of infection (surgery, trauma, or significant infection) within 90 days of screening, or history of skin abscesses within 90 days of screening.

3.6. Clinically significant lower respiratory tract infection not resolved within 4 weeks prior to screening, as determined by the Investigator.

3.7. History and / or presence of COVID-19:

3.7.1. Patient who has had a severe course of COVID-19 (ie, hospitalization, ECMO, mechanically ventilated).

3.7.2. Patient has clinical signs and symptoms consistent with COVID-19, eg, fever, dry cough, dyspnea, sore throat, fatigue or confirmed current infection by appropriate laboratory test within the last 4 weeks prior to screening or on admission to the Clinical Unit.

3.7.3. Patient has confirmed COVID-19 infection by PCR test before randomization.

3.8. History of cancer within the last 10 years (20 years for breast cancer) except for basal and squamous cell carcinoma of the skin or in situ carcinoma of the cervix treated and considered cured. Any history of lymphoma is not allowed.

3.9. Disease history suggesting abnormal immune function.

3.10. Have received live or live-attenuated vaccine in the 4 weeks prior to dosing.

3.11. High-sensitivity C-reactive protein above upper limit of laboratory reference range at screening and on admission to the Clinical Unit.

3.12. Has a body temperature of >37.7°C on Day -1, or as judged by the Investigator.

3.13. Has an absolute neutrophil count <LLN at the Screening Visit.

3.14. Has an absolute lymphocyte count <LLN at the Screening Visit.

4. History or presence of gastrointestinal, hepatic or renal disease or any other condition known to interfere with absorption, distribution, metabolism or excretion of drugs.

5. Any clinically important illness, medical/surgical procedure or trauma within 4 weeks of the first administration of IMP.

6. Suspicion of Gilbert's syndrome.

7. Any laboratory values with the following deviations at the Screening Visit and on admission to the Clinical Unit. Abnormal values may be repeated once at the discretion of the Investigator:

7.1. Alanine aminotransferase >1.5 ULN.

7.2. Aspartate aminotransferase >1.5 ULN.

7.3. Creatinine >ULN.

7.4. White blood cell count <LLN.

7.5. Hemoglobin <LLN.

8. Any clinically important abnormalities in clinical chemistry, hematology or urinalysis results, other than those described under exclusion criteria numbers 3 and 7, as judged by the Investigator.

9. Abnormal vital signs, after at least 10 minutes supine rest, deviations at the Screening Visit and on admission to the Clinical Unit, as defined below. Abnormal values may be repeated once at the discretion of the Investigator:

9.1. Systolic BP <90 mmHg or >140 mmHg (or <89 mmHg or >139 mmHg in Germany)

9.2. Diastolic BP <50 mmHg or >90 mmHg.

9.3. Pulse <45 or >85 bpm (or <50 or >90 bpm in Germany).

10. Any clinically important abnormalities in rhythm, conduction or morphology of the resting ECG and any clinically important abnormalities in the 12 Lead ECG as considered by the Investigator that may interfere with the interpretation of QTc interval changes, including abnormal ST-T-wave morphology, particularly in the protocol defined primary lead or left ventricular hypertrophy at the Screening Visit and Day -1, as defined below.

10.1. Prolonged QTcF >450 ms.

10.2. Shortened QTcF <340 ms.

10.3. Family history of long QT syndrome.

10.4. PR (PQ) interval shortening <120 ms (PR >110 ms but <120 ms is acceptable if there is no evidence of ventricular pre-excitation).

10.5. PR (PQ) interval prolongation (>240 ms) intermittent second (Wenckebach block while asleep is not exclusive) or third-degree AV block, or AV dissociation.

10.6. Persistent or intermittent complete BBB. Patients with IVCD and QRS >110 ms but <120 ms are acceptable if there is no evidence of eg, ventricular hypertrophy or pre-excitation.

11. Known or suspected history of drug abuse within the past 2 years as judged by the Investigator.

12. Current smokers or those who have smoked or used nicotine products (including e-cigarettes) within the previous 6 months or has smoking history of >5 packyears.

13. History of alcohol abuse or excessive intake of alcohol within the past 2 years as judged by the Investigator (in Germany only: excessive intake of alcohol defined as the regular consumption of more than 3 units [24 g] of alcohol per day for men or 2 units [16 g] of alcohol per day for women).

14. Positive screen for drugs of abuse, alcohol or cotinine (nicotine) at the Screening Visit and/or on admission to the Clinical Unit.

15. History of severe allergy/hypersensitivity or ongoing clinically important allergy/hypersensitivity, as judged by the Investigator or history of hypersensitivity to drugs with a similar chemical structure or class to AZD0449.

16. Excessive intake of caffeine-containing drinks or food (eg, coffee, tea, chocolate,) as judged by the Investigator.

17. Use of drugs with enzyme inducing properties such as St John's Wort within 3 weeks before the first administration of IMP.

18. Exacerbation of asthma symptoms within 6 months prior to Screening and Day -1 and requiring the use of oral or IV steroids, antibiotics, Accident and Emergency visit, or hospital admission.

19. Use of the following medicines within the specified time before Screening:

19.1. Long-acting β_2 agonists; none for 4 weeks prior to Screening.

19.2. Anti-IgE or anti−IL-5 or anti-IL4R therapy; for 6 months prior to Screening.

19.3. Inhaled corticosteroids (ICS) >500 µg per day of beclometasone dipropionate (BDP) or equivalent within 16 weeks prior to Screening.

19.4. Any ICS at any dose at screening or within 4 weeks prior to screening or at randomization.

19.5. Oral or injectable steroids for treatment of asthma or respiratory tract infection within 6 months prior to Screening.

19.6. Intranasal steroids within 4 weeks prior to Screening.

19.7. Leukotriene antagonists within 2 weeks prior to Screening.

19.8. Xanthines (excluding caffeine), anticholinergics, or cromoglycate within 1 week prior to Screening.

19.9. Short acting bronchodilator other than for rescue and within 12 hours of Screening and Day -1 assessments.

20. Use of any prescribed or nonprescribed medication including, but not limited to antacids, analgesics other than paracetamol/acetaminophen, herbal remedies, megadose vitamins (intake of 20 to 600 times the recommended daily dose) and minerals during the 2 weeks before the first administration of IMP or longer if the medication has a long half-life. Short-acting inhaled beta agonist for the purposes of rescue only is allowed for Part 2a and Part 3a (Patients with mild asthma).

21. Plasma donation within 1 month of the Screening Visit or any blood donation/blood loss >500 mL during the 3 months before the Screening Visit.

22. Female patients who are pregnant, breastfeeding, or are planning a pregnancy during the study period or within 1 month after the last dose of IMP.

23. Non-sterilized male patients who are sexually active with a WOCBP and who do not agree to a highly effective method of contraception from Day 1 to 3 months after the last dose of the IMP. WOCBP who are sexually active with a fertile male partner and who do not agree to a highly effective method of contraception that is described in Section 5.2.3.1.

24. Has received another new chemical entity (defined as a compound which has not been approved for marketing) within 3 months of the first administration of IMP in this study. The period of exclusion begins 3 months after the final dose or 1 month after the last visit whichever is the longest.
    Note: Patients consented and screened, but not randomized in this study or a previous Phase I study, are not excluded.

25. Patients who have previously received AZD0449.

26. Involvement of any AstraZeneca or Clinical Unit employee or their close relatives.

27. Judgment by the Investigator that the patient should not participate in the study if they have any ongoing or recent (ie, during the screening period) minor medical complaints that may interfere with the interpretation of study data or are considered unlikely to comply with study procedures, restrictions and requirements.

28. Patients who cannot communicate reliably with the Investigator.

29. Vulnerable patients, eg, kept in detention, protected adults under guardianship, trusteeship, or committed to an institution by governmental or juridical order.

In addition, any of the following is regarded as a criterion for exclusion from the genetic research:

30. Previous bone marrow transplant.

31. Nonleukocyte depleted whole blood transfusion within 120 days of the date of the genetic sample collection.

5.1.2.2.2. Healthy volunteers (Part 2b and Part 3b)

1. History of any clinically important disease or disorder which, in the opinion of the Investigator, may either put the volunteer at risk because of participation in the study, or influence the results or the volunteer's ability to participate in the study.

2. History of any respiratory disorders such as asthma, COPD or IPF.

3. Healthy volunteer has an increased risk of infection:

3.1. History and/or presence of TB; positive result for IGRA (ie, QuantiFERON TB-Gold), volunteers who have resided in regions where TB and mycosis are endemic during 90 days before screening, or who intend to visit such a region during the duration of the study ie, desert areas, Eastern Europe, Central and South America, Africa (except Egypt), Russia, Asia, Indonesia. The test may be repeated if the initial test result is indeterminate.

3.2. History of herpes zoster infection.

3.3. Any positive result for serum hepatitis B surface antigen (or anti-HBc antibody in Germany only), hepatitis C antibody, HIV and/or TB at the Screening Visit.

3.4. Is in high risk-group for HIV infection within the last 6 months (ie, men who have had unprotected sex with men, women who have had sex without a condom with men who have sex with men, people who have had sex without a condom with a person who has lived or travelled in Africa, people who inject drugs, people who have had sex without a condom with somebody who has injected drugs, people who have caught another sexually transmitted infection, people who have received a blood transfusion while in Africa, Eastern Europe, the countries of the former Soviet Union, Asia or Central and South America).

3.5. Other latent or chronic infections (eg, recurrent sinusitis, genital or ocular herpes, urinary tract infection) or at risk of infection (surgery, trauma, or significant infection) within 90 days of screening, or history of skin abscesses within 90 days of screening.

3.6. Clinically significant lower respiratory tract infection not resolved within 4 weeks prior to screening, as determined by the Investigator.

3.7. History and / or presence of COVID-19:

3.7.1. Healthy volunteer who has had a severe course of COVID-19 (ie, hospitalization, ECMO, mechanically ventilated).

3.7.2. Healthy volunteer has clinical signs and symptoms consistent with COVID-19, eg, fever, dry cough, dyspnea, sore throat, fatigue or confirmed current infection by appropriate laboratory test within the last 4 weeks prior to screening or on admission to the Clinical Unit.

3.7.3. Healthy volunteer has confirmed COVID-19 infection by PCR test before randomization.

3.8. History of cancer within the last 10 years (20 years for breast cancer) except for basal and squamous cell carcinoma of the skin or in situ carcinoma of the cervix treated and considered cured. Any history of lymphoma is not allowed.

3.9. Disease history suggesting abnormal immune function.

3.10. Have received live or live-attenuated vaccine in the 4 weeks prior to dosing.

3.11. High-sensitivity C-reactive protein above upper limit of laboratory reference range at screening and on admission to the Clinical Unit.

3.12. Has a body temperature of >37.7°C on Day -1, or as judged by the Investigator.

3.13. Has an absolute neutrophil count <LLN at the Screening Visit.

3.14. Has an absolute lymphocyte count <LLN at the Screening Visit.

4. History or presence of gastrointestinal, hepatic or renal disease or any other condition known to interfere with absorption, distribution, metabolism or excretion of drugs.

5. Any clinically important illness, medical/surgical procedure or trauma within 4 weeks of the first administration of IMP.

6. Any laboratory values with the following deviations at the Screening Visit and on admission to Clinical Unit. Abnormal values may be repeated once at the discretion of the Investigator:

6.1. Alanine aminotransferase >ULN.

6.2. Aspartate aminotransferase >ULN.

6.3. Creatinine >ULN.

6.4. White blood cell count <LLN.

6.5. Hemoglobin <LLN.

7. Any clinically important abnormalities in clinical chemistry, hematology or urinalysis results, other than those described under exclusion criteria numbers 3 and 6, as judged by the Investigator.

8. Abnormal vital signs, after at least 10 minutes supine rest, as defined below. Abnormal values may be repeated once at the discretion of the Investigator:

8.1. Systolic BP <90 mmHg or >140 mmHg (or <89 mmHg or >139 mmHg in Germany).

8.2. Diastolic BP <50 mmHg or >90 mmHg.

8.3. Pulse <45 or >85 bpm (or <50 or >90 bpm in Germany).

9. Any clinically important abnormalities in rhythm, conduction or morphology of the resting ECG and any clinically important abnormalities in the 12-lead ECG as considered by the Investigator that may interfere with the interpretation of QTc interval changes, including abnormal STTwave morphology, particularly in the protocol defined primary lead or left ventricular hypertrophy at the Screening Visit and Day 1, as defined below.

9.1. Prolonged QTcF >450 ms.

9.2. Shortened QTcF <340 ms.

9.3. Family history of long QT syndrome.

9.4. PR (PQ) interval shortening <120 ms (PR >110 ms but <120 ms is acceptable if there is no evidence of ventricular pre-excitation).

9.5. PR (PQ) interval prolongation (>220 ms) intermittent second (Wenckebach block while asleep is not exclusive) or third-degree AV block, or AV dissociation.

9.6. Persistent or intermittent complete BBB, IBBB, or IVCD with QRS >110 ms. Healthy volunteers with QRS >110 ms but <115 ms are acceptable if there is no evidence of eg, ventricular hypertrophy or pre excitation.

10. Known or suspected history of drug abuse as judged by the Investigator.

11. Current smokers or those who have smoked or used nicotine products (including e-cigarettes) within the previous 6 months or has smoking history of >5 packyears.

12. History of alcohol abuse or excessive intake of alcohol as judged by the Investigator (in Germany only: excessive intake of alcohol defined as the regular consumption of more than 3 units [24 g] of alcohol per day for men or 2 units [16 g] of alcohol per day for women).

13. Positive screen for drugs of abuse, cotinine (nicotine) or alcohol at the Screening Visit and/or on admission to the Clinical Unit.

14. History of severe allergy/hypersensitivity or ongoing clinically important allergy/hypersensitivity, as judged by the Investigator or history of hypersensitivity to drugs with a similar chemical structure or class to AZD0449.

15. Excessive intake of caffeine-containing drinks or food (eg, coffee, tea, chocolate,) as judged by the Investigator.

16. Use of drugs with enzyme inducing properties such as St John's Wort within 3 weeks before the first administration of IMP.

17. Use of any prescribed or nonprescribed medication including antacids, analgesics (other than paracetamol/acetaminophen), herbal remedies, megadose vitamins (intake of 20 to 600 times the recommended daily dose) and minerals during the 2 weeks before the first administration of IMP or longer if the medication has a long half-life.

18. Plasma donation within 1 month of the Screening Visit or any blood donation/blood loss >500 mL during the 3 months before the Screening Visit.

19. Female healthy volunteers who are pregnant, breastfeeding, or are planning a pregnancy during the study period or within 1 month after the last dose of IMP.

20. Non-sterilized male healthy volunteers who are sexually active with a WOCBP and who do not agree to a highly effective method of contraception from Day 1 to 3 months after

the last dose of the IMP. WOCBP who are sexually active with a fertile male partner and who do not agree to a highly effective method of contraception that is described in Section 5.2.3.1.

21. Has received another new chemical entity (defined as a compound which has not been approved for marketing) within 3 months of the first administration of IMP in this study. The period of exclusion begins 3 months after the final dose or 1 month after the last visit whichever is the longest.
    Note: Healthy volunteers consented and screened, but not randomized in this study or a previous Phase I study, are not excluded.
22. Healthy volunteers who have previously received AZD0449.
23. Involvement of any AstraZeneca or Clinical Unit employee or their close relatives.
24. Judgment by the Investigator that the healthy volunteers should not participate in the study if they have any ongoing or recent (ie, during the screening period) minor medical complaints that may interfere with the interpretation of study data or are considered unlikely to comply with study procedures, restrictions and requirements.
25. Healthy volunteers who cannot communicate reliably with the Investigator.
26. Vulnerable healthy volunteers, eg, kept in detention, protected adults under guardianship, trusteeship, or committed to an institution by governmental or juridical order.

In addition, any of the following is regarded as a criterion for exclusion from the genetic research:

27. Previous bone marrow transplant.
28. Nonleukocyte depleted whole blood transfusion within 120 days of the date of the genetic sample collection.

5.2. Restrictions During the Study

5.2.1. Part 1a/b

The following restrictions apply for the specified times during the study period:

1. For Part 1a, on Day 1, healthy volunteers will be fasted for 10 hours before IMP administration until 2 hours after IMP administration, when healthy volunteers will receive a light breakfast. No fluids will be allowed apart from water which can be given until 1 hour before IMP administration and then from 1 hour after IMP administration.
2. Healthy volunteers should not engage in any strenuous activity from 72 hours before (first) admission to the Clinical Unit until after their Follow-up Visit.

3. Prior to (each) treatment period healthy volunteers should abstain from alcohol for 72 hours before admission to the Clinical Unit until after their last PK sampling visit. Healthy volunteers should also abstain from alcohol for 72 hours before their Follow-up Visit.
4. Prior to (each) treatment period healthy volunteers should abstain from caffeine-containing foods and beverages for 24 hours before admission to the Clinical Unit until discharge from the Clinical Unit.
5. Healthy volunteers should abstain from grapefruit or grapefruit juice, Seville oranges, quinine (eg, tonic water) from 7 days before admission to the Clinical Unit until after their Follow-up Visit.
6. During admission periods, healthy volunteers will receive a standard diet, which excludes all alcohol and grapefruit-containing products. No additional food or beverages must be consumed while in the Clinical Unit.
7. During the healthy volunteers' outpatient periods, healthy volunteers should abstain from consuming high energy drinks (eg, Red Bull), and food containing poppy seeds and any over-the-counter (OTC) medication or herbal preparations until after their Follow-up Visit has been completed. Healthy volunteers should also limit their caffeine intake to equivalent of 3 cups of coffee per day (1 cup=12 oz soda, 6 oz coffee, or 8 oz tea). Healthy volunteers should consume no more than 2 units of alcohol per day and completely abstain from alcohol from 72 hours before their next admission to the Clinical Unit.
8. Healthy volunteers will be required to abstain from blood or plasma donation until 3 months after the final medical examination at the study Follow-up Visit.
9. Healthy volunteers must observe and comply with study site COVID-19 procedures.
10. For medication restrictions, please refer to Section 7.7.

5.2.2. Part 2a/b and Part 3a/b

The following restrictions apply to all subjects (patients with asthma and healthy volunteers) for the specified times during the study period:

1. On Day 1 and 12 for Part 2a/b and Part 3a/b, subjects will be fasted for 10 hours before IMP administration until 2 hours after IMP administration, when subjects will receive a light breakfast. On Day 3 to 11 for Part 2a/b and Part 3a/b, subjects will be fasted for 10 hours before IMP administration until 1 hour after IMP administration, when subjects will receive a light breakfast. No fluids will be allowed apart from water which can be given until 1 hour before IMP administration and then from 1 hour after IMP administration.

2. Subjects should not engage in any strenuous activity from 72 hours before (first) admission to the Clinical Unit until after their Follow-up Visit (Part 2a)/last day of study (Part 2b, 3a, and 3b).

3. Subjects should abstain from alcohol for 72 hours before admission to the Clinical Unit until after their last PK sampling visit. Subjects should also abstain from alcohol for 72 hours before their Follow-up Visit (Part 2a)/last day of study (Part 2b, 3a, and 3b).

4. Subjects must not consume any food or beverages for 1 hour prior to each FeNO measurement. The intake of foods with high nitrate content must be limited as far as possible (eg, vegetable juices, salads, lettuce, radishes, celery, broccoli, cauliflower, spinach, rocket, beets, parsley, leeks, cabbage, fennel, turnips, carrots, cured meats, bacon and carbonated drinks) within 6 hours prior to each FeNO measurement.

5. Subjects should abstain from caffeine-containing foods and beverages for 24 hours before admission to the Clinical Unit until discharge from the Clinical Unit. Decaffeinated beverages are allowed.

6. Subjects should abstain from grapefruit or grapefruit juice, Seville oranges, quinine (eg, tonic water) from 7 days before admission to the Clinical Unit until after their Follow-up Visit (Part 2a)/last day of study (Part 2b, 3a, and 3b).

7. During admission, subjects will receive a standard diet, which excludes all alcohol and grapefruit-containing products. No additional food or beverages must be consumed while in the Clinical Unit.

8. Subjects will be required to abstain from blood or plasma donation until 3 months after the final medical examination at the study Follow-up Visit (Part 2a)/last day of study (Part 2b, 3a, and 3b).

9. Subjects must observe and comply with study site COVID-19 procedures.

10. For medication restrictions, please refer to Section 7.7.

11. If permitted by local relevant regulatory authorities and considered feasible and safe to do so, the extended safety monitoring period may be conducted as non-residential visits for subjects participating in Part 2b, Part 3a and 3b. Please refer to Section 5.2.1 for restrictions on non-residential/outpatient visits.

5.2.3. Reproductive Restrictions

Female subjects who are enrolled in this study must have a negative serum pregnancy at the Screening Visit, and a negative urine pregnancy test performed on admission to the Clinical Unit, and a date of last menstruation consistent with non-pregnancy prior to randomization. A pregnancy test will also be conducted at the study Follow-up Visit/last day of study.

5.2.3.1. Women of Childbearing Potential

WOCBP defined as women between menarche and menopause who have not been permanently or surgically sterilized and are capable of procreation.

In study Part 1b (second IV cohort), Part 2a/b, and Part 3a/b, WOCBP subjects should be stable on their chosen method of birth control for at least 1 month before first dosing.

A highly effective method of contraception is defined as one that can achieve a failure rate of less than 1% per year when used consistently and correctly. Women of childbearing potential who are sexually active with a non-sterilized male partner must agree to use one highly effective method of birth control, as defined below, from enrolment throughout the study and until at least 1 month after last dose of study intervention. Cessation of contraception after this point should be discussed with a responsible physician. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method are not acceptable methods of contraception. Female condom and male condom should not be used together. All WOCBP must have a negative serum pregnancy test result at Visit 1.

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation.
    - Oral
    - Intravaginal (eg, NuvaRing®, etonogestrel/ethinyl estradiol vaginal ring)
    - Transdermal (eg, Norelgestromin/ethinyl estradiol transdermal system, eg, Ortho Evra®)
- Progestogen-only hormonal contraception associated with inhibition of ovulation.
    - Oral (eg, Cerazette®, progestin/desogestrel)
    - Injectable (eg, Depo-Provera®)
    - Implantable (eg, Etonogestrel implants, Implanon®, Norplan®)
- Intrauterine device (IUD).
- Intrauterine hormone-releasing system (IUS) (eg, levonorgestrel-releasing IUS, Mirena®).
- Bilateral tubal occlusion.
- Vasectomised partner (only acceptable provided that partner is the sole sexual partner of the subject and that the vasectomised partner has received medical assessment of the surgical success).

- Sexual abstinence is defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. It is only acceptable if it is the subject's preferred and usual lifestyle.

In addition, one barrier method must also be used, ie, condom (**without** foam/gel/film/cream/suppository or fat- or oil-containing lubricants); or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. Double barrier methods (a combination of male condom with either a cap, diaphragm or sponge with spermicide) are not considered to be highly effective methods of contraception.

Pregnancy Testing

In study Part 1b (second IV cohort), Part 2a/b, and Part 3a/b, WOCBP can be included only after a negative highly sensitive serum pregnancy test. Additionally urine pregnancy testing will be done as per the Schedule of Assessments (Table 3.2-3, Table 3.2-4, Table 3.2-5, Table 3.2-6, Table 3.2-7).

Pregnancy

If a subject becomes pregnant during the study this should be reported to the Investigator. The Investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study. The pregnancy will be followed up and the status of mother and child will be reported to the Sponsor after delivery.

A pregnancy notification form and follow-up will be completed.

5.2.3.2. Women of Non-Childbearing Potential

In all study parts of the study women of non-childbearing potential can be enrolled in the study. Women of non-childbearing potential are defined as female subjects who are permanently surgically sterilized or postmenopausal.

Permanent sterilization includes hysterectomy and/or bilateral oophorectomy and/or bilateral salpingectomy at least 6 weeks before screening but excludes bilateral tubal ligation. Bilateral oophorectomy alone is acceptable only when the reproductive status of the woman has been confirmed by follow-up hormone level assessment. Women who have undergone tubal occlusion should be managed on the study as if they are of WOCBP.

Females are considered postmenopausal if they have had amenorrhea for at least 12 months or more following cessation of all exogenous hormonal treatments and without an alternative medical cause, and the FSH levels are in the postmenopausal range.

5.2.3.3. Male Subjects

Restrictions for Male Subjects

There is no information about the effects that AZD0449 could have on the development of the fetus in humans. Therefore, it is important that WOBCP partners of male subjects do not become pregnant during the study and should follow highly effective contraceptive methods (see list in Section 5.2.3.1) for a total period of 3 months after the male subject has received his last dose of IMP.

As a precaution, all non-sterilized male subjects should:

- Use a condom without spermicide (Germany only) or condom and spermicide to prevent pregnancy and drug exposure of a partner from the day of the first administration of the IMP until 3 months after the last administration of the IMP.
- In addition to a condom with/without spermicide, a second highly effective method of contraception (see list in Section 5.2.3.1) or true sexual abstinence* should be used with female partners of childbearing potential until 3 months after the last administration of the IMP. Double barrier methods (a combination of male condom with either a cap, diaphragm or sponge with spermicide) are not considered to be highly effective methods of contraception.

* True abstinence refers to: When this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a study, and withdrawal are not acceptable methods of contraception.

Male subjects/patients who have been sterilized are required to use one barrier method of contraception (eg, condom with spermicide) from the time of IMP administration until 3 months after the last dose of the IMP. The subject must have received medical assessment of the surgical success.

Sperm Donation

Male subjects should not donate sperm for the duration of the study and for at least 3 months after the last dose of IMP.

Pregnancy of a female partner of a male subject

Subjects will be instructed that if their partner becomes pregnant during the study this should be reported to the Investigator. The Investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study. In the event that a subject's partner is subsequently found to be pregnant after the volunteer is included in the study, then

consent will be sought from the partner and if granted any pregnancy will be followed and the status of mother and child will be reported to the Sponsor after delivery.

A pregnancy notification form and follow-up will be completed.

5.3. Replacement of Subjects

Subjects who are withdrawn from the study due to AEs or changes in safety parameters may be replaced to ensure that adequate cohort sizes are maintained and if the Sponsor's responsible physician and the PI agree it is safe to do so. Subjects who withdraw or are withdrawn from the study for other reasons may also be replaced following discussion with the Sponsor. Replacement subjects will be allocated the same treatment as the subject being replaced.

6. STUDY STOPPING RULES

If rules specified in this Section are fulfilled, dosing **must** be stopped (please refer to Section 3.3 for further information).

6.1. Stopping for an Individual Subject, at Any Time in the Study

Dosing for any individual subject **must** be stopped if the subject experiences a possible drug-related SAE or a possibly drug-related significant non-serious AE, which in the opinion of the SRC or PI warrants discontinuation of the subject from the active CSP for his or her well-being.

The subject is free to withdraw their consent to participate in the study at any time. If the subject withdraws their consent, the subject's participation in the study will be stopped.

6.2. Stopping Rules for a Whole Cohort, Dose Escalation, Progression to Next Study Part and Termination of Study

Stopping rules as detailed below (general, cardiovascular, laboratory and AZD0449 specific) are applicable for each of the following situation:

- Stopping dosing for a whole cohort
  - When subjects in a cohort are dosed staggered
  - During multiple dosing
- Stopping dose escalation
- Stopping progression to the next part of the study
- Final stop of study

General Criteria

The study will be placed on temporary hold (defined as treatment stopped for enrolled subjects; and stop of enrolment of subjects into the study) pending further safety data analysis if any of the following criteria occur in subjects receiving AZD0449:

- A "serious" adverse reaction (ie, a SAE considered at least possibly related to the IMP administration) in one subject;
- A "severe" non-serious adverse reactions (ie, severe non-serious AEs considered as, at least, possibly related to the AZD0449 administration) in 2 subjects in the same cohort, independent of within or not within the same system organ class.

The risk to all participating subjects will be evaluated thoroughly before a decision as to whether to terminate the study prematurely or continue dosing in agreement with the regulatory authorities.

The SRC will carefully review the totality of data, taking into account moderate non-serious AEs at least possibly related to the IMP administration in unblinded fashion, the number of subjects in which they occur, concurrency of more than one within the same subject and potential safety signals identified for other IMPs in the same class (mechanistic and/or chemical). Although changes outside normal ranges that apply for healthy volunteers are most relevant, changes from baseline measurements will also considered observing any trends.

Cardiovascular Criteria

- Two or more subjects, that receive AZD0449, have QTc prolongation defined as QTcF >500 ms, or a prolongation from baseline of >60 ms, confirmed (persistent for at least 5 minutes) and determined post-dose either during continuous 12-lead ECG monitoring or on a repeat 12-lead ECG.
- Two or more subjects, who receive AZD0449, have tachycardia defined as resting supine HR >125 bpm persisting for at least 10 minutes.
- Two or more subjects, who receive AZD0449, have symptomatic bradycardia defined as resting supine HR <45 bpm or asymptomatic bradycardia defined as resting supine HR <30 bpm while awake persisting for at least 10 minutes.
- Two or more subjects, who receive AZD0449, develop hypertension defined as an increase in resting supine systolic BP >40 mmHg to above 180 mmHg and persisting for at least 10 minutes.

Laboratory Findings

- One or more subjects, who receive AZD0449, fulfill Hy's Law (HL) defined as "An increase in AST or ALT ≥3 x ULN and Total bilirubin (TBL) ≥2 x ULN, where no other reason can be found to explain the combination of increases, eg, elevated serum alkaline phosphatase (ALP) indicating cholestasis, viral hepatitis, another drug." The elevations do not have to be at the same time or within a specified time frame (see Appendix 15.3 for follow-up procedures).
- Two or more subjects, who receive AZD0449, have confirmed >3 x ULN of either ALT or AST, or >2 x ULN for bilirubin or ALP.
- Two or more subjects, who receive AZD0449, have confirmed leukocyte count <2.0 x 10^9/L.

- Two or more subjects, who receive AZD0449, have confirmed neutrophil count $<1.0 \times 10^9$/L.
- Two or more subjects, who receive AZD0449, have confirmed platelet count $<75 \times 10^9$/L.
- Two or more subjects, who receive AZD0449, have confirmed serum creatinine increase to >1.5 x ULN.

COVID-19

Patients who test positive for having active COVID-19 infection will be discontinued from the study and followed up until the outcome of the AE is established.

Study Specific Stopping Criteria Related to AZD0449

Please refer to Section 3.4.1 where rationale for exposure limits is explained in detail. The total and free exposure limits have been considered and the most conservative approach has been chosen.

When emerging PK data will be reviewed, the maximum exposure observed in individual subjects within a cohort rather than the mean exposure should be taken into account.

Pulmonary Criteria

- Two or more subjects/patients who receive AZD0449 in a cohort, or 3 or more subjects/patients in total who received AZD0449, have a fall in FEV_1 ≥30% compared with the baseline value at pre-dose on Day 1, within 1 hour after administration of the IMP.

If bronchoconstriction occurs leading to a reduction of 12% (200 cc) in FEV_1, the subject should be treated with bronchodilators and measurements should be repeated until FEV_1 values have normalized.

Dose and PK Criteria for Stopping Dose Escalation in Part 1a

When emerging PK data will be reviewed, the maximum exposure observed in individual volunteers within a cohort rather than the mean exposure will be taken into account. Dose escalation will be stopped if any of the following criteria has been met or if the escalated dose level is predicted to meet any of the following criteria:

- The NOAEL-based maximum dose has been reached (CCI AZD0449 delivered dose).
- The upper end of the estimated human PD dose range has been reached (ED_{96}) (CCI AZD0449 delivered dose).

- Any individual volunteer reaches the C_{max} of 188 nmol/L.
- Any individual volunteer reaches the AUC of 1200 nmol*h/L.
- A C_{max} of 188 nmol/L is predicted to be reached in the next cohort.
- An AUC of 1200 nmol*h/L is predicted to be reached in the next cohort.

Dose and PK-criteria for Stopping Dose Escalation in Part 2a/b (MAD)

When emerging PK data will be reviewed, the maximum exposure observed in individual patients within a cohort rather than the mean exposure will be taken into account. Dose escalation will be stopped if any of the following criteria has been met or if the escalated dose level is predicted to meet any of the following criteria:

- The NOAEL-based maximum dose has been reached (CCI AZD0449 delivered dose).
- The upper end of the estimated human PD dose range has been reached (ED_{96}) (CCI AZD0449 delivered dose).
- Any individual patient reaches the C_{max} of 188 nmol/L.
- Any individual patient reaches the $AUC_{(0-24)}$ (steady state) of 1200 nmol*h/L.
- A C_{max} of 188 nmol/L is predicted to be reached in the next cohort.
- An $AUC_{(0-24)}$ (steady state) of 1200 nmol*h/L is predicted to be reached in the next cohort.

7. TREATMENTS

7.1. Identity of the Investigational Medicinal Products

The IMPs used in this study are described in Table 7.1-1.

Table 7.1-1 Identity of the Investigational Medicinal Products

AZD0449 for Inhalation	
Supplier:	AstraZeneca AB, R & D Gothenburg
Formulation 1:	AZD0449 nebulizer suspension CCI
Formulation 2:	AZD0449 nebulizer suspension CCI
Formulation 3:	AZD0449 nebulizer suspension CCI
Packaging Unit:	Type I glass vials
Dose:	Dose range CCI delivered dose
Route of Administration:	Inhalation
Specific Device for Drug Administration:	Jet Nebulizer (Provo.X system)
Regimen:	Single and multiple ascending dose
Special Handling Requirements:	Sonication of suspension before use
Placebo for AZD0449 for Inhalation	
Supplier:	AstraZeneca AB, R & D Gothenburg
Formulation:	Placebo for AZD0449 nebulizer suspension
Strength/Concentrations:	N/A
Dose:	N/A
Route of Administration:	Inhalation
Specific Device for Drug Administration:	Jet Nebulizer (Provo.X system)
Regimen:	Single and multiple ascending dose
Special Handling Requirements:	No
AZD0449 for Intravenous Administration	
Supplier:	AstraZeneca AB, R & D Gothenburg
Formulation:	AZD0449 solution for infusion CCI
Strength/Concentrations:	CCI
Packaging Unit:	Type I glass vials
Dose:	Cohort 1: CCI total dose; cohort 2: CCI total dose
Route of Administration:	Intravenous
Specific Device for Drug Administration:	Infusion pump set
Regimen:	Two single doses
Special Handling Requirements:	Sonication of solution before use

AZD0449 for Inhalation via DPI	
Formulation:	AZD0449 inhalation powder: CCI
Dose:	Dose range CCI delivered dose
Route of Administration:	Inhalation via DPI
Specific Device for Drug Administration:	SD3FL version of Genuair
Regimen:	Repeated inhaled administration
Special Handling Requirements:	No

Placebo for Inhalation via DPI	
Formulation:	Placebo for AZD0449 inhalation powder
Strength/Concentrations/Dose:	N/A
Route of Administration:	Inhalation via DPI

Specific Device for Drug Administration:	SD3FL version of Genuair
Regimen:	Repeated inhaled administration
Special Handling Requirements:	No

Details of the batch numbers will be included in the trial master file and the final CSR.

7.2. Supply of Investigational Medicinal Product

AZD0449 will be supplied by AstraZeneca as non-subject specific open-label bulk to the Clinical Units.

A technical agreement between the Investigator and AstraZeneca will be in place to cover all pharmacy related activities, detailing roles and responsibilities before receipt of the IMPs at the Clinical Units.

A release document signed by a legally authorized Qualified Person (QP) at the Clinical Units will be placed in the appropriate section of the Trial Master File to document labeling and dispensing of the IMP to the subject.

7.3. Storage and Handling Procedures

The IMP will be stored in a secure facility under appropriate storage conditions. Details of storage conditions will be provided on the label of the IMP.

AstraZeneca will be permitted upon request to audit the supplies, storage, dispensing procedures and records provided that the blind of the study is not compromised.

7.4. Labeling

Labels will be prepared in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines. The labels will fulfill GMP Annex 13 requirements and medical device directive for labeling.

7.5. Drug Accountability, Dispensing and Destruction

The IMP provided for this clinical study will be used only as directed in the CSP.

In accordance with Good Clinical Practice (GCP), the Clinical Units will account for all supplies of the IMP. Details of receipt, storage, assembly/dispensing and return will be recorded.

Since compound inhalation may lead to contamination of the plasma PK samples, appropriate measures will be taken to avoid such contamination. Due to the low limit of quantification of the analytical assay, even smallest amounts may pose a problem. Contamination is theoretically possible through aerosol depositing or through compound spilling onto eg, vials, blood sampling equipment, skin, clothes or gloves. Remedial measures will include:

- The study site team will be made aware of the risk of sample contamination in the project-specific training
- Administration of IMP will take place in a room separate from the room where blood samples will be drawn. During administration patients and study personnel will wear disposable gowns and gloves which will be discarded immediately after each administration in the room used for inhalation, to avoid subsequent contamination of blood samples.

All used and unused supplies of the IMP will be destroyed by each Clinical Unit at the end of the study. The certificate of delivery and destruction must be signed, in accordance with instruction by AstraZeneca. Destruction must not take place unless the responsible person at AstraZeneca has approved it.

7.6. Dose and Treatment Regimens

7.6.1. Part 1a

Each volunteer will receive a single inhaled dose of AZD0449 or placebo administered with a nebulizer.

The dose will be administered after an overnight fast of at least 10 hours.

Volunteers will be allowed to drink water to prevent dehydration until 1 hour before IMP administration.

Water will be allowed ad libitum from 1 hour after IMP administration and a light breakfast will be provided 2 hours after IMP administration.

Other restrictions, including posture control are described in Section 5.2. Data of volunteers may be excluded from the PK analysis set as described in Section 10.3.2.

7.6.2. Part 1b

In the first IV cohort, each volunteer will receive a single IV dose of CCI AZD0449 administered over 60 minutes (see Section 3.5.2). In the second IV cohort, each volunteer will receive a single IV dose of CCI AZD0449 administered over 48 minutes (see Section 3.5.2).

Other restrictions, including posture control are described in Section 5.2. Data of volunteers may be excluded from the PK analysis set as described in Section 10.3.2.

7.6.3. Part 2a/b

Each subject will receive a multiple inhaled dose of AZD0449 or placebo administered with a nebulizer.

Other restrictions, including posture control are described in Section 5.2. Data of subjects may be excluded from the PK analysis set as described in Section 10.3.2.

7.6.4. Part 3a/b

Each subject will receive a multiple inhaled dose of CCI AZD0449 or placebo administered with a DPI.

Other restrictions, including posture control are described in Section 5.2. Data of subjects may be excluded from the PK analysis set as described in Section 10.3.2.

7.7. Concomitant and Post-study Treatment(s)

Apart from paracetamol/acetaminophen no concomitant medication or therapy will be allowed.

A Short Acting Beta Agonist (SABA), may be used for the purposes of rescue only and not as a regular use medication.

The subjects should be instructed that no other medication is allowed, including herbal remedies, vitamin supplements and over-the-counter products, without the consent of the Investigator. Contraceptive medication will be permitted as described in Section 5.2.3.

Medication, which is considered necessary for the subject's safety and well-being, may be given at the discretion of the Investigator during the residential period.

When any medication is required, the Investigator should prescribe it. Following consultation with AstraZeneca Lead Physician, the Investigator should determine whether or not the subject should continue in the study. Administration of concomitant medications that may influence the measurement of the PK and/or PD endpoints may be documented as a protocol deviation after consultation of the Investigator with AstraZeneca Lead Physician.

7.8. Treatment Compliance

Dosing will take place at the Clinical Unit. Subject compliance will be assured by direct supervision and witnessing of IMP administration.

Administration of IMP will be recorded in DataLabs®.

7.9. Randomization

7.9.1. Subject Enrolment and Randomization

The PI will ensure:

- Signed informed consent is obtained from each potential subject before any study specific procedures are performed.
- Each potential subject is assigned a unique enrolment number at the Screening Visit upon signing the Informed Consent Form (ICF).
- The eligibility of each subject is in accordance with the inclusion and exclusion criteria.
- Each eligible subject is assigned a unique randomization code (subject number).

Randomization codes will be assigned strictly sequentially as subjects become eligible for randomization, starting from eg, 101 (no leading zero(s)).

When using unique enrolment number, the specific format must be followed (ie, reduced enrolment number, eg, "1001" in DataLabs and on labels, full enrolment number, eg, "E0001001" for outputs).

If a subject withdraws his/her participation in the study, then his/her enrolment/randomization code cannot be reused.

Replacement subjects will be allocated the same treatment as the subject being replaced, in order to ensure that the minimum number of subjects are dosed with AZD0449 or placebo. For each cohort, an additional set of random numbers will be generated within the AstraZeneca randomization system (AZRand) according to the same treatment allocation ratio. The unblinded Pharmacist at the Clinical Unit will allocate a replacement number such that the replacement subject receives a random number having the same treatment allocation.

7.9.2. Procedures for Randomization

Upon completion of the randomization requirements specifications form, the randomization will be produced by Parexel according to AZRand.

The randomization will be completed for each part and cohort using consecutive randomization codes.

Once a randomization number has been allocated to one subject, it may not be assigned to another subject. If subjects withdraw prematurely from the study and are replaced under the direction of the Sponsor, then a new randomization number will be assigned. The replacement

subjects will be assigned to the same treatment as the discontinued subject using a randomization number that corresponds to the specific treatment.

7.9.3. Procedures for Handling Incorrectly Randomized Subjects

Subjects who fail to meet the inclusion criteria or meet any exclusion criterion should not, under any circumstances, be randomized into the study. There can be no exceptions to this rule.

Where a subject, who does not meet the selection criteria, is randomized in error and this is identified before dosing, the subject should be withdrawn from the study. If a subject is withdrawn before dosing they will be replaced.

If a subject, who does not meet the selection criteria, has been dosed before the error is identified, the subject should be advised to continue safety assessments to ensure their safety. The PI will inform the AZ Lead Physician of the error and a joint decision made as to whether the subject should be replaced.

7.10. Blinding and Procedures for Unblinding the Study

7.10.1. Methods for Ensuring Blinding

The randomization list should be kept in a secure location until the data base is lock or equivalently, clean file is declared.

The pharmacokineticist will be unblinded to perform the PK analyses after each cohort, for the SRC meeting.

The following personnel will have access to the randomization list:

- The Clinical Unit personnel carrying out the labeling and packaging of subject specific treatments
- The pharmacy personnel preparing IMP at the site
- The personnel performing the bioanalyses of the plasma/urine samples

The randomization list should be kept in a secure location until the end of the study.

7.10.1.1. Part 1a

This study is single-blind with regard to treatment (AZD0449 or placebo) at each dose level.

AZD0449 and placebo will be matched for formulation and amount. Volunteers randomized to placebo will receive the same nebulized volume as volunteers on active drug.

7.10.1.2. Part 1b

Part 1b will be open-label.

7.10.1.3. Part 2a/b

This study is single-blind with regard to treatment (AZD0449 or placebo) at each dose level.

AZD0449 and placebo will be matched for formulation and amount. Subjects randomized to placebo will receive the same nebulized volume as subjects on active drug.

7.10.1.4. Part 3a/b

This study is single-blind with regard to treatment (AZD0449 or placebo).

AZD0449 and placebo will be matched for formulation and amount. The same DPI will be used for subjects randomized to placebo as subjects on active drug.

7.10.2. Methods for Unblinding the Study

The treatment code should not be broken except in medical emergencies when the appropriate management of the subject requires knowledge of the treatment randomization. The Investigator documents and reports the action to AstraZeneca, without revealing the treatment given to subject to the AstraZeneca staff. Under some conditions, the SRC may be required to review "unblinded" data and an adequate procedure should be followed to maintain the blinding or allow breaking the blind for non-emergency reasons.

In the event of a medical emergency when management of a subject's condition requires knowledge of the trial medication, the treatment received may be revealed by personnel authorized by the PI. If possible, such emergencies are to be discussed with AstraZeneca before disclosure of the treatment allocation. Reasons for breaking a code will be clearly explained and justified in DataLabs. The date on which the code was broken together with the identity of the person responsible will also be documented.

8. MEASUREMENTS AND METHODS OF ASSESSMENT

8.1. Appropriateness of Measurements

Standard measures to assess PK, safety and tolerability apply during the study. For the single doses of AZD0449 planned to be given during this study, no safety issues are expected.

The timing of assessments are indicated in the Schedule of Assessments, Table 3.2-1 (Part 1a), Table 3.2-2 and Table 3.2-3 (Part 1b), Table 3.2-4, and Table 3.2-5 (Part 2a/b), Table 3.2-6 and Table 3.2-7 (Part 3a/b).

8.2. Safety and Eligibility Measurements

Safety and tolerability variables will include:

- Adverse events
- Additional safety monitoring
- Vital signs (systolic and diastolic BP, pulse, respiratory rate and body temperature)
- Electrocardiograms (12-lead ECGs and telemetry)
- Physical examination
- Spirometry
- Pulse oximetry
- Laboratory assessments (hematology, clinical chemistry [including plasma glucose and triglycerides] and urinalysis)

Serology, urine drugs of abuse, alcohol and cotinine will be assessed for eligibility. Follicle-stimulating hormone (female volunteers only in Part 1a and Part 1b [first IV cohort], and postmenopausal patients only in Part 1b [second IV cohort], Part 2a/b, and Part 3a/b), pregnancy testing (female subjects only) and use of concomitant medication will also be assessed and reported.

8.2.1. Adverse Events

See Section 11.2.3.

8.2.2. Vital Signs

The following variables will be collected after the subject has rested in the supine position for at least 10 minutes, in accordance with the site's standard operating procedure (SOPs):

- Systolic BP (mmHg)

- Diastolic BP (mmHg)
- Pulse (bpm)
- Respiratory rate (supine)
- Oral or tympanic body temperature

8.2.3. Electrocardiography

8.2.3.1. Twelve lead Safety Electrocardiogram

At the time-points specified in the Schedule of Assessments (Section 3.2.2), 12-lead ECGs will be obtained after the subject rested in the supine position for at least 10 minutes (using the Clinical Unit's own ECG device when not performing dECGs and using the same device as used for the dECGs when time-points coincide).

The PI will judge the overall interpretation as normal or abnormal and this evaluation will be reported in DataLabs. If abnormal, it will be further documented as to whether or not the abnormality is clinically significant by the PI. For all abnormalities (regardless of clinical significance) the specific type and nature of the abnormality will be documented in DataLabs. Clinically significant findings should also be documented on the AE page of the case report form (CRF) if applicable.

The PI may add extra 12-lead resting safety ECGs assessments if there are any abnormal findings or if the PI considers it is required for any other safety reason. These assessments should be entered as an unscheduled assessment.

All ECG readings will be digitally stored as source documents.

8.2.3.2. Electronic Capture of 12-lead Continuous Digital Electrocardiogram

Continuous 12-lead digital ECG recordings will be performed using the site´s Mortara Telemetry Surveyor equipment. At the time-points specified in the Schedule of Assessments (Section 3.2.2), 12-lead continuous dECG files will be extracted over at least 5 minutes from the Mortara Telemetry Surveyor or H12+ continuous files and transmitted to the AstraZeneca central dECG repository, according to AstraZeneca ECG Center´s standard procedures, settings, recording and transmission of dECGs.

The same recording device will be used for each subject at all time-points, when possible. Date and time settings must be checked on the Mortara Telemetry Surveyor or H12+ at the start of each study day and aligned with an official timekeeper.

Skin preparation must be thorough and electrode positions must be according to standard 12-lead ECG placement.

Electrode positions will be marked with an indelible pen at the start of each study day to ensure exact reposition. Permanent electrodes will be applied at least 30 minutes before first study recording and left in place for the duration of each relevant study day.

Subjects will rest in a supine position for at least 10 minutes (can be reduced **accordingly** at collection time-points within the first hour after dosing) before the start of each recording. The subject should be in the same supine body position (maximum 30 degrees flexion of the hip and feet not in contact with the footboard) at each recording time-point during the study.

The metadata for all dECG files will be checked by the responsible personnel at the study site to ensure that the files transferred to the AstraZeneca central dECG files repository can be approved by the AZ ECG Center to be made accessible to the ECG Scientific Advisors for analysis. As standard, 10-second ECGs will be extracted by the EClysis© system twice per minute from the continuous recording and initially automatically analysed by the software.

Lead V2 will be analysed and reported as primary. Lead V5 will be analysed, for all visits, as backup for the individual where analysis in lead V2 is not deemed possible for pre-dose, for significant parts of visits or for whole visits.

The ECG Scientific Advisor will perform all required manual corrections to the ECG annotations provided automatically by EClysis©.

To provide a decision basis for dose escalation, the ECG Scientific Advisor(s) will perform a preliminary analysis of the first 24 hours of dECG recordings in lead V2, with the main focus on QT changes, wave morphology changes and dysrhythmia.

The AZ ECG Center Cardiologist will review the data, perform an evaluation and interpretation of the findings and will provide a safety report for the SRC meeting.

The AZ ECG Center Cardiologist will review the totality of the data and perform all necessary adjustments before locking the EClysis© data into a read-only state, before the data will be exported.

The numerical values for ECG intervals and amplitudes will be exported and made accessible on the AstraZeneca dECG Central repository to accredited data management specialists for conversion into SAS® files.

The following dECG variables will be reported by the AstraZeneca ECG Center: RR, PR, QRS and QT intervals from the lead defined as the primary analyses lead. Derived parameters (QTcF, HR and others, as applicable) are calculated by the study statistician or delegate.

8.2.3.3. Real-Time ECG (Cardiac Telemetry)

A minimum of 2-lead real-time telemetry ECG will be performed at the time-points specified in the Schedule of Assessments (Section 3.2.2).

The telemetry monitoring system will be reviewed by the Investigator and paper printouts of any clinically important events will be stored as source data.

8.2.4. Physical Examination

Full

The complete physical examinations will include an assessment of the general appearance, skin, cardiovascular, respiratory, abdomen, head, and neck (including ears, eyes, nose, and throat), lymph nodes, thyroid, musculoskeletal and neurological systems.

Brief (Abbreviated)

The brief physical examinations will include an assessment of the general appearance, skin, cardiovascular system, respiratory and abdomen.

8.2.5. Spirometry

Spirometry measurements, FEV_1 and forced vital capacity (FVC) (maximal volume of air exhaled in liters with maximally forced expiratory effort from a position of maximal inspiration) will be performed at the time-points outlined the Schedule of Assessments (Section 3.2.2), and in accordance with the site's SOPs.

8.2.6. Pulse Oximetry

Pulse oximetry will be performed and SpO_2 will be measured at the time-points outlined the Schedule of Assessments (Section 3.2.2), and in accordance with the site's SOPs.

8.2.7. Laboratory Assessments

Table 8.2-1 Hematology

Hematology	
White blood cell (WBC) count	Neutrophils absolute count
Red blood cell (RBC) count	Lymphocytes absolute count
Hemoglobin (Hb)	Monocytes absolute count
Hematocrit (HCT)	Eosinophils absolute count
Mean corpuscular volume (MCV)	Basophils absolute count
Mean corpuscular hemoglobin (MCH)	Platelets
Mean corpuscular hemoglobin concentration (MCHC)	Reticulocytes absolute count

Table 8.2-2 Clinical Chemistry

Clinical Chemistry	
Sodium	Alkaline phosphatase (ALP)
Potassium	Alanine aminotransferase (ALT)
Urea	Aspartate aminotransferase (AST)
Uric acid	Gamma glutamyl transpeptidase (GGT)
Creatinine	Total Bilirubin (TBL)
Albumin	Unconjugated bilirubin
Calcium	Triglycerides
Phosphate	Follicle-stimulating hormone (FSH) [a] (post-menopausal females only)
Glucose (fasting in plasma)	Thyroid-stimulating hormone (TSH) [a]
High-Sensitivity C-reactive protein (hs-CRP)	

a) Screening only

Table 8.2-3 Urinalysis

Urinalysis
Glucose
Protein
Blood
Microscopy (if positive for protein or blood): RBC, WBC, Casts (Cellular, Granular, Hyaline)

Table 8.2-4 Pregnancy Testing

Pregnancy test (females only)
Human beta chorionic gonadotrophin

Table 8.2-5 Serology

Serology	
Human immunodeficiency virus (HIV) I and II	Hepatitis C Virus antibody
Hepatitis B surface antigen (HBsAg)	Anti-HBc antibody [a]
QuantiFERON® TB	

a) Germany only

Table 8.2-6 Drugs of Abuse, Alcohol and Cotinine

Drugs of Abuse and Alcohol	
Amphetamine	Benzodiazepines
Ethanol	Methadone Metabolites
Cannabinoids	Barbiturates
Cocaine	Phencyclidine
Opiates	Urine Creatinine
Cotinine	
Tricyclic anti-depressants (TCA)	

Table 8.2-7 COVID-19 Testing

COVID-19 Testing
Antibody, PCR, or alternative COVID-19 test will be conducted subject to local availability and site guidance

8.2.8. Concomitant Medication

See Section 7.7.

8.3. Pharmacokinetics

8.3.1. Sample Collection and Handling

Blood samples for the determination of plasma concentrations of AZD0449 will be collected as specified in the Schedule of Assessments (Section 3.2.2). CCI [redacted]

Samples will be collected, handled, labeled, stored and shipped as detailed in the Laboratory Manual.

8.3.2. Pharmacokinetic Drug Assays

Blood samples for determination of AZD0449 and CCI [redacted] concentrations in plasma will be analysed by Covance Laboratories Ltd on behalf of AstraZeneca, using validated assays. Additional analyses may be conducted on the biological samples to further investigate the presence and/or identity of drug metabolites.

CCI [redacted]

Placebo samples will not be analysed, unless there is a need to confirm that correct treatment has been given to study subjects.

Full details of the analytical methods and analyses performed will be described in a separate bioanalytical report.

CCI [redacted]

CCI [redacted]

[redacted]

8.5. Pharmacodynamic Assessments

8.5.1. Fractional Exhaled Nitric Oxide Part 2a and Part 3a

The FeNO assessments will be performed at the time-points specified in the Schedule of Assessments (Section 3.2.2). See Section 5.2.2 for restrictions that needs to be met before FeNO assessment can be performed.

During inflammation associated with bronchial asthma, nitric oxide (NO) will be released by cells within the bronchial tree, including eosinophil leucocytes, via activation of inducible nitric oxide synthase (iNOS). Since NO is a gas, it will be exhaled and is believed to be a valuable biomarker of airway inflammation.

The measurement of exhaled NO is performed during regular slow exhalation. The maneuver will be described detail in study specific manual.

CCI [redacted]

[redacted]

[redacted]

[redacted]

8.6. Exploratory Assessments

CCI [redacted]

[redacted]

CCI

CCI [redacted]

[redacted]

[redacted]

[redacted]

8.7. Procedures for Handling of Biological Samples

Samples will be disposed of, on instruction from AstraZeneca, after the CSR has been finalized, unless samples are retained for additional or future analyses.

8.7.1. Storage and Destruction of Biological Samples

Samples will be disposed of, on instruction from AstraZeneca, after the CSR has been finalized, unless samples are retained for additional or future analyses.

8.7.1.1. Pharmacokinetic Samples and CCI [redacted]

Pharmacokinetic and CCI [redacted] samples will be disposed of after the bioanalytical report finalization or 6 months after issuance of the draft bioanalytical report (whichever is earlier), unless requested for future analyses.

Pharmacokinetic samples may be disposed of or anonymized by pooling. Additional analyses may be conducted on the anonymized, pooled PK samples to further evaluate and validate the analytical method. Any results from such analyses may be reported separately from the CSR.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples. The results from the evaluation will not be reported in the CSR but separately in a bioanalytical report.

CCI [redacted]

[redacted]

CCI [redacted]

CCI [redacted]

[redacted]

8.7.2. Labeling and Shipment of Biohazard Samples

Samples will be labeled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria) (for International Airline Transportation Association [IATA] guidance, see Appendix 15.2 of this CSP).

Any samples identified as Infectious Category A materials will not be shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labeling, shipment and containment provisions are approved.

8.7.3. Chain of Custody of Biological Samples

A full chain of custody will be maintained for all samples throughout their life cycle.

The PI will ensure full traceability of collected biological samples from the subjects while in storage at the Clinical Unit until shipment and will keep documentation of receipt of arrival.

The sample receiver will keep full traceability of samples while in storage and during use, until used, disposed of, or until further shipment or disposal (where appropriate) and will keep documentation of receipt of arrival.

Samples retained for further use will be registered in the AstraZeneca bio-bank system during the entire life cycle.

8.7.4. Withdrawal of Informed Consent for Donated Biological Samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed if not already analysed and the action documented. As collection of donated biological samples is an integral part of the study, consent withdrawal implies that the subject is withdrawn from further study participation.

If a subject only withdraws consent to use the donated samples for the optional genetic and biomarker analyses, the subject's participation in the study will not be influenced.

AstraZeneca ensures the laboratory holding the samples is informed about the withdrawn consent immediately and that samples are disposed of or destroyed, the action documented, and the signed document returned to the Clinical Unit.

9. DATA QUALITY ASSURANCE AND DATA MANAGEMENT

9.1. Quality Control and Source Data Verification

Source data verification will be conducted with due regard to subject confidentiality.

The Clinical Unit will allow the study monitor and Sponsor representative direct access to all study documents, medical files and source documents to enable verification of the study data, while maintaining the anonymity of the subject and confidentiality of the data.

Internal quality control will be performed at all stages of the study by the Clinical Unit.

9.2. Audit/Inspections

The Clinical Unit facilities and all study data/documentation may be audited/inspected by independent auditor/inspector/any representatives of regulatory authorities. The Investigator must allow the applicable persons access to all relevant facilities and data/documents. The Investigator must be available to discuss any findings/issues.

If an audit was performed, the audit certificate will be included in the CSR.

9.3. Study Monitoring

The conduct of the study will be monitored by an independent Parexel monitor or a subcontracted monitor to ensure compliance with applicable regulatory requirements and GCP. The summary of the documentation of the monitoring visits will form part of the study documentation and will be archived as such.

9.4. Data Collection

The DataLabs system is an electronic source data capturing and information management system. The system combines all aspects of source data capturing with process control and clinical study management. All clinical and laboratory data, except those which are paper-based, will be collected in DataLabs. Only paper-based data will be subject to data entry. For electronic source data, no data entry will be performed.

The responsible study monitor will check data at the monitoring visits to the Clinical Unit. The Investigator will ensure that the data collected are accurate, complete and legible. Data will be monitored within DataLabs by the study monitor before being exported. Any changes made during monitoring will be documented with a full audit trail within DataLabs.

9.4.1. Case Report Forms and Source Documents

All data obtained using paper collection methods during the clinical study will be recorded in DataLabs. All source documents from which DataLabs entries are derived should be placed in the subject's personal records.

The original DataLabs entries for each subject will be checked against source documents by the study monitor. Instances of missing or uninterpretable data will be discussed with the Investigator for resolution.

9.4.2. Access to Source Documents

During the course of the clinical study, a study monitor will make Clinical Unit visits to review protocol compliance, compare DataLabs entries and individual subject's personal records, assess IMP accountability and ensure that the clinical study is being conducted according to pertinent regulatory requirements. DataLabs entries will be verified against source documents. The review of medical records will be handled confidentially to ensure subject anonymity.

Checking of the DataLabs entries for completeness and clarity and verifying with source documents, will be required to monitor the clinical study for compliance with GCP and other regulations. Moreover, regulatory authorities of certain countries, IECs may wish to carry out source data inspections on-site, and the Sponsor's clinical quality assurance group may wish to carry out audits. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and subject confidentiality. The Investigator assures the Sponsor of the necessary support at all times.

9.5. Data Management

Parexel will utilize standardized and validated procedures and systems to collect, process and file the clinical data of this study. Any system used will be compliant with FDA 21 CFR Part 11 requirements.

A data management plan (DMP) will be prepared to describe the processes and data-flow within the clinical study. Timelines, versions for the computer systems and the coding will be defined in the DMP, and if applicable, Sponsor-specific requests will also be documented within. The DMP will be finalized before first dose where possible but before database lock.

A data validation specification (DVS) will be created to outline the validation checks to be performed during the study. The DVS must be finalized before data validation.

After the data has been monitored by the responsible study monitor all data received will be reviewed, logged and filed.

The raw data intended for further processing will be checked by standard routines or according to the DVS and queries will be generated and sent to the Investigator for review and resolution. Corrections resulting from these queries will be confirmed on the data clarification forms (DCFs). This process will be repeated until no further discrepancies are found. The data will then be declared as clean. Applicable documentation will be stored in the study files.

Only trained study staff will have access to the clinical database and every change in data will have a full audit trail.

10. STATISTICAL ANALYSES

10.1. Overview

The statistical methodology below describes the statistical analysis as it is foreseen when the study is being planned.

If circumstances should arise during the study rendering the analysis inappropriate, or if in the meantime improved methods of analysis should come to light, eg triggered by changes to the design of the study, different analyses may be performed. All further details of the statistical analyses and methods will be contained in a separate SAP which will be finalized before the final unblinding of the data. Any deviations from the statistical methodology defined in this protocol, reasons for such deviations and all alternative/additional statistical analyses that may be performed will be described in the CSR.

10.2. General Statistical Methodology

All results will be presented by treatment (dose level of AZD0449 or pooled placebo) and overall, where applicable, with descriptive statistics appropriate to the nature of the variables for each study part separately. For continuous variables, the number of non-missing observations, mean, SD, median, minimum and maximum, will be presented; for categorical variables: counts (n) and percentages (%) (where specified) will be presented. These summaries will be provided by time-point of assessment as appropriate. If at a given time-point, n<3, then only n, minimum and maximum will be presented. If n=3, then only n, mean, median, minimum and maximum will be presented. The other descriptive statistics will be left blank.

All original and derived parameters as well as demographic and disposition data will be listed and described using summary statistics. All safety data (scheduled and unscheduled) will be presented in the data listings.

The following rules will apply to any repeated safety assessments occurring within each treatment period:

- If the repeated measurement of a specific parameter occurs before IMP administration (Day 1), then the last obtained value before dosing will be used in the descriptive statistics and in the calculation of changes from baseline;
- If the repeated measurement of a specific parameter occurs after IMP administration (Day 1), then the first (non-missing) value after dosing will be used in descriptive statistics and in the calculation of changes from baseline.

The planned sequence for measurement of multiple assessments at the same time-point is described in Section 3.2.3.

For safety assessments performed at screening and the Follow-up Visit/last day of study, the following rules will apply for any repeated assessments:

- If the repeated assessment occurs at the Screening Visit the last available value will be used in the summary statistics;
- If the repeated assessment occurs at the Follow-up Visit/last day of study, the first non-missing assessment will be used in the summary statistics.

All statistical analyses and production of tables, figures and listings will be performed using SAS® version 9.4. or later. A complete set of raw data listings will be appended to the final CSR. All tables, figures and listings will be presented in portable document format (PDF) documents without any manual editing, ie, they will appear unmodified as programmed by means of the statistical package.

10.2.1. Missing Data

There will be no imputation of missing data except of data analysis mentioned below in this section. All subjects will be included into the safety analyses as far as the data permit.

The AE related missing data will be handled as described in Section 10.6.4.1. Concentrations that are below limit of quantification (BLQ) in the PK data will be handled as described in Section 10.6.5.2.

The analysis of the change from baseline to Day 12 in 2-hour post-dose FeNO and $AUC_{(0-12)}$ will be based on a mixed effect model of repeated measures (MMRM) analysis. This analysis will be conducted using data for observed cases (OC) with no data imputation. In addition, further analysis of covariance (ANCOVA) (see Section 10.6.6.1) will be conducted for the change from baseline to Day 12 in 2-hour post-dose FeNO and $AUC_{(0-12)}$ using the last observation carried forward (LOCF) method. Under the LOCF methodology, the last post-baseline observation, ie, FeNO assessment or $AUC_{(0-12)}$, before the missing value will be carried forward to the missing time-points.

10.3. Study Analyses Sets

10.3.1. Safety Analysis Set

The Safety analysis set (SAF) will include all subjects who received at least 1 dose of AZD0449 and for whom any safety post-dose data are available.

Unless otherwise stated the SAF will be used for the presentation of all demographic and disposition data, as well as all safety analyses. Exposure to IMP will also be presented using the SAF.

10.3.2. Pharmacokinetic Analysis Set

The PK analysis set (PKS) will consist of all subjects who received at least 1 dose of AZD0449 and who have evaluable PK data, with no major protocol deviations thought to impact on the analysis of the PK data. All protocol deviations that occur during the study will be considered for their severity/impact and will be taken into consideration when subjects are assigned to the PK analysis sets.

Data from subjects for whom the pre-dose concentration is >5% of C_{max} for the first dose of AZD0449 on Day 1 of the dosing period may be excluded from the statistical analysis and corresponding figures. If there are no other reportable PK data for a subject, then they may be excluded from the PK analysis set. The exclusion of any subjects or time-points from the calculation of the PK parameters and/or PK summary statistics will be agreed at the blinded review meeting and will be documented by the PK Scientist including the reason(s) for exclusion.

The available concentration data and PK parameter data for any subjects excluded from the PK analysis set will be listed only. Concentration data for subjects excluded from the PK analysis set will be presented in the individual figures of concentration versus time plots.

10.3.3. Randomized Set

The Randomized Set (RS) will consist of all subjects randomized into the study.

10.3.4. Enrolled Analysis Set

The Enrolled Analysis Set (EN) will include all subjects who signed informed consent.

10.3.5. Full Analysis Set

The Full Analysis Set (FAS) will consist of all subjects randomized into the study, receiving at least 1 dose of IMP and having at least one 2-hour post-dose FeNO assessment after the first IMP intake.

10.3.6. Per Protocol Set

The Per Protocol analysis set (PP) will consist of all subjects who belong to the FAS and who have no major protocol deviations that might impact results of FeNO.

10.4. Determination of Sample Size

This is a Phase I study to investigate the safety and tolerability of a novel compound. The sample size was chosen to obtain reasonable evidence of safety and tolerability without exposing undue numbers of subjects to the compound at this phase of clinical development.

Previous experience in Phase I studies has shown that the sample size being proposed is reasonable to accomplish the objectives of the study. Thus, no formal sample size calculation was done for Part 1 and the non-PoM cohorts in Part 2a/b and Part 3.

For Part 3a (PoM cohort):

- Endpoint: change from baseline in exhaled FeNO (log scale) while receiving multiple inhaled doses of AZD0449, in active and placebo arms.
- Treatment difference (Treatment – Placebo) of change from baseline of mean Log FeNO, equivalent to a 25% reduction of the ratio of geometric mean.

For Part 3a, assuming a log mean baseline FeNO level of 4.24 with SD of 0.38 (geometric coefficient of variation [GCV]=39.4%) and a correlation between baseline and last day of study data of 0.7, as suggested by previous studies [19], the required sample size for a 25% absolute reduction in the ratio of geometric means (a 0.288 reduction of the mean log FeNO levels) is 18 evaluable patients per arm using a one-sided test at 5% significance level.

The current design presents an opportunity to perform a sample size calculation at an IA taking place when approximately half the data from Part 3a is available. If the actual estimate of baseline FeNO SD is higher than expected, reaching SD=0.55, the sample size of the PoM cohort in Part 3a will be potentially increased up to n=26 per arm.

Should the variation be even higher, requiring adjustments beyond this, an amendment will be submitted.

10.4.1. Interim Analysis

As mentioned above, an unblinded IA will be performed when approximately 50% of the patients have completed Part 3a. The collected data will be used to inform various decisions or actions such as assessing the efficacy of AZD0449 in an unblinded manner and to perform an unblinded sample size re-estimation. Based on the results from the IA (PK, PD [FeNO] and safety), the PoM cohort size will be decided. Further details will be included in the SAP for Part 3. The latter will detail the nature and the exact criteria of the decisions as well as handling of the data and actual decision makers.

10.5. Protocol Deviations

Protocol deviations are considered any deviation from the CSP relating to a subject, and include the following:

- Inclusion/exclusion criteria deviations.
- Dosing deviations (eg, incorrect treatment received, subject was not fasted as per the CSP requirements before and after dosing).
- Time window deviations for safety and/or PK assessments.
- Subjects receiving prohibited concomitant medications.
- Other procedural and study conduct deviations recorded by the Clinical Unit on a CSP deviation log.

The criteria for the assessment and reporting of protocol deviations will be stipulated in a separate study specific protocol deviation specification (PDS) document. This will include a Windows Allowance Document (WAD), which stipulates tolerance windows for safety, PK and PD assessments. Measurements performed within these tolerance windows will not be considered as protocol deviations and will not be reported.

All protocol deviations will be discussed at the Data Review Meeting (DRM) before database hard lock in order to define the analysis sets for the study. The precise reasons for excluding patients from the study populations will be fully agreed on in the DRM report.

Important protocol deviations will be summarized and listed by subject for all randomized subjects.

Protocol deviations will be handled in accordance with Parexel SOPs.

For handling of CSP amendments, see Section 12.6.

10.6. Statistical Methods

10.6.1. Subject Disposition

Subjects and/or data excluded from the PK analysis set will be listed including the reason for exclusion.

Subject disposition will be summarized separately for screening failures and randomized subjects for each part of the study. Screening failures data will be based on the EN set and will include the following information: number of subjects screened, number and percentage of subjects with screen failure including the reason withdrawal. Percentage will be based on the number of screening failures.

Disposition summaries of randomized subjects will be presented by treatment group and overall and include the following information: number of subjects randomized, number and percentage of subjects who received/ did not receive the treatment, completed the treatment period/study and the number and percentage of subjects who were withdrawn after randomization (including reasons for withdrawal). Disposition data will be presented based on all subjects randomized.

Subject discontinuations will be listed including the date of study exit, duration of treatment and reason for discontinuation.

An inform consented/randomization listing will be presented and include the following: each subject's randomization number, date of informed consent, date of randomization and the dose/treatment to which the subject has been randomized.

Number and percentage of subjects in each analysis set will be presented.

10.6.2. Demographic and Baseline Data

Demographic variables (age, gender, race, ethnicity, height, weight and BMI) will be listed by subject. Demographic characteristics (age, gender, race and ethnicity) and subject characteristics (height, weight and BMI) will be summarized by treatment (dose level of AZD0449 and pooled placebo), for all subjects in the SAF, for each part of the study. The denominator for percentages will be the number of subjects in the SAF for each treatment or for all subjects as applicable, in that study part.

Medical history data will be listed by subject including visit, description of the disease/procedure, Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC), MedDRA Preferred Term, start date, and stop date (or ongoing if applicable), for each study part.

10.6.3. Prior and Concomitant Medication and Drug Administration

10.6.3.1. Prior and Concomitant Medication

Prior medications are those that started and stopped before the first dose of IMP; all medications taken after first dosing are considered as concomitant (including medications that started before dosing and continued after). Prior medication started within 3 months before the first dose of IMP will be recorded also in the concomitant medication module of DataLabs.

Prior and concomitant medication medications will be listed by subject and will include the following information: reported name, preferred term, the route of administration, dose, frequency, start date/time, duration and indication, for each part of the study. Prior and

concomitant medication will be coded according to the World Health Organization Drug Dictionary (WHO DD).

The duration will be calculated as:

Duration = end date/time – start date/time

The duration may be presented in hours or days in the listing depending on the applicability to the emerging data. For medications with partial or completely missing start date/times and/or end date/times, the duration will not be calculated.

Medications with missing or partial start date/time and/or end date/time such that it is not possible to classify as prior or concomitant will be considered as concomitant in the listings.

10.6.3.2. Drug Administration

Drug administration dates and times will be listed for each subject for all parts of the study.

Exposure in days and treatment compliance will be summarized using descriptive statistics by treatment (dose level of AZD0449 and pooled placebo) for all subjects in the SAF for Part 2a/b and Part 3a/b. As well as number and percent of subjects in the following categories:

- <70% of compliance
- ≥70% and ≤130% of compliance
- >130% of compliance

10.6.4. Analysis of Safety Data

All safety data (scheduled and unscheduled) will be presented in the data listings. If not otherwise stated, continuous variables will be summarized using descriptive statistics (n, mean, SD, minimum, median, maximum) by treatment, for each study part. Categorical variables will be summarized in frequency tables (frequency and proportion) by treatment and study part. All summaries will be based on the SAF unless otherwise stated.

10.6.4.1. Adverse Events

All AEs will be coded using MedDRA vocabulary and will be listed for each subject.

Adverse events with missing start dates/times will be handled as follows:

- Adverse events with unknown start times, but with start date known, will be imputed with a time of 00:00, unless the start date corresponds to any given dosing date. In this case, the start time will be imputed with the time of dosing. If this

results in a start date/time after end date/time of the AE, then the time will also be imputed with 00:00.

- Adverse events with completely unknown start dates will be imputed with the date and time of dosing, unless the end date is known and before dosing; in that case the start date will be imputed as the date of screening and a time of 00:00.
- Adverse events with partially known start dates/times will be treated as follows:

– If only the day is missing, then the day will be imputed with the first day of the month, unless the month and year in which the AE started is a month and year in which IMP was administered, then the day will be imputed with the first day on which IMP was administered in that month. If this results in a start date after the end date, then the day will be imputed with the first day of the month.

– If only the month is missing and the year is a year in which IMP was administered, then the month will be imputed with the first month in which IMP was administered. If this results in a start date after the end date of the AE, then the month will be imputed with January (JAN). If the known year part is not a year in which IMP was administered, then the month will also be imputed with JAN.

– If both the day and month is missing and the year is a year in which IMP was administered, then the day and month will be imputed with the day and month of dosing. If this results in a start date after end date, then the day and month will be imputed with 01JAN. If the year is not a year in which IMP was administered, then the day and month will also be imputed with 01JAN.

– If only the year is missing, then the year will be imputed with the year of dosing.

Adverse events with Unknown Intensity, Relationship and Seriousness:

- For the purposes of the AE summaries, AEs with Unknown Intensity will be treated as 'severe' for the tabulations. Adverse events with unknown relationship will be treated as 'related' for the tabulations. Adverse events with unknown seriousness will be treated as serious for the tabulations.

There will be no imputation of AE data for the data listings. All data will be listed as recorded in the CRF.

Adverse events will be summarized by each dose of AZD0449 or pooled placebo, and pooled AZD0449 doses, for each study part. Tabulations will include causality and severity (mild, moderate and severe). All tabulations will be presented by SOC and Preferred Term. Furthermore, listings of SAEs and AEs that led to withdrawal will be made and the number of

subjects who had any AEs, SAEs, AEs that led to discontinuation (DAEs) will be summarized. The AEs that occur before first dosing will be excluded from the summary tables.

The following information will be included in the listings: verbatim term, MedDRA SOC, Preferred Term and lowest level term, start date/time, end date/time, time from last dose, causality, action taken, whether the AE was classified as serious and the outcome.

All tabulations will include the number and percentage of subjects.

10.6.4.2. Vital Signs

The results of the vital signs measurements, systolic and diastolic BP, pulse, respiratory rate and oral/tympanic body temperature, will be listed by subject and time-point including the date/time of the assessment, flags for measurements that are outside the reference range (L or H, if applicable), changes from baseline and repeat/unscheduled measurements. The baseline for vital signs measurements will be the last measurement before the first administration of IMP. Descriptive statistics (n, mean, SD, minimum, Q1, median, Q3, maximum) will be presented by treatment (each dose level of AZD0449, pooled placebo, and pooled AZD0449) and time-point for both observed values and changes from baseline, for each part of the study.

10.6.4.3. Resting 12-lead Electrocardiogram

The twelve-lead ECG overall interpretation (normal/abnormal), specification of abnormality and clinical significance will be listed by subject and time-point based on the SAF for each part of the study.

The number and percentage of subjects with clinically significant 12-lead ECG findings will be summarized by treatment group (dose level of AZD0449, pooled placebo, and pooled AZD0449) by each day for Part 1a/b, Part 2a/b, and Part 3a/b based on the SAF.

10.6.4.4. 12-lead Continuous Digital Electrocardiogram

The following parameters will be derived from the dECG:

- QTcF will be calculated as $QTcF = \frac{QT}{\sqrt[3]{RR}}$ where the QT interval is in milliseconds and the RR interval is in seconds
- Heart rate will be calculated, based on the RR interval as $HR = \frac{60}{\text{RR Interval}}$, where the RR interval is in seconds

The dECG data will be smoothed on an individual basis before performing the derivations above and before calculation of any changes from baseline or descriptive statistics. For each subject it will be done as follows: the mean value of all the measurements will be taken for target time-point recordings. At least 4 measurements with the time between the first and last

record greater than 2.75 minutes for a target time-point should be present or else, the smoothed value at the corresponding target time-point will be set to missing.

Digital ECG results will be listed by treatment (dose level of AZD0449, pooled placebo, and pooled AZD0449) for each subject and time-point and will include all individual and smoothed values of PR, RR, QRS, QT interval and the derived values of QTcF and HR. The changes from baseline for smoothed and derived parameters will be listed as well.

Descriptive statistics of smoothed PR, RR, QRS, QT values and derived QTcF and HR values as well as change from baseline will be summarized by treatment group (dose level of AZD0449, pooled placebo, and pooled AZD0449) and time-point, for each study part. The baseline for the dECG measurements will be the smoothed pre-dose assessment on Day 1 for each part of the study.

Outliers with respect to QTcF will also be tabulated for the following categories:

- Absolute value >450 ms and ≤480 ms
- Absolute value >480 ms and ≤500 ms
- Absolute value >500 ms
- Increase from baseline >30 ms and ≤60 ms
- Increase from baseline >60 ms

All calculations of dECG parameters and reporting described in this section will be performed by Parexel.

10.6.4.5. Real Time ECG (Cardiac Telemetry)

The results of cardiac telemetry will be listed by treatment group, subject and timepoint based on the SAF.

10.6.4.6. Physical Examination

The baseline/screening results of the physical examination will be documented in medical history for each subject.

Any new or aggravated clinically relevant abnormal medical physical examination finding compared to the baseline assessment will be reported as an AE.

10.6.4.7. Spirometry

For spirometry variables, the baseline value for each study part is defined as the pre-dose values recorded on Day 1 of each study period (or Day -1, if not recorded on Day 1). If a subject's baseline value is missing, then the value at screening will be used. Note that within a study

part, for all FEV_1 endpoints, the same baseline FEV_1 value will be used; similarly, for the FVC endpoints, the same baseline FVC value will be used.

Spirometry values (FEV_1 and FVC) will be listed by treatment (dose level of AZD0449 and pooled placebo), subject and time-point including absolute values, absolute and percentage changes from baseline. Summary tabulations for absolute values and changes from baseline will be presented by treatment (each dose level of AZD0449, or pooled placebo, and pooled AZD0449 and time-point for the SAF. For the purpose of the summary tabulations, where more than one FEV_1 or FVC measurements are assessed at a given time-point, the average FEV_1 or FVC value at that time-point will be summarized.

10.6.4.8. Pulse Oximetry

Peripheral capillary oxygen saturation will be listed by treatment (dose level of AZD0449 and pooled placebo), subject and timepoint including absolute values, changes from baseline and percentages changes from baseline. The baseline for the measurements will be the pre-dose assessment on Day 1 (in each treatment period). Changes from baseline will be calculated and presented for pre-dose to each post-dose timepoint in each of these periods. Summary tabulations for absolute values and changes from baseline will be presented by treatment (each dose level of AZD0449, or pooled placebo, and pooled AZD0449) or by route of administration (following inhaled and IV doses), and timepoint for the SAF.

10.6.4.9. Laboratory Assessments

Hematology and clinical chemistry values will be listed by subject and time-point including changes from baseline and repeat/unscheduled measurements. Summary tabulations will be presented by treatment (each dose level of AZD0449 or pooled placebo, and pooled AZD0449) or by route of administration (following inhaled and IV doses) for the SAF, for each study part. The baseline for the measurements will be the last assessment available before the first IMP administration. Changes from baseline will be calculated and presented for all post-baseline timepoints including the Follow-up Visit/last day of study. Shift tables showing changes with respect to the normal ranges between baseline and any post-baseline visit will also be presented.

Any laboratory parameters with results from the laboratory given as '<xx' or '>xx' in the database will be imputed with the absolute value of the number without the sign (eg, <2.2 will be imputed as 2.2) for the descriptive statistics and changes from baseline.

The listings will include the following information: test name, date of measurement, reference range, result and flags for any measurements that are outside the reference range (eg, AstraZeneca, program or laboratory ranges) as well as assessment of clinically relevant abnormality assessed by Investigator. Data listings for subjects that shows elevations in liver

biochemistry (ie, occurrences of AST or ALT ≥3 x ULN together with total bilirubin ≥2 x ULN) will be presented to evaluate HL. Clinical laboratory data will be reported in the units provided by the clinical laboratory for the SRC meeting (if applicable), and in System International units in the CSR.

Additional listings will be presented for the following:

- Urinalysis (macroscopic and microscopic, if applicable)
- Pregnancy testing (including FSH)

10.6.5. Pharmacokinetic Analysis

10.6.5.1. Pharmacokinetic Parameters

Where possible the following PK parameters will be determined from the AZD0449 and, in Part 2b (cohort 3) and Part 3a/b, CCI [redacted] plasma concentrations on Day 1 following the first dose and on Day 12 following once daily multiple dosing as appropriate for each Study Part.

10.6.5.1.1. Plasma Parameters

Following Inhaled Dosing (Parts 1a, Part 2, and Part 3)	
C_{max}	Maximum observed plasma concentration
t_{max}	Time of maximum observed concentration (first occurrence)
$AUC_{(0-t)}$	Area under the plasma concentration-curve from time zero to the time of last quantifiable concentration
$AUC_{(0-12)}$	Area under the plasma concentration-time curve from time zero to 12 hours post-dose
$AUC_{(0-24)}$	Area under the plasma concentration-time curve from time zero to 24 hours post-dose
AUC	Area under the concentration-time curve from time zero to infinity
λz	Terminal elimination rate constant
$t_{1/2}\lambda z$	Terminal elimination half-life
CL/F	Apparent total clearance of drug from plasma (Extravascular administration [parent drug only])
Vz/F	Apparent volume of distribution during the terminal phase (Extravascular administration [parent drug only])
C_{max}/D	Dose normalized Cmax
AUC/D	Dose normalized AUC
$AUC_{(0-t)}/D$	Dose normalized $AUC_{(0-t)}$
t_{last}	Time of last quantifiable concentration

Following Intravenous Dosing (Part 1b)	
C_{max}	Maximum observed plasma concentration
t_{max}	Time of maximum observed concentration (first occurrence)
$AUC_{(0-t)}$	Area under the plasma concentration-curve from time zero to the time of last quantifiable concentration
$AUC_{(0-12)}$	Area under the plasma concentration-time curve from time zero to 12 hours post-dose
$AUC_{(0-24)}$	Area under the plasma concentration-time curve from time zero to 24 hours post-dose
AUC	Area under the concentration-time curve from time zero to infinity
λz	Terminal elimination rate constant
$t_{1/2}\lambda z$	Terminal elimination half-life
CL	Total clearance of drug from plasma (IV administration)
Vz	Volume of distribution during the terminal phase (IV administration)
C_{max}/D	Dose normalized Cmax
AUC/D	Dose normalized AUC
$AUC_{(0-t)}/D$	Dose normalized $AUC_{(0-t)}$
t_{last}	Time of last quantifiable concentration

The following PK parameters will be calculated for diagnostic purposes and listed but not summarized:	
t lower	Start of exponential fit of terminal phase
t upper	End of exponential fit of terminal phase
λz N	Number of data points included in the log-linear regression analysis
Rsq-adj	Regression coefficient adjusted for λzN, Goodness-of-fit statistic for calculation of λz
$\%AUC_{extr}$	Percentage of AUC extrapolated

Additional PK parameters may be determined where appropriate.

10.6.5.2. Calculation or Derivation of Pharmacokinetic Parameters

The PK analyses of the plasma concentration data for AZD0449 and CCI [redacted] as appropriate, will be performed by Covance, Clinical Pharmacokinetic Alliance (CPKA) on behalf of AstraZeneca R&D.

Standard Operating Procedures and Work Instructions will be used as the default methodology if not otherwise specified.

The actual sampling times will be used in the final plasma PK parameter calculations. If actual times are missing, nominal times may be used.

Nominal sampling times will be used for interim plasma PK parameter calculations for the purpose of the SRC meetings.

Pharmacokinetic parameters will be derived using non-compartmental methods with Phoenix® WinNonlin® Version 8.1, or higher. All descriptive and inferential statistical computations will be performed using SAS® Version 9.4, or higher.

Plasma concentrations BLQ from the time of pre-dose sampling (t=0) up to the time of the first quantifiable concentration will be set to a value of 0. After this point, BLQ plasma concentrations will be set to missing for all concentration profiles. Also, if 2 or more consecutive BLQ concentrations are followed by quantifiable concentrations in the terminal portion of the concentration-curve, the profile will be deemed to have terminated and therefore these quantifiable values will be set to missing for the calculation of the PK parameters unless there is a scientific rationale not to do so, this is documented in the PK analysis notes.

If an entire concentration-time profile is BLQ, the profile is excluded from the PK analysis.

Terminal elimination half-life ($t_{1/2}\lambda z$) will be calculated as (ln2)/λz, where λz will be estimated by log-linear least squares regression of the terminal part of the concentration-time curve. For the determination of λz, the start of the terminal elimination phase for each subject will be defined by visual inspection and will be the first point at which there is no systematic deviation from the log-linear decline in plasma concentrations (t lower). The last point (t upper) will be the time of the last quantifiable plasma concentration. A minimum of 3 data points not including C_{max} and including t_{last} will be used in calculating λz, and the duration of time over which λz is calculated is recommended to be at least 3 times the subsequently estimated terminal half-life. Where an elimination half-life is estimated over less than 3 times the subsequently estimated terminal half-life, it will be flagged and commented upon in the study report by agreement with the Sponsor. The Rsq-adj value will be calculated to show the goodness-of-fit of the log-linear regression taking into consideration the number of points used in the estimation (λzN). To achieve a good precision in the estimation, the Rsq-adj value should be high, a value of $\geq$0.8 being indicative of good correlation. Any λz with a Rsq-adj of <0.8 will be flagged in the data listings along with any parameters derived from λz (eg, $t_{1/2}\lambda z$, AUC, CL, CL/F, Vz, Vz/F and $\%AUC_{extr}$) and agreement reached with the Sponsor regarding any exclusion of data from the summaries.

AUCs (including AUC, $AUC_{(0-t)}$, $AUC_{(0-12)}$, $AUC_{(0-24)}$) will be calculated using the linear trapezoidal method when concentrations are increasing and the logarithmic trapezoidal method when concentrations are decreasing (linear up, log down). AUC is estimated by $AUC_{(0-t)}+C_{last}/\lambda z$ where C_{last} is the observed last quantifiable drug concentration. The AUC values where percentage extrapolation ($\%AUC_{extr}$) is greater than 20% will be flagged in the data

listings and agreement reached with the Sponsor on whether exclusions from the summaries are required.

The minimum requirement for the calculation of AUC will be the inclusion of at least 3 consecutive plasma concentrations above the lower limit of quantification (LLOQ), with at least one of these concentrations following C_{max}.

C_{max}, t_{max} and t_{last} will be taken directly from the concentration-time profiles.

Clearance (CL) and apparent clearance (CL/F) will be determined from the ratio of dose/AUC. Volume of distribution (Vz) and apparent volume of distribution based on the terminal phase (Vz/F) will be determined from the ratio of dose/λz*AUC.

Dose normalized parameters will be determined by dividing the parameter by the dose.

10.6.5.3. Presentation and Statistical Analysis of Pharmacokinetic Data

A listing of PK blood sample collection times as well as derived sampling time deviations and all reportable concentrations will be presented for AZD0499 and CCI [redacted] as appropriate, for all subjects in the SAF.

Plasma concentrations will be summarized for the PK analysis set for each analyte and timepoint by treatment (dose level of AZD0449) Study Part and Study Day using protocol scheduled times and appropriate descriptive statistics (ie, n, n below LLOQ, geometric mean [gmean], gmean+geometric standard deviation [gSD], gmean-gSD, geometric coefficient of variance expressed as a percentage [gCV%], arithmetic mean [mean], arithmetic SD [SD], median, minimum and maximum).

The gmean is calculated as exponential (μ), where μ is the arithmetic mean calculated using log-transformed data.

The gCV% is calculated as 100 x sqrt[exp(s2)-1], where s is the SD of the log-transformed data.

The gmean±gSD (gmean-gSD and gmean+gSD) are calculated as exp[μ±s].

Individual concentrations with time deviations of greater than ±10% from the protocol scheduled time will be used in the PK analysis but will be flagged for exclusion from the summary tables and corresponding figures.

Individual concentrations below the LLOQ of the bioanalytical assay will be reported as NQ in the listings with the LLOQ defined in the footnotes of the relevant TFLs. Individual plasma concentrations that are not reportable will be reported as NR and those that are missing will be

reported as NS (No Sample) in the listings. Plasma concentrations that are NQ, NR or NS will be handled as follows for the provision of descriptive statistics:

- Any values reported as NR or NS will be excluded from the summary tables and corresponding figures
- At a timepoint where less than or equal to 50% of the concentration values are NQ, all NQ values will be set to the LLOQ, and all descriptive statistics will be calculated accordingly
- At a timepoint where more than half of the values are NQ, the gmean, gmean+gSD, gmean-gSD and gCV% will be set to NC. The maximum value will be reported from the individual data, and the minimum and median will be set to NQ
- If all values are NQ at a timepoint, no descriptive statistics will be calculated for that timepoint. The gmean, minimum, median and maximum will be reported as NQ and gmean±gSD and gCV% as NC

The number of values below LLOQ (n <LLOQ) will be reported for each timepoint. together with the total number of collected values (n).

Three observations >LLOQ are required as a minimum for a plasma concentration to be summarized. Two values >LLOQ are presented as a minimum and maximum with the other summary statistics as NC.

Plasma concentrations that are NQ will be handled as follows for display in figures:

- For gmean concentration-time plots: NQ concentrations will be handled as described for the descriptive statistics. If this handling results in a geometric mean of "NQ", then the value plotted at that timepoint will be zero for linear plots and set to missing for semi-logarithmic plots. Any gmean±gSD error bar values that are negative will be truncated at zero on linear concentration-time plots and omitted from semi-logarithmic plots.
- For individual plots and combined individual plots: NQ values prior to the first quantifiable concentration in that profile will be set to zero (linear plots only); after the first quantifiable concentration of the profile any NQ values will be set to missing.

All reportable PK parameters will be listed for AZD0449 and CCI [redacted] as appropriate, for all subjects (Safety analysis set). A separate listing will be provided for the diagnostic parameters.

Plasma PK parameters will be summarized for the PK analysis set by analyte, treatment (dose level of AZD0449), Study Part, and Study Day using the following descriptive statistics:

- C_{max}, AUC, $AUC_{(0-12)}$, $AUC_{(0-24)}$ and $AUC_{(0-t)}$ will present n, gmean, gmean+gSD, gmean-gSD, gCV(%), median, min and max.
- C_{max}/D, AUC/D, $AUC_{(0-t)}$/D and λz will present n, gmean, gCV%, mean, SD, median, min and max.
- $t_{1/2}$λz, CL/F, CL, Vz/F, Vz, will present n, gmean, gCV%, mean, SD, median, min and max.
- t_{max} and t_{last} will present only n, median, min and max.
- diagnostic parameters (t upper, t lower, λzN, Rsq-adj and $\%AUC_{extr}$) will be listed only and not summarized.

For the calculation of summary statistics of PK parameters, all not reportable (NR) and not calculated (NC) values will be set to missing. Three reportable values are required as a minimum for a PK parameter to be summarized. Two values are presented as a minimum and maximum with the other summary statistics as NC. If one or more values for a given parameter is zero (or imputed with zero), then no geometric statistics will be calculated for that parameter and the results for geometric statistics will be set to "NA", not applicable.

PK concentration and parameter data for patients excluded from the PKS will be included in the data listings, but not in the descriptive or inferential statistics or in mean figures or combined individual figures.

Individual plasma concentrations versus actual elapsed time after dose will be plotted on both the linear and semi-logarithmic scale separately for AZD0449 and CCI [redacted] with the Day 1 and Day 12 data overlaid on the same plot for study parts 2 and 3.

Combined individual plasma concentration versus actual elapsed times after dose will be plotted on both the linear and semi-logarithmic scale separately for AZD0449 and CCI [redacted] Plots will be grouped by dose level of AZD0449 for each Study Part and Study Day.

Gmean (±gSD) plasma concentration versus nominal sampling time will be plotted separately for each analyte on both the linear and semi-logarithmic scale (no error bars) for each Study Part and Study Day with all dose levels overlaid on the same plot for Part 1a Day 1 and Part 2a/b Day 1 and Day 12, with Day 1 and Day 12 overlaid on the same plot for Part 3a/b.

All plots will be based on the PKS, with the exception of individual plots by subject, which will be based on the SAF.

Precision and Rounding Rules

PK concentration data will be presented in the listings to the same number of significant digits as the data received from the bioanalytical laboratory (usually to 3 significant figures) and against the same units as received. PK concentration descriptive statistics will all be presented to 4 significant figures with the exception of the min and max which will be presented to 3 significant figures and n and n<LLOQ which will be presented as integers. For plasma PK parameters, the listings will be presented according to the following rules:

- C_{max}– will be presented to the same number of significant figures as received from the bioanalytical laboratory
- t_{max}, t_{last}, t lower and t upper– will be presented as received in the data, usually to 2 decimal places
- C_{max}/D, AUC, AUC/D, $AUC_{(0-t)}$, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$/D, $\%AUC_{extr}$, λz, $t_{1/2}$λz, CL/F, CL, Vz/F, Vz, Rsq-adj will be presented to 3 significant figures
- λzN – will be presented as an integer (no decimals)

For PK concentration data all descriptive statistics will be presented to 4 significant figures with the exception of the minimum and maximum which will be presented to 3 significant figures and n and n <LLOQ, which will be presented as integers.

For PK parameter data the descriptive statistics will be presented according to the following rules:

- C_{max}, C_{max}/D, AUC, AUC/D, $AUC_{(0-t)}$, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$/D, $t_{1/2}$λz, CL/F, CL, Vz/F, and Vz, – all descriptive statistics will be presented to 4 significant figures with the exception of the minimum and maximum which will be presented to 3 significant figures and n which will be presented as integers
- λz – will be presented to 5 significant figures with the exception of the minimum and maximum which will be presented to 3 significant figures and n which will be presented as an integer
- t_{max} and t_{last} – all descriptive statistics will be presented as received in the data, usually to 2 decimal places with the exception of n which will be presented as an integer

10.6.5.4. Statistical Analysis of Pharmacokinetic Data

The power model will be used for the analysis of dose-proportionality following a single dose and following multiple dosing as appropriate for Part 1a, Part 2a/b and Part 3. The power model

is denoted as y=α*doseβ, where "y" refers to the PK parameter, AUC (AUC or $AUC_{(0-t)}$) and C_{max} from the last day of dosing, under consideration.

Dose-proportionality will be assessed following a single dose and following multiple dosing via least squares linear regression of the log-transformed PK parameters versus the log-transformed dose, ie, log (PK parameter)=α+βlog(Dose). An estimate of the slope and intercept of the regression line and the corresponding 2-sided 95% confidence interval (CI) for the slope will be obtained and tabulated for AUC and C_{max}.

If AUC cannot be fully characterized, then $AUC_{(0-t)}$ may be used instead for the analysis.

The power model will be fitted by restricted maximum likelihood (REML) using SAS Proc Mixed. Both the intercept and slope will be fitted as fixed effects.

Scatter plots for each of the log-transformed PK parameters C_{max}, $AUC_{(0-t)}$ and AUC versus log-transformed dose will be presented with the regression line from the analysis overlaid on the same plot for Part 1a Day 1, and Part 2a/b Day 1, Day 12.

In addition, figures of dose normalized C_{max}, $AUC_{(0-t)}$ and AUC versus dose, showing individual values and geometric mean will be presented separately for each PK parameter for Part 1a Day 1 and for Part 2 Day 1 and Day 12, to visually demonstrate dose-proportionality.

Significant figures or decimal places for statistical outputs, eg, the slope estimate intercept and 95% CIs will be presented to 4 decimal places (p-values that are smaller than 0.0001 will be presented as "<0.0001").

10.6.6. Analysis of Pharmacodynamic Data

10.6.6.1. Fractional Exhaled Nitric Oxide - Part 2a and Part 3a

The change from baseline in 2 hours post-dose FeNO level to Day 12 (Part 2a and Part 3a) will be analysed based on a MMRM using all post-baseline 2 hours post-dose FeNO assessments. Baseline will be defined as mean FeNO value at Day -1. The model will include the fixed, categorical effects of treatment, day, and treatment-by-day interaction, as well as the continuous, fixed covariate of baseline FeNO value. Patient will be included as a random effect. This analysis will be based on the FAS. Analyses will be performed on the log-transformed FeNO data to normalize the skewed distribution of this endpoint.

The within-patient correlation will be modeled using the unstructured covariance matrix. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom. The analysis will be performed using only the OC without imputation of missing values. If the model does not converge, then the compound symmetry covariance structure will be used. REML method will be used for estimation.

Treatment effect and treatment differences will be estimated using contrasts of the Least-Square (LS) means on the correspondent treatment-by-day interaction, along with their SE and 2-sided 90% CI, and the p-value corresponding to the between-treatment group difference.

The same model will be used to evaluate treatment effect between the groups in Part 2a and Part 3a. The following treatment groups will be compared at Part 2a:

- AZD0449 CCI
- pooled placebo

The following treatment groups will be compared using Part 3a data:

- DPI AZD0449 CCI
- Placebo

In addition, treatment effect of the change from baseline in 2 hours post-dose FeNO level at Day 12 will be analysed using ANCOVA with treatment as a fixed effect, and baseline as a covariate. The LOCF method will be used to deal with missing values. No multiplicity adjustments will be applied.

In addition, as sensitivity analysis, the change from baseline in FeNO area under the effect-time curve to Day 12 ($AUC_{(0-12)}$) will be analysed using the same methods as the change from baseline in 2 hours post-dose FeNO level to 12 days.

Fractional exhaled nitric oxide will be listed by subject, day and time-point including absolute values, absolute and percentage changes from baseline. Summary tabulations as well as geometric mean for absolute values and changes from baseline will be presented by treatment (dose level of AZD0449, and pooled placebo), day and time-point for the FAS and PPS.

FeNO $AUC_{(0-12)}$ will be listed by subject and study day including absolute values and change from baseline. Descriptive statistics of FeNO $AUC_{(0-12)}$ including change from baseline at each study day will be presented by treatment (dose level of AZD0449, and pooled placebo) and day for the FAS and PP.

Graphs will be produced to illustrate the analysis.

CCI

CCI

10.6.7. Analysis of Exploratory Data

CCI

11. ADVERSE EVENTS

11.1. Definitions

11.1.1. Definition of Adverse Events

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product.

An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, ECG).

In clinical studies, an AE can include an undesirable medical condition occurring at any time after the subject has signed informed consent, including run-in or washout periods, even if no specific treatment has been administered.

The term AE is used generally to include any AE whether serious or non-serious.

11.1.2. Definitions of Serious Adverse Event

A SAE is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up/safety monitoring period), that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

For further guidance on the definition of a SAE, see Appendix 15.1 of this CSP.

11.1.3. Other Significant Adverse Events

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs or where relevant DAEs and withdrawal from the study. Based on the expert's judgment, significant AEs of particular clinical importance may, after consultation with the Global Safety Physician, be considered other significant AEs

(OAEs) and reported as such in the CSR. A similar review of other data from laboratory tests, vital signs, ECGs and other safety assessments will be performed for identification of OAEs.

Examples of these are marked hematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment.

11.2. Recording of Adverse Events

11.2.1. Time Period for Collection of Adverse Events

Adverse Events will be collected from the start of randomization throughout the treatment period up to and including the Follow-up Visit/last day of study.

Serious adverse events will be recorded from the time of informed consent.

11.2.2. Follow-up of Unresolved Adverse Events

Any AEs that are unresolved at the subject's last visit in the study are followed up by the Investigator for as long as medically indicated, but without further recording in the DataLabs.

AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

11.2.3. Variables

The following variables will be collected for each AE:

- Adverse event diagnosis/description
- The date and time when the AE started and stopped
- Intensity
- Whether the AE is serious or not
- Investigator causality rating against the IMP (yes or no)
- Action taken with regard to investigational product
- Adverse event caused subject's withdrawal from study (yes or no)
- Outcome

Additional variables will be collected for all SAEs including treatment given for the event.

The following intensity ratings will be used:

1. mild (awareness of sign or symptom, but easily tolerated)

2. moderate (discomfort sufficient to cause interference with normal activities)

3. severe (incapacitating, with inability to perform normal activities)

It is important to distinguish between serious and severe AEs.

Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 11.1.2.

An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE.

11.2.4. Causality Collection

The Investigator will assess causal relationship between investigational product and each AE, and answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?"

For SAEs causal relationship will also be assessed for other medication, any additional drug and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as "yes".

A guide to the interpretation of the causality question is found in Appendix 15.1 of this CSP.

11.2.5. Adverse Events Based on Symptoms and Signs

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: *"Have you had any health problems since you were last asked?"* or revealed by observation will be collected and recorded in the DataLabs.

When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms.

However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

11.2.6. Adverse Events Based on Examinations and Tests

The results from CSP-mandated laboratory tests, vital signs, ECGs and other safety assessments will be summarized in the CSR.

Deterioration as compared to baseline in CSP-mandated laboratory values, vital signs, ECGs and other safety assessments should therefore only be reported as AEs if they fulfill any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product

or are considered to be clinically relevant as judged by the investigator (which may include but not limited to considerations as to whether treatment or non-planned visits were required or other action was taken with the investigational product).

If deterioration in a laboratory value or vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result or vital sign will be considered as additional information.

Wherever possible the reporting Investigator should use the clinical, rather than the laboratory term (eg, anemia versus low hemoglobin value).

In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

Hy's Law

Cases where a subject shows elevation in liver biochemistry may require further evaluation and occurrences of AST or ALT ≥3 x ULN together with total bilirubin ≥2 x ULN may need to be reported as SAEs. Please refer to Appendix 15.3 for further instruction on cases of increases in liver biochemistry and evaluation of HL.

11.3. Reporting of Serious Adverse Events

All SAEs must be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the DataLabs.

If any SAE occurs in the course of the study, then Investigators or other site personnel will inform appropriate AstraZeneca representatives immediately, or **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative will work with the Investigator to ensure that all the necessary information is provided to the AstraZeneca patient safety data entry site **within 1 calendar day** of initial receipt for fatal and life-threatening events and **within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up will be undertaken immediately.

Investigators or other site personnel will inform AstraZeneca representatives of any follow-up information on a previously reported SAE immediately, or **no later than 24 hours** of when he or she becomes aware of it.

The reference document for definition of expectedness/listedness is the Investigator's Brochure (IB) for the AstraZeneca drug [2].

12. ETHICAL AND REGULATORY REQUIREMENTS

12.1. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (version 1996) and are consistent with International Council for Harmonisation (ICH) GCP and the AstraZeneca policy on Bioethics and Human Biological Samples.

12.2. Subject Data Protection

The ICF will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

All clinical study findings and documents will be regarded as confidential. The PI and members of his/her research team must not disclose such information without prior written approval from the Sponsor.

The anonymity of participating subjects must be maintained. Subjects will be specified in outputs and other documents containing subject data by their subject number, not by name. Documents that identify the subject (eg, signed ICF) will be maintained in confidence by the PI.

Study data will be stored in accordance with local and global data protection laws.

12.3. Ethics and Regulatory Review

The study will be submitted to the national regulatory agency for review and approval, by Parexel in accordance with local regulatory procedures.

The study will be submitted to the IEC for ethical review and approval by the PI in accordance with local procedures.

Parexel will provide the IEC and PI with safety updates/reports according to local requirements, including Suspected Unexpected Adverse Reactions (SUSARs), where relevant.

AstraZeneca will provide the Regulatory Authority with safety updates/reports according to local requirements, including SUSARs, where relevant.

Compensation will be reasonable and related to the nature and degree of inconvenience and discomfort as a result of participation in the study. Information on how subjects will be compensated is contained in the ICF.

12.4. Insurance

The Sponsor has covered this clinical study by means of an insurance of the clinical study according to national requirements. The name and address of the relevant insurance company, the certificate of insurance, the policy number and the sum insured are provided in the Investigator's Site File.

12.5. Informed Consent

The subjects shall be informed of the nature, significance, implications and risks of the study, and informed consent will be freely given and evidenced in writing, dated and signed or otherwise marked, by the subject as evidence to indicate his/her free informed consent, before the start of the study.

The nature of the informed consent will comply with the Declaration of Helsinki (version 1996), the current requirements of GCP (CPMP/ICH/135/95) and local regulation which ever offers the greater subject protection.

12.6. Changes to the Protocol and Informed Consent Document

Study procedures will not be changed without the mutual agreement of the PI and AstraZeneca.

If there are any substantial changes to the CSP, then these changes will be documented in a CSP amendment and where required in a new version of the CSP.

If a CSP amendment requires a change to the ICF, the IEC should approve the revised ICF before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by the IEC.

13. LEGAL AND ADMINISTRATIVE ASPECTS

13.1. Archiving of Study Documents

All source documents generated in connection with the study will be retained in the limited access file storage area, respecting the privacy and confidentiality of all records that could identify the subjects. Direct access is allowed only for authorized people for monitoring and auditing purposes. Source documents will be handled, stored and archived according to in house procedures.

The Investigator's Site File will be archived by the contract research organization (CRO) for 15 years after completion of the study.

13.2. Publication of Study Results

All of the study information and data collected during the study are confidential and the property of AstraZeneca. After completion of the study, AstraZeneca may prepare a joint publication with the Investigator. The Investigator must undertake not to submit any data from this CSP for publication without prior consent of AstraZeneca at a mutually agreed time.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

13.3. Clinical Study Report

An integrated CSR will be prepared in accordance with the standards of the ICH guideline for structure and content of clinical study reports (ICH E3). Copies of the CSR will be provided to the IEC and the national Regulatory Authority in accordance with regulatory requirements and Parexel SOPs. In the event of premature termination of the study or other conditions specified in ICH E3, an abbreviated CSR may be prepared.

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15. APPENDICES

15.1. Additional Safety Information

Further Guidance on the Definition of a Serious Adverse Event

Life-threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalization

Outpatient treatment in an emergency room is not in itself a SAE, although the reasons for it may be (eg, bronchospasm, laryngeal edema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important Medical Event or Medical Intervention

Medical and scientific judgment should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalization, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent 1 or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgment must be used.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring IV hydrocortisone treatment.
- Hepatotoxicity caused by paracetamol/acetaminophen overdose requiring treatment with N-acetyl cysteine.
- Intensive treatment in an emergency room or at home for allergic bronchospasm.
- Blood dyscrasias (eg, neutropenia or anemia requiring blood transfusion) or convulsions that do not result in hospitalization.
- Development of drug dependency or drug abuse.

A Guide to Interpreting the Causality Question

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the IMP.

- Time Course / Exposure to suspect drug:
  Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?
- Consistency with known drug profile:
  Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR, could the AE be anticipated from its pharmacological properties?
- Dechallenge experience:
  Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?
- No alternative cause:
  The AE cannot be reasonably explained by other etiology such as the underlying disease, other drugs, other host or environmental factors.
- Rechallenge experience:
  Did the AE reoccur if the suspected drug was reintroduced after having been stopped? *Note: AstraZeneca would not normally recommend or support a rechallenge.*
- Laboratory tests:
  A specific laboratory investigation (if performed) has confirmed the relationship?

In difficult cases, other factors could be considered such as:

- Is this a recognized feature of overdose of the drug?
- Is there a known mechanism?

Causality of 'related' is made if following a review of the relevant data, there is evidence for a 'reasonable possibility' of a causal relationship for the individual case. The expression 'reasonable possibility' of a causal relationship is meant to convey, in general, that there are facts (evidence) or arguments to suggest a causal relationship.

The causality assessment is performed based on the available data including enough information to make an informed judgment. With limited or insufficient information in the case, it is likely that the event(s) will be assessed as 'not related'.

Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

15.2. International Airline Transportation Association 6.2 Guidance Document

Labeling and Shipment of Biohazard Samples

International Airline Transportation Association classifies biohazardous agents into 3 categories (http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm). For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and Categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A pathogens are for example, Ebola and Lassa Fever viruses. Category A pathogens:

- Are to be packed and shipped in accordance with IATA Instruction 602.

Category B Infectious Substances are infectious substances that do not meet the criteria for inclusion in Category A. Category B pathogens are for example, hepatitis A, B, C, D and E viruses and HIV types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- Are to be packed in accordance with UN3373 and IATA Instruction 650.

Exempt refers to all other materials with minimal risk of containing pathogens.

- Clinical trial samples will fall into Category B or Exempt under IATA regulations.
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging. (http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm)
- Biological samples transported in dry ice require additional dangerous goods specification for the dry ice content.
- International Airline Transportation Association compliant courier and packaging materials should be used for packing and transportation. Packing should be done by an IATA certified person, as applicable.
- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and

packaging/containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.

15.3. Actions Required in Cases Increases in Liver Biochemistry and Evaluation of Hy's Law

15.3.1. Introduction

This Appendix describes the process to be followed in order to identify and appropriately report Potential Hy's Law (PHL) cases and Hy's Law (HL) cases. It is not intended to be a comprehensive guide to the management of elevated liver biochemistries.

During the course of the study the Investigator will remain vigilant for increases in liver biochemistry. The Investigator is responsible for determining whether a subject meets potential PHL criteria at any point during the study.

All sources of laboratory data are appropriate for the determination of PHL and HL events; this includes samples taken at scheduled study visits and other visits including central and all local laboratory evaluations even if collected outside of the study visits; for example, PHL criteria could be met by an elevated ALT from a central laboratory **and/or** elevated TBL from a local laboratory.

The Investigator will also review Adverse Event (AE) data (for example, for AEs that may indicate elevations in liver biochemistry) for possible PHL events.

The Investigator participates, together with AstraZeneca clinical project representatives, in review and assessment of cases meeting PHL criteria to agree whether HL criteria are met. HL criteria are met if there is no alternative explanation for the elevations in liver biochemistry other than Drug-Induced Liver Injury (DILI) caused by the Investigational Medicinal Product (IMP).

The Investigator is responsible for recording data pertaining to PHL/HL cases and for reporting Serious Adverse Events (SAEs) and AEs according to the outcome of the review and assessment in line with standard safety reporting processes.

15.3.2. Definitions

Potential Hy's Law (PHL)

Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥3× Upper Limit of Normal (ULN) **together with** Total Bilirubin (TBL) ≥2×ULN at any point during the study following the start of study medication irrespective of an increase in Alkaline Phosphatase (ALP).

Hy's Law (HL)

AST or ALT ≥3×ULN **together with** TBL ≥2×ULN, where no other reason, other than the IMP, can be found to explain the combination of increases, eg, elevated ALP indicating cholestasis, viral hepatitis, another drug.

For PHL and HL the elevation in transaminases must precede or be coincident with (ie, on the same day) the elevation in TBL, but there is no specified timeframe within which the elevations in transaminases and TBL must occur.

15.3.3. Identification of Potential Hy's Law Cases

In order to identify cases of PHL it is important to perform a comprehensive review of laboratory data for any subject who meets any of the following identification criteria in isolation or in combination:

- ALT ≥3×ULN
- AST ≥3×ULN
- TBL ≥2×ULN

The Investigator will without delay review each new laboratory report and if the identification criteria are met will:

- Notify the AstraZeneca representative
- Determine whether the subject meets PHL criteria (see Section 2 Definitions within this Appendix for definition) by reviewing laboratory reports from all previous visits
- Promptly enter the laboratory data into the laboratory CRF

15.3.4. Follow-Up

15.3.4.1. Potential Hy's Law Criteria Not Met

If the subject does not meet PHL criteria the Investigator will:

- Inform the AstraZeneca representative that the subject does not meet PHL criteria
- Perform follow-up on subsequent laboratory results according to the guidance provided in the Clinical Study Protocol.

15.3.4.2. Potential Hy's Law Criteria Met

If the subject does meet PHL criteria the Investigator will:

- Notify the AstraZeneca representative who will then inform the central Study Team.
- Within 1 day of PHL criteria being met, the Investigator will report the case as an SAE of PHL; serious criteria 'Important medical event' and causality assessment 'yes/related' according to CSP process for SAE reporting.

- For subjects that met PHL criteria prior to starting IMP, the Investigator is not required to submit a PHL SAE unless there is a significant change# in the subject's condition.
- The Study Physician contacts the Investigator, to provide guidance, discuss and agree an approach for the study subjects' follow-up (including any further laboratory testing) and the continuous review of data.
- Subsequent to this contact the Investigator will:

– Monitor the subject until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated. Completes follow-up SAE Form as required.

– Investigate the aetiology of the event and perform diagnostic investigations as discussed with the Study Physician.

– Complete the 3 Liver CRF Modules as information becomes available.

A 'significant' change in the subject's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or total bilirubin) in isolation or in combination, or a clinically relevant change in associated symptoms. The determination of whether there has been a significant change will be at the discretion of the Investigator, this may be in consultation with the Study Physician if there is any uncertainty.

15.3.5. Review and Assessment of Potential Hy's Law Cases

The instructions in this Section should be followed for all cases where PHL criteria were met.

As soon as possible after the biochemistry abnormality was initially detected, the Study Physician contacts the Investigator in order to review available data and agree on whether there is an alternative explanation for meeting PHL criteria other than DILI caused by the IMP, to ensure timely analysis and reporting to health authorities within 15 calendar days from date PHL criteria was met. The AstraZeneca Global Clinical Lead or equivalent and Global Safety Physician will also be involved in this review together with other subject matter experts as appropriate.

According to the outcome of the review and assessment, the Investigator will follow the instructions below.

Where there is an agreed alternative explanation for the ALT or AST and TBL elevations, a determination of whether the alternative explanation is an AE will be made and subsequently whether the AE meets the criteria for a SAE:

- If the alternative explanation is **not** an AE, record the alternative explanation on the appropriate CRF.
- If the alternative explanation is an AE/SAE: update the previously submitted Potential Hy's Law SAE and AE CRFs accordingly with the new information (reassessing event term; causality and seriousness criteria) following the AZ standard processes.

If it is agreed that there is **no** explanation that would clarify the ALT or AST and TBL elevations other than IMP:

- Send updated SAE (report term 'Hy's Law') according to AstraZeneca standard processes.
  - The 'Medically Important' serious criterion should be used if no other serious criteria apply.
  - As there is no alternative explanation for the HL case, a causality assessment of 'related' should be assigned.

If, there is an unavoidable delay, of over 15 calendar days in obtaining the information necessary to assess whether or not the case meets the criteria for HL, then it is assumed that there is no alternative explanation until such time as an informed decision can be made:

- Provides any further update to the previously submitted SAE of PHL, (report term now 'Hy's Law case') ensuring causality assessment is related to IMP and seriousness criteria is medically important, according to CSP process for SAE reporting.
- Continue follow-up and review according to agreed plan. Once the necessary supplementary information is obtained, repeat the review and assessment to determine whether HL criteria are still met. Update the previously submitted PHL SAE report following CSP process for SAE reporting, according to the outcome of the review and amending the reported term if an alternative explanation for the liver biochemistry elevations is determined.

15.3.6. Actions Required for Repeat Episodes of Potential Hy's Law

This section is applicable when a subject meets PHL criteria on study treatment and has already met PHL criteria at a previous on study treatment visit.

The requirement to conduct follow-up, review and assessment of a repeat occurrence(s) of PHL is based on the nature of the alternative cause identified for the previous occurrence.

The Investigator should determine the cause for the previous occurrence of PHL criteria being met and answer the following question:

> Was the alternative cause for the previous occurrence of PHL criteria being met found to be the disease under study, eg, chronic or progressing malignant disease, severe infection or liver disease

If **No**: follow the process described in Section 15.3.4.2 for reporting PHL as an SAE

If **Yes**: Determine if there has been a significant change in the subject's condition# compared with when PHL criteria were previously met

If there is no significant change no action is required

If there is a significant change follow the process described in Section 15.3.4.2 for reporting PHL as an SAE

> # A 'significant' change in the subject's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or total bilirubin) in isolation or in combination, or a clinically relevant change in associated symptoms. The determination of whether there has been a significant change will be at the discretion of the Investigator, this may be in consultation with the Study Physician if there is any uncertainty.

15.3.7. Laboratory Tests

Hy's Law lab kit for local laboratories (18 December 2018)

Additional standard chemistry and coagulation tests	GGT LDH Prothrombin time INR
Viral hepatitis	IgM anti-HAV IgM and IgG anti-HBc HBsAg HBV DNA* Anti-HCV HCV RNA* IgM anti-HEV HEV RNA
Other viral infections	IgM & IgG anti-CMV IgM & IgG anti-HSV IgM & IgG anti-EBV
Alcoholic hepatitis	Carbohydrate deficient transferrin (CD-transferrin)
Autoimmune hepatitis	Antinuclear antibody (ANA) Anti-Liver/Kidney Microsomal Ab (Anti-LKM) Anti-Smooth Muscle Ab (ASMA)

Metabolic diseases	alpha-1-antitrypsin Ceruloplasmin Iron Ferritin Transferrin Transferrin saturation

* HCV RNA and HBV DNA are only tested when anti-HCV is positive or inconclusive

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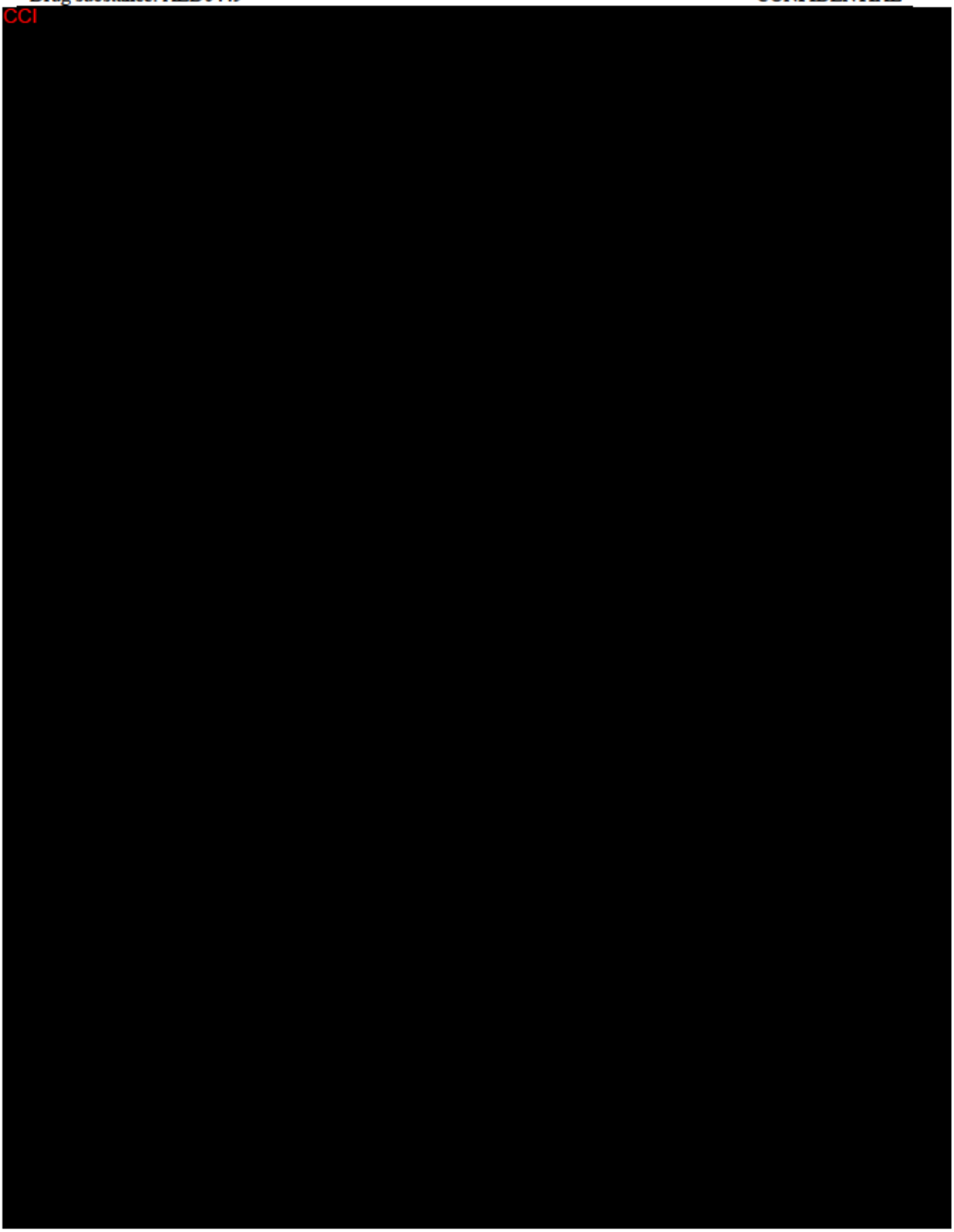

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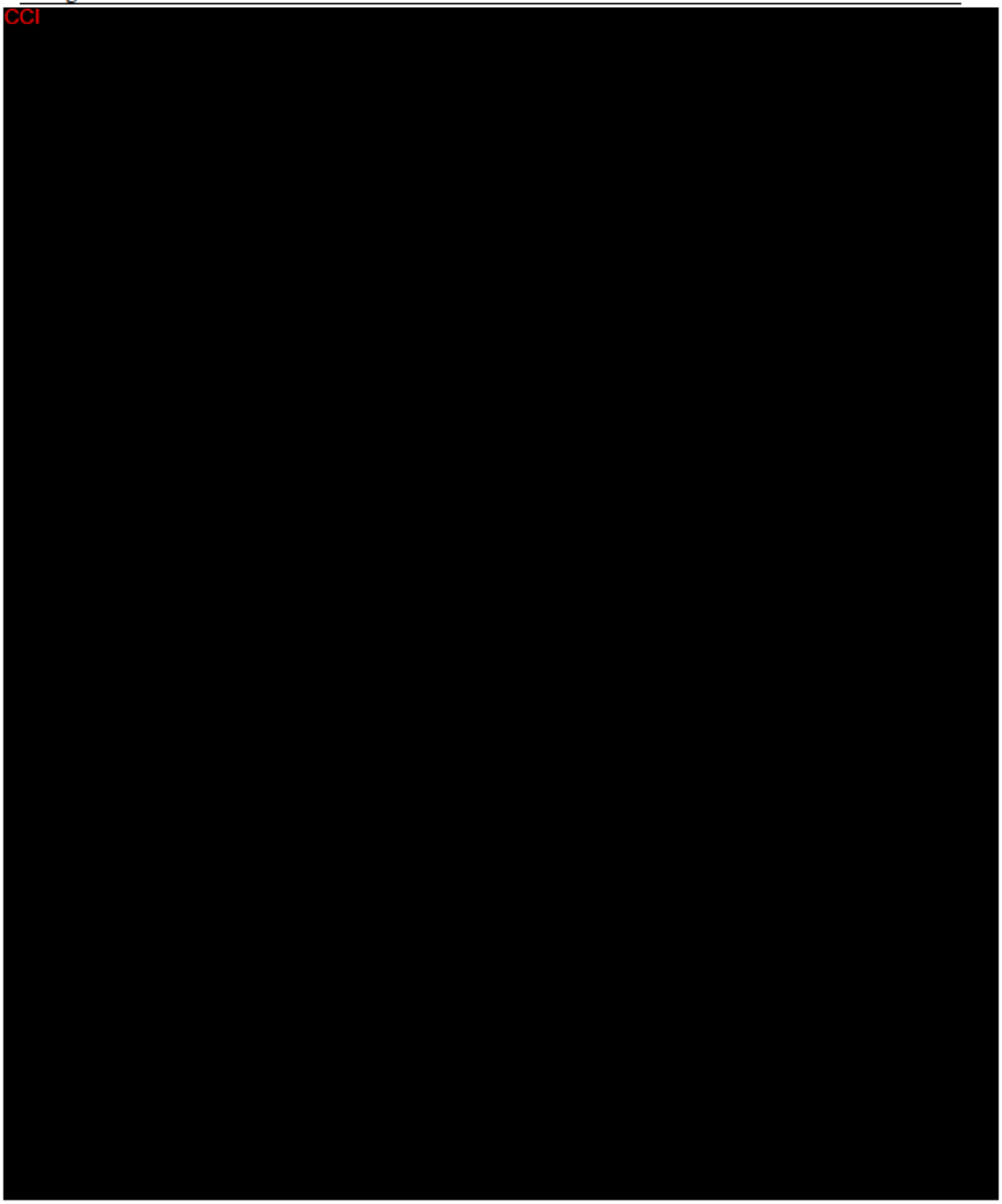

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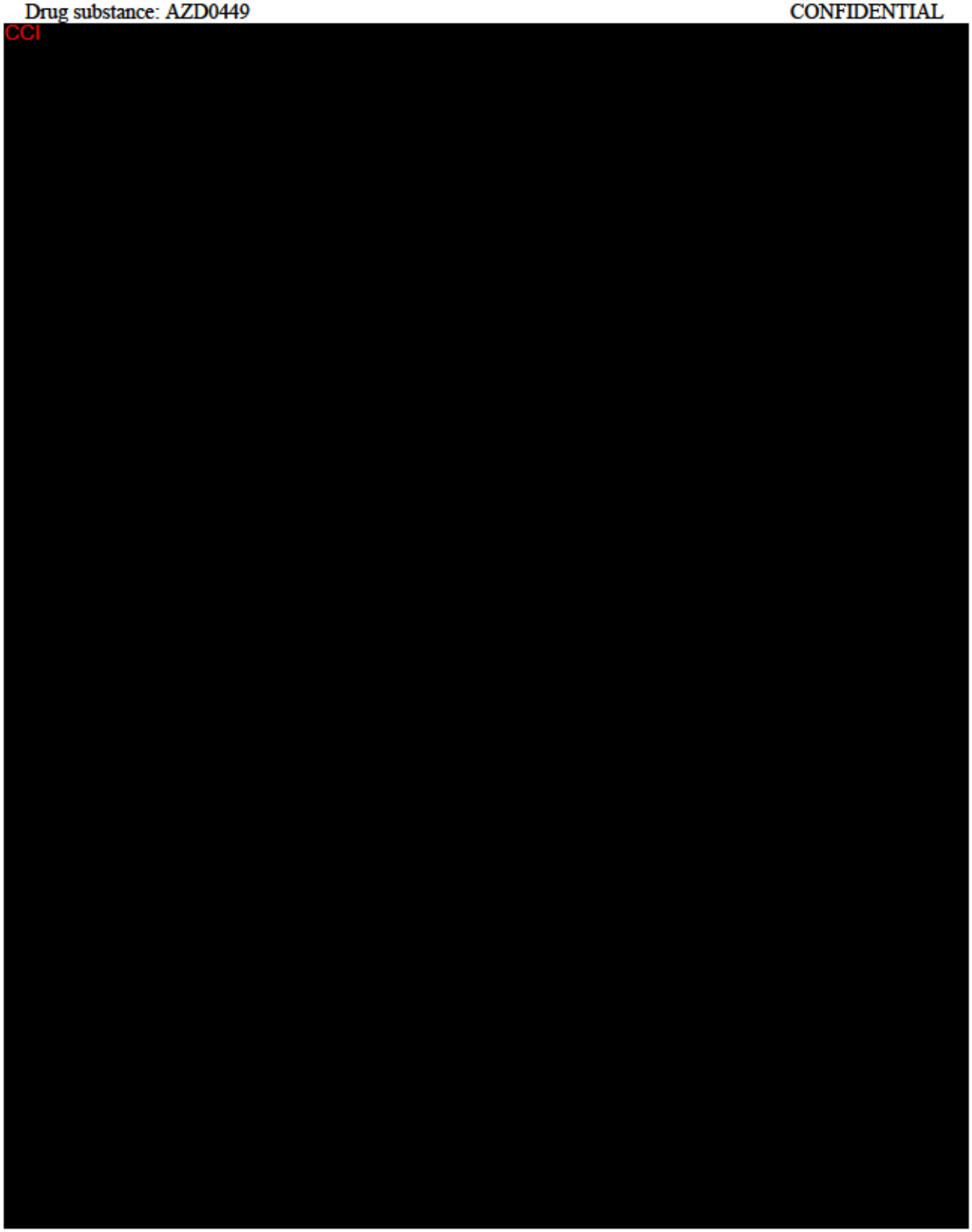

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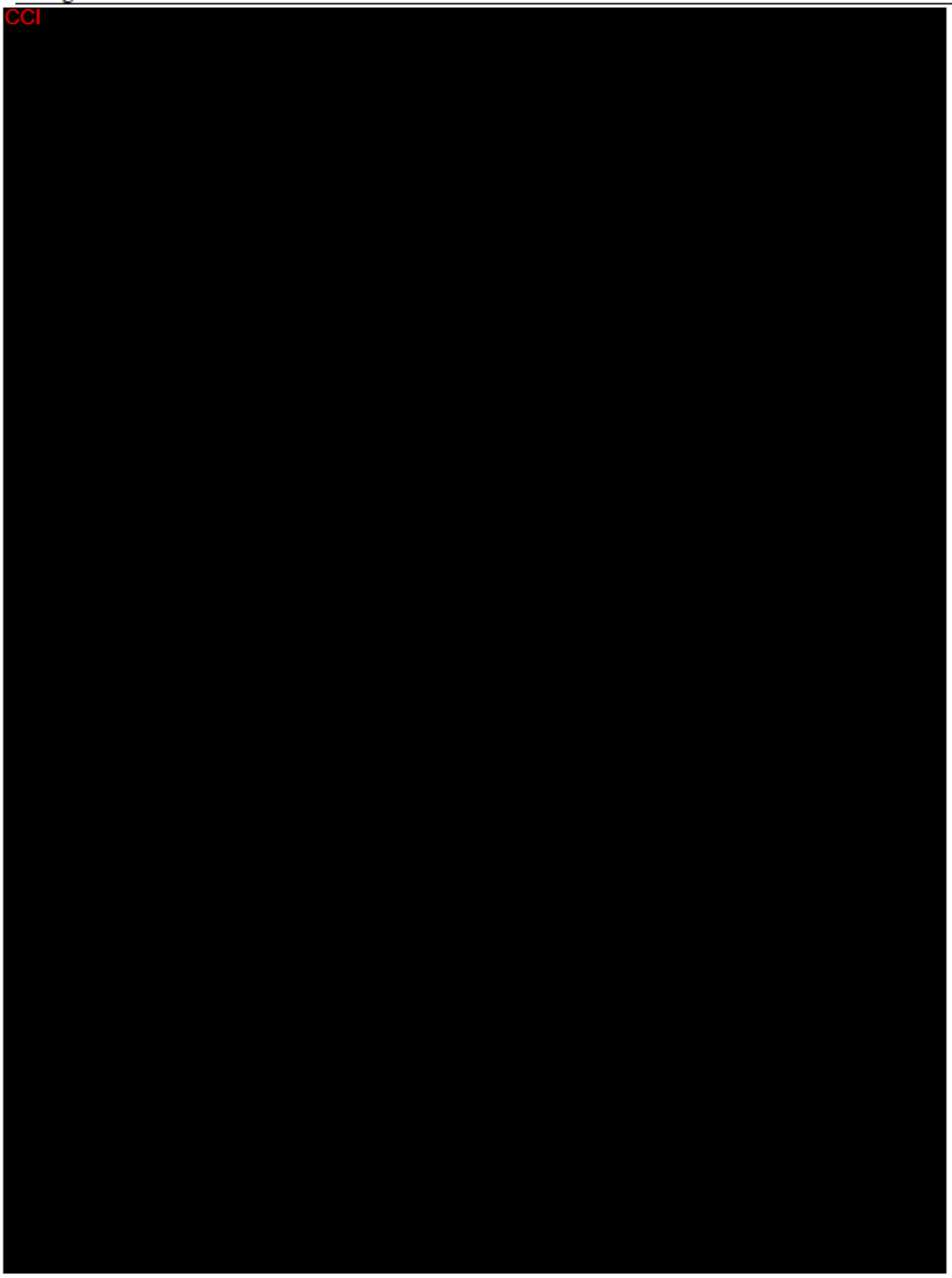

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