2 SYNOPSIS

Title of Study:	A Fixed-sequence, Open-label Study to Assess the Effect of Multiple Doses of AZD4831 on the Pharmacokinetics of Oral Midazolam (a CYP450 3A Probe) in Healthy Subjects	
Study Numbers:	Parexel Study No.: CCI [redacted] Sponsor Study No.: D6580C00012	
Investigational Medicinal Products:	Test Product: AZD4831 Additional Test Product: Midazolam	
Indication Studied:	Cardiovascular Disease	
Development Phase:	Phase I	
Sponsor:	AstraZeneca AB 151 85 Södertälje Sweden	
Principal Investigator:	PPD	
Study Centre:	Parexel Early Phase Clinical Unit – London	
Publication:	None	
Study Duration:	First subject first visit: 05 Oct 2021	Last subject last visit: 29 Nov 2021

Study Objective(s):

Primary objective(s):

- To assess the effect of AZD4831 on the pharmacokinetics (PK) of midazolam.

Secondary objective(s):

- To assess the PK of AZD4831 and midazolam.
- To examine the safety and tolerability of AZD4831 alone and in combination with midazolam.

Title of Study:	A Fixed-sequence, Open-label Study to Assess the Effect of Multiple Doses of AZD4831 on the Pharmacokinetics of Oral Midazolam (a CYP450 3A Probe) in Healthy Subjects

Study Design:

This was an open-label, fixed-sequence, cross-over study to assess the PK of midazolam in healthy subjects when administered alone and in combination with AZD4831 after multiple once daily doses of AZD4831 for 10 consecutive days. Subjects received midazolam (2 mg) as a single dose on 2 separate dosing occasions, 10 days apart (Days 1 and 11). The study was performed at a single Clinical Unit. The study comprised:

- A 28-day Screening Period.
- Two treatment periods:
  - Treatment Period 1: Midazolam only (Day 1).
  - Treatment Period 2: AZD4831 only (Days 2 to 10, once daily dosing) and AZD4831 plus midazolam (Day 11).
- A final Follow-up Visit after the last administration of investigational medicinal product (IMP) (Day 20 [± 1 day]).

Subjects were admitted to the Clinical Unit on Day -1. As per protocol, subjects had the option to be discharged on Day 3 following the second dose of AZD4831, after which they could return to the Clinical Unit for outpatient dosing with AZD4831 on Days 4 to 9. Subjects could be re-admitted on Day 10 and remain in the Clinical Unit until Day 12. However, given the COVID-19 pandemic, the other option was adopted, ie, all subjects remained in-house for both Treatment Periods 1 and 2, and were discharged on Day 12.

Each subject was involved in the study for approximately 7 weeks.

Participants were to receive a [redacted] mg single oral dose of AZD4831 under fasted conditions once daily for 10 consecutive days (Days 2 to 11). When plasma samples for the participants were analysed, only half the expected concentration of AZD4831 was found. An investigation showed that due to incorrectly labelled boxes, [redacted] mg was administered instead of the planned [redacted] mg. It was considered that there was no risk to participant safety as a lower dose was administered. Data from this study were analysed for the scientific value this data can provide.

Study Subjects:

Planned for Inclusion:	**Enrolled:**	**Completed Study:**
14 subjects	14 subjects	14 subjects

Main Inclusion Criteria:

This study was to be conducted in healthy subjects (males and females of non-childbearing potential), 18 to 55 years of age, who had a body mass index (BMI) between 18.5 and 30 kg/m^2 (inclusive), and weighed between 50 and 100 kg (inclusive).

Investigational Medicinal Products:

Formulation(s):	**Strength/Concentrations:**	**Batch/Manufacturing Lot Number(s):**	**Expiry Date(s):**
AZD4831 tablet	[redacted] mg	[redacted]	31 Mar 2022
Midazolam solution	2 mg/mL	033514	31 Aug 2023

Title of Study:	A Fixed-sequence, Open-label Study to Assess the Effect of Multiple Doses of AZD4831 on the Pharmacokinetics of Oral Midazolam (a CYP450 3A Probe) in Healthy Subjects

Duration of Treatment:

Each subject received a single dose of midazolam 2 mg on 2 separate occasions, 10 days apart (Days 1 and 11) and AZD4831 CC mg once daily over a period of 10 days (Days 2 to 11)

Treatment Compliance:

Dosing took place at the Parexel Early Phase Clinical Unit.

The administration of all IMPs was recorded in ClinBase™.

Compliance was assured by direct supervision and witnessing of IMP administration. After IMP administration, a check of the subject's mouth and hands was performed.

Criteria for Evaluation:

Pharmacokinetic Parameters:

The following PK parameters were determined for midazolam, using plasma concentrations for Treatment Period 1 (reference treatment) and Treatment Period 2 (test treatment), and for AZD4831 using plasma concentrations for Treatment Period 2.

- Primary PK parameters (midazolam): AUCinf, AUClast, Cmax
- Secondary PK parameters (midazolam): tmax, t½λz, CL/F, Vz/F
- Secondary PK parameters (AZD4831): AUCtau, Cmax, tmax, t½λz, CL/F, Ctrough (pre-dose concentrations on Day 3 up to Day 10), C24h

Safety Variables:

- Adverse events (AEs).
- Laboratory assessments (haematology, clinical chemistry, and urinalysis).
- Vital signs.
- Pulse oximetry.
- Resting 12-Lead electrocardiograms (ECGs).
- Physical examination.

Statistical Methods:

Determination of Sample Size:

The number of subjects was based on the desire to gain sufficient precision for the 90% confidence interval (CI) of the geometric mean ratio (GMR) of AUCinf while exposing as few subjects as possible to study procedures. Based on an estimate of the within-subject coefficient of variation of 25% for AUCinf, 12 evaluable subjects were expected to give a relative precision of 1.56 (ratio between the upper and lower limits of the 90% CI) with a probability of 80%. This corresponded to a 90% CI of 0.8 to 1.25 if the observed GMR was 1.00.

A total of 14 subjects were included in the study to ensure at least 12 evaluable subjects at the end of the last treatment period.

Presentation and Analysis of Pharmacokinetic Data:

The plasma concentrations of AZD4831 and midazolam and the PK parameters were listed and presented in tabular and graphical form as appropriate according to the most recent version of the AstraZeneca Corporate Clinical Study Report High Level Document (CSRHLD) reporting standards, which includes applicable descriptive statistics, handling of individual concentrations below the lower limit of quantification for listings, descriptive statistics and figures, and precision and rounding rules for concentrations and PK parameter data.

The primary PK outcome variables, AUCinf, AUClast, and Cmax of midazolam, were analysed separately using a mixed-effects analysis of variance model following a natural logarithmic transformation of AUCinf, AUClast, and Cmax as the response variables and treatment as fixed effect. For each primary PK variable, least square (LS) means (with 2-sided 95% CIs) for midazolam in the presence of AZD4831 (test treatment) and in the absence of AZD4831 (reference treatment), as well as LS mean differences (with 2-sided 90% CIs) for test treatment versus reference treatment were estimated. Results were back transformed to the original scale and were presented as geometric means and GMRs, with corresponding CIs.

The interpretation was based on the 90% CIs of GMR for AUCinf and Cmax. If the CIs for AUCinf were between 0.8 and 1.25, and for Cmax between 0.7 and 1.43, then it was concluded that AZD4831 had no effect on CYP3A4.

Presentation and Analysis of Safety Data:

All safety data (scheduled and unscheduled) were presented in the data listings. Continuous variables were summarised using descriptive statistics (n, mean, SD, min, median, max) by treatment period. Categorical variables were summarised in frequency tables (frequency and proportion) by treatment/dose group. The analyses of the safety variables were based on the safety analysis set.

Adverse events were summarised by Preferred Term and System Organ Class using Medical Dictionary for Regulatory Activities (MedDRA) vocabulary. Furthermore, listings of serious adverse events (SAEs) and AEs leading to the discontinuation of the IMP (DAEs) were made and the number of subjects who had any AEs, SAEs, DAEs, and AEs with severe intensity were summarised. Adverse events that occurred before dosing were reported separately.

Tabulations and listings of data for vital signs, clinical laboratory tests, and ECGs (listings only), were presented. Any new or aggravated clinically relevant abnormal medical physical examination finding compared to the baseline assessment was to be reported as an AE. Data were summarised for the observed values at each scheduled assessment, together with the corresponding changes (and/or percentage change) from the baseline when baseline was defined. Clinical laboratory data were be reported in the units provided by the clinical laboratory for the Safety Review Committee meeting, and in Système International units.

Out-of-range values for the safety laboratory tests were flagged in individual listings as well as summarised descriptively using agreed reference ranges (eg, AstraZeneca, programme, or laboratory ranges).

Protocol Deviations:

Title of Study:	A Fixed-sequence, Open-label Study to Assess the Effect of Multiple Doses of AZD4831 on the Pharmacokinetics of Oral Midazolam (a CYP450 3A Probe) in Healthy Subjects

All subjects had an important protocol deviation related to dosing. Due to mislabelling, subjects were administered [redacted] mg AZD4831 instead of the planned [redacted] mg (See Study Design). No other important protocol deviations were reported.

Pharmacokinetic Results:

The geometric LS mean ratio (in the presence versus absence of [redacted] mg AZD4831) for AUCinf and AUClast of 2 mg oral midazolam were 94.70% (90% CI, 85.86 - 104.44) and 93.85% (90% CI, 85.08 - 103.54), respectively. For both parameters, the 90% CI spanned 100% and were fully within the 80% to 125% limits.

The administration of AZD4831 with midazolam resulted in a lowering of peak exposure by approximately 13%. The geometric LS mean ratio of Cmax was 87.28%. The 90% CIs (78.57 - 96.96) did not span 100% but were fully contained within the 70% to 143% limits.

Since the 90% CI of the GMR of AUCinf spanned 100% and was fully contained within the 80% to 125% limits, and that of Cmax was contained within the 70% to 143% limits, it was concluded that there was no interaction between AZD4831 and CYP3A probe midazolam.

Following administration of [redacted] mg AZD4831, steady state was attained by Day 10 of dosing.

Safety Results:

There were no AEs with an outcome of death, SAEs, or AEs leading to IMP discontinuation reported during the study. AEs were experienced by 4 subjects following administration of midazolam 2 mg alone, 5 subjects following administration of AZD4831 [redacted] mg alone, and 5 subjects following co-administration of midazolam and AZD4831. All AEs were reported by a single subject each, except for somnolence reported by 3 subjects following co-administration of midazolam and AZD4831, and toothache, reported by 2 subjects following administration of AZD4831 [redacted] mg alone. The majority of the reported AEs were considered mild in intensity. Adverse events considered related to the IMP, as assessed by the Investigator, were pollakiuria, reported for 1 subject following administration of midazolam [redacted] mg alone, and headache, feeling hot, and palpitations, reported for 1 subject each following administration of AZD4831 [redacted] mg alone. All these related events were mild in intensity and resolved before the end of the study without intervention. The majority of the reported AEs were resolved before the end of the study. The AEs of cough, insomnia, and oropharyngeal pain, all reported in 1 subject were not yet resolved before the end of the study.

Discussion and Conclusion:

- The geometric LS mean ratio (in the presence versus absence of [redacted] mg AZD4831) for AUCinf and Cmax of 2 mg oral midazolam were 94.70% (90% CI, 85.86 - 104.44) and 87.28% (90% CI, 78.57 - 96.96), respectively. Since the 90% CI of the GMR of AUCinf spanned 100% and was fully contained within the 80% to 125% limits, and that of Cmax was contained within the 70% to 143% limits, it was concluded that there was no interaction between AZD4831 and CYP3A probe midazolam.
- Given the non-clinical data, if [redacted] mg AZD4831 were used in this study as planned, similar results could be anticipated. Despite an in vitro IC_{50} for CYP3A4 inhibition of ~5 µmol/L, there was no observed increase in midazolam exposure, point estimate was actually below 100%. In addition, applying mechanistic drug-drug interaction assessment using a [redacted] mg dose instead of [redacted] mg AZD4831, predicts only a minor change in the AUC ratio. Thus, AZD4831 ([redacted] mg or [redacted] mg) is unlikely to cause a clinically significant change in the exposure of midazolam or other CYP3A4 probes/substrates at the anticipated therapeutic exposures.
- Following administration of [redacted] mg AZD4831, steady state was attained by Day 10 of dosing.
- AZD4831 administered alone and in combination with midazolam demonstrated an acceptable safety profile and was well tolerated in the studied population.

Version and date of report: Version 1.0, 27 July 2022

Title of Study:	A Fixed-sequence, Open-label Study to Assess the Effect of Multiple Doses of AZD4831 on the Pharmacokinetics of Oral Midazolam (a CYP450 3A Probe) in Healthy Subjects
This study was conducted in compliance with International Council for Harmonisation Good Clinical Practice guidelines. Due to incorrectly labelled boxes, subjects were administered with [redacted] mg investigational product instead of the planned [redacted] mg. The [redacted] mg tablet was released per standard requirement.	