2. SYNOPSIS

Study centers

The study was conducted at 7 study centers in the US.

Publications

None at the time of writing this report.

Objectives and criteria for evaluation

The study objectives and criteria for evaluation are presented in Table S1.

Table S1 Objectives and Endpoints

Objectives	Endpoints[a]
Primary	
• To assess the safety and tolerability of AZD8233 following SC administration of multiple ascending doses.	• Adverse events. • Vital signs (SBP, pulse rate, and oral body temperature). • 12-lead dECG. • Telemetry. • Physical examination. • Injection site reaction examinations. • Laboratory assessments (hematology, clinical chemistry, coagulation, renal safety biomarkers, immune activation response, complement activation panel, and urinalysis for hematuria).
Secondary	
• To characterize the PK of AZD8233 following SC administration of multiple ascending doses.	• Plasma parameters: t_{lag}, t_{max}, C_{max}, $AUC_{(0\text{-}last)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}48)}$, AUC, AUC_{τ}, C_{trough}, CL/F, Vz/F, $t_{1/2}$, MRT. • Urine parameters: Ae, Fe, CL_R, diagnostic parameters.
• To assess the effect of AZD8233 on levels of PCSK9 following SC administration of multiple ascending doses.	• Level of PCSK9 in plasma.
• To assess the effect of AZD8233 on levels of LDL-C following SC administration of multiple ascending doses.	• Level of LDL-C.
• To assess the effects of AZD8233 on the lipid profile (total cholesterol, HDL-C, triglycerides) following SC administration of multiple ascending doses.	• Lipid profile.

Ae: amount excreted in urine; AUC: area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC_{τ}: Area under the plasma concentration-time curve in the dosing interval; $AUC_{(0\text{-}24)}$: area under the plasma concentration-time curve from time zero to 24 hours after dosing; $AUC_{(0\text{-}48)}$: area under the plasma concentration-time curve from time zero to 48 hours after dosing $AUC_{(0\text{-}last)}$: area under the plasma concentration curve from time zero to time last value above the limit of quantification; CL/F: apparent total body clearance of drug from plasma after extravascular administration; CL_R: renal clearance of drug from plasma, estimated by dividing $Ae_{(0\text{-}24)}$ by $AUC_{(0\text{-}24)}$; C_{max}: observed maximum plasma concentration; C_{trough}: observed trough plasma drug concentration; CSR: Clinical Study Report; dECG: digital electrocardiogram; Fe: fraction excreted unchanged in urine; HDL-C: high-density lipoprotein cholesterol; λz: terminal rate constant, estimated by log-linear least-squares regression of the terminal part of the concentration-time curve; LDL-C: low-density lipoprotein cholesterol; PCSK9: proprotein convertase subtilisin/kexin type-9; MRT: mean residence time of the unchanged drug in the systemic circulation; PK: pharmacokinetic(s); SBP: systolic blood pressure; SC: subcutaneous; t1/2: half-life associated with the terminal slope (λz) of a semi-logarithmic concentration-time curve.; t_{lag}: time delay between drug administration and the first observed concentration in plasma; t_{max}: time to reach peak or maximum observed concentration or response following drug administration; Vz/F: apparent volume of distribution during the terminal phase after extravascular administration.

[a] Exploratory results are not reported in the CSR synopsis but can be found in the CSR.

Study design

This was a randomized, single-blind, placebo controlled, multiple ascending dose (MAD) study to assess the safety and tolerability and characterize the pharmacokinetics (PK) of AZD8233 following subcutaneous (SC) administration of ascending doses on scheduled days. The study also assessed the pharmacodynamics (PD) of AZD8233 by investigating the effect of AZD8233 on low-density lipoprotein cholesterol (LDL-C) and proprotein convertase subtilisin/kexin type-9 (PCSK9).

Subjects came to the study Clinical Units for a maximum of 17 visits, including:

- A Screening Period ≤ 28 days before the Baseline Visit up to Day -1.
- An active Treatment Period, that lasted for 58 days (up to Visit 9).
- Follow-up Period, including Visits 10 to 17 (weeks 2, 4, 6, 8, 10, 12, 14, and 16 post last dose).

The study was conducted in 3 cohorts: Cohort 1 ([redacted] Cohort 2 ([redacted] and Cohort 3 ([redacted]). Each subject received AZD8233 or placebo SC on Days 1, 8, 29, and 57. The safety and tolerability data up to 7 days post second dose was reviewed by a Safety Review Committee (SRC) to decide the dose level for the following cohort.

Eligible subjects who signed the informed consent form (ICF) were randomly assigned using an Interactive voice/Web response system (IxRS) to receive AZD8233 or placebo in a ratio of 8:3.

The subjects, Clinical Unit Investigators, and staff (except for the pharmacy staff) were blinded. Dosing was done by blinded study staff at the Clinical Units, who remained blinded until dosing, follow-up, and SRC review were completed.

Target population and sample size

[redacted]

Investigational product and comparator(s): dosage, mode of administration and batch numbers

Table S2 Study Treatments

	AZD8233	AZD8233 Placebo-to-Match
Supplier:	AstraZeneca	Hospira (sourced by AstraZeneca)
Batch/lot numbers:	CCI	CCI
Formulation:	AZD8233 solution for SC injection	Saline solution (sodium chloride) for injection
Strength/concentrations:	CCI	CCI
Dose:	Cohort 1: CCI; Cohort 2: CCI; Cohort 3: CCI	N/A
Route of administration:	Subcutaneous injection	
Methods of ensuring blinding:	This study was single-blind with regard to treatment (AZD8233 or placebo) at each dose level. AZD8233 and placebo were matched for appearance and amount. Subjects randomized to placebo received the same volume of injection as subjects on active drug. At each Clinical Unit, the unblinded pharmacist was required to spend the IMP or placebo.	
Specific device for drug administration:	Syringes for injection provided by clinical CRO	
Regimen:	Multiple doses: Days 1, 8, 29, and 57	
Special handling requirements:	Provided in a separate document	

CRO: Contract Research Organization; IMP: investigational medicinal product; N/A: not applicable.

Duration of treatment

Each subject was involved in the study for up to 28 weeks. The active treatment period of the study lasted for 58 Days (up to Visit 9)

Statistical methods

Presentation and analysis of pharmacokinetic data:

AZD8233 plasma concentrations, amount excreted in urine (Ae), and fraction of dose excreted (per collection interval and cumulative) and PK parameters were summarized by dose level using descriptive statistics (eg, n, n below lower limit of quantification [LLOQ], arithmetic mean, standard deviation [SD], geometric mean [GeoM], geometric coefficient of variation CV%], minimum, median, and maximum) based upon the PK analysis set.

Dose-proportionality of AZD8233 and AZD8233 full length antisense oligonucleotide (ASOs) was assessed graphically and analyzed using an analysis of covariance (ANCOVA)/regression model using the logarithm of AUC0-48 (Day 1), AUCτ (Day 57), and Cmax; Days 1 and 57 as the dependent variable and the logarithm of the dose as the independent variable.

Presentation and analysis of pharmacodynamic data:
All PD data collected were listed for each subject and summarized descriptively by treatment and time point/visit including actual changes and percentage change from baseline. Statistical analysis comparisons of 2 and 4 week post last dose versus baseline were performed using the T-test and ANCOVA. Figures of the mean response (actual concentrations and percent change from baseline versus time) were used to visualize the average PD response over time.

Presentation and analysis of safety and tolerability data:
All safety data (scheduled and unscheduled) were presented in data listings. Continuous variables were summarized using descriptive statistics (n, mean, SD, minimum, median, maximum) by treatment. Categorical variables were summarized in frequency tables (frequency and proportion) by treatment. The analysis of the safety variables was based on the safety analysis set, which included all subjects who received at least one dose of AZD8233 or placebo and for whom any safety post-dose data were available.

Adverse events were coded using Medical Dictionary for Regulatory Activities (MedDRA) vocabulary and were summarized by preferred term (PT) and system organ class (SOC). Furthermore, listings of serious adverse events (SAEs), adverse events (AEs) that led to discontinuation of the investigational medicinal product (IMP), and AEs that led to withdrawal from the study were made and the number of subjects who had any AE, SAEs, AEs that led to discontinuation of the IMP, withdrawal from the study, and AEs by intensity were summarized. The AEs that occurred before dosing were reported separately. Any injection site reaction was treated as an adverse event of special interest (AESI) and was listed separately. Tabulations and listings of data for vital signs, clinical laboratory tests and electrocardiograms (ECGs), were presented.

Study population

A total of 102 subjects were enrolled and 34 subjects were randomized: 8 subjects to receive CCI AZD8233, 9 subjects to receive CCI, 8 subjects to receive CCI and 9 subjects to receive placebo. All cohorts were balanced in terms of demographic and baseline characteristics.

All randomized subjects received treatment, of which 32 (94.1%) subjects completed the treatment and study follow-up. Two (5.9%) subjects discontinued treatment, 1 subject due to an AE of positive coronavirus disease 2019 (COVID-19) viral test, and 1 subject was discontinued due to the COVID-19 pandemic (it was decided that the subject would not be returning to the Clinical Unit due to the subject's underlying medical condition putting him at an increased risk for COVID-19). Both these subjects received the planned doses up to Day 29 before discontinuation).

Summary of pharmacokinetic results

AZD8233 in plasma

AZD8233 was rapidly absorbed following SC administration with a median tmax of 1.50 to 2.00 hours post-dose on Day 1 and 1.25 to 2.52 hours postdose on Day 57 with no apparent trend with dose or study Day. Plasma concentrations on Day 1 remained quantifiable until between 12.00 and 60.00 hours postdose.

Following Cmax, plasma concentrations declined generally in a biphasic manner with a geometric mean t½λz ranging between 2.847 and 4.498 hours on Day 1 and between 2.898 and 4.813 hours on Day 57.

Inter-subject variability (based upon geometric CV%) was generally low (< 25%) to moderate (25% to 40%) at the CCI dose groups and high (> 40%) at the CCI dose group for the AUC estimates (AUC, AUCτ, $AUC_{(0\text{-last})}$, $AUC_{(0\text{-}48)}$, and $AUC_{(0\text{-}24)}$) and moderate (25 to 40%) to high (> 40%) for Cmax in all dose groups on both Days 1 and 57. The highest inter-subject variability was observed in the CCI dose group.

There appeared to be minimal accumulation observed at all dose levels following multiple AZD8233 doses; with geometric mean accumulation ratios ($Rac_{(AUC)}$ and $Rac_{(Cmax)}$) ranging from 0.9540 to 1.172. There was no evidence of substantial time-dependent changes in AZD8233 exposure across all dose levels; geometric mean TCP values ranged from 0.9646 to 1.024.

The overall increase in systemic exposure to AZD8233 with dose was supra-proportional for both $AUC_{(0\text{-}48)}$ on Day 1 and AUCτ on Day 57 with a slope of 1.355 and 1.417 respectively and 90% CI which did not include unity. The 6-fold increase in dose from CCI AZD8233 resulted in a 10.9-fold increase in geometric mean $AUC_{(0\text{-}48)}$ and a 14.3-fold increase in geometric mean Cmax on Day 1. A similar trend was observed following multiple AZD8233 doses with a 11.7-fold increase in geometric mean AUCτ and a 11.2-fold increase in geometric mean Cmax on Day 57. The more than proportional increase was mainly observed between the CCI dose range. The 2-fold increase in dose from CCI resulted in an approximately dose proportional increase exposure, with 1.99-fold and 2.33-fold increases in $AUC_{(0\text{-}48)}$ and Cmax respectively on Day 1 and 1.89-fold and 2.01-fold increases in AUCτ and Cmax respectively on Day 57.

AZD8233 full length ASOs in plasma

Full length ASOs appeared rapidly with a median t_{max} of 1.250 to 2.375 hours postdose on Day 1 and 1.567 to 2.042 hours postdose on Day 57. There was no apparent trend with dose or study Day. Plasma concentrations on Day 1 and Day 57 remained quantifiable throughout the dosing interval at all dose levels and until the follow up visits at 6 to 16 weeks postdose after the Day 57 dose.

Following Cmax, plasma concentrations declined generally in a biphasic manner. The profiles showed a rapid distribution phase, which generally mirrored the concentration profiles of the parent compound, and slow elimination phase. The geometric mean t½λz ranged between 123.2 and 145.5 hours (5 and 6 days) on Day 1, however, these values are not representative of the terminal phase since data were only available until 168 hours postdose (Day 8 predose). On Day 57, the geometric mean t½λz estimates were between 439.8 to 650.8 hours (18 and 27 days) on Day 57.

Inter-subject variability (based upon geometric CV%) was generally low (< 25%) to moderate (25% to 40%) at the CCI dose level and high (> 40%) at the CCI dose level for the AUC estimates (AUC, AUCτ, $AUC_{(0\text{-last})}$, $AUC_{(0\text{-}48)}$, and $AUC_{(0\text{-}24)}$) on both Day 1 and Day 57. The inter-subject variability in Cmax was moderate (25 to 40%) at the CCI dose level and high (> 40%) at the CCI dose levels on both Days 1 and 57.

There appeared to be minimal accumulation observed at all dose levels following multiple AZD8233 doses; with geometric mean accumulation ratios ($Rac_{(AUC)}$ and $Rac_{(Cmax)}$) ranging from 0.9148 to 1.116. There was no evidence of substantial time-dependent changes in AZD8233 full length ASOs exposure across all dose levels; geometric mean TCP values ranged from 1.085 to 1.251. Trough values of AZD8233 full length ASOs were generally constant across dosing days suggesting that steady state was achieved for AZD8233 full length ASOs by Day 8.

The overall increase in systemic exposure to AZD8233 full length ASOs with dose was supra-proportional for both $AUC_{(0\text{-}48)}$ on Day 1 and AUCτ on Day 57 with a slope of 1.323 and 1.287 respectively and 90% CI which did not include unity. The 6-fold increase in dose from CCI AZD8233 resulted in a 10.3-fold increase in geometric mean $AUC_{(0\text{-}48)}$ and a 13.1-fold increase in geometric mean Cmax on Day 1. A similar trend was observed following multiple AZD8233 doses with a 9.4-fold increase in geometric mean AUCτ and a 10.7-fold increase in geometric mean Cmax on Day 57. The more than proportional increase was mainly observed between the CCI dose range. The 2-fold increase in dose from CCI resulted in an approximately dose proportional increase in exposure, with a 1.95-fold and 2.20-fold increases in $AUC_{(0\text{-}48)}$ and Cmax respectively on Day 1 and 1.72-fold and 1.88-fold increases in AUC□ and Cmax respectively on Day 57.

The increase in C_{trough} on Day 57 followed a similar pattern, although the overall increase 8.4-fold in Ctrough between the CCI dose levels was less marked than the increased in AUCτ and C_{max}. The 2-fold increase in dose from CCI resulted in a 2.3-fold increase in C_{trough} and the 3-fold increase in dose from CCI resulted in a 3.7-fold increase in C_{trough}.

AZD8233 full length ASOs in urine

Following single dose administration of AZD8233 at the CCI dose levels, the geometric mean $Ae_{(0\text{-last})}$ for AZD8233 of full length ASOs in urine on Day 1 was between 0.01842 mg and 0.3566 mg which represented between CCI of the dose. The geometric mean Ae(0-last) after multiple dosing (Day 57) ranged between 0.09081 and 0.5073, representing CCI of the dose. Overall CLR ranged between 0.03450 L/h and 0.1697 L/h across both days, which was small compared to CL/F which ranged between 12.22 L/h and 27.31 L/h and suggests that the clearance of full length ASOs are predominantly non-renal. The fe% was higher at the CCI dose level on Day 1, consistent with the supraproportional increase in systemic exposure. However, on Day 57 fe% did not show a consistent trend with dose.

Summary of pharmacodynamic results

There was a significant decrease in plasma PCSK9 levels within 48 hours of the first dose. The differences between baseline for PCSK9 results overall and 2 and 4 weeks post last dose were all highly significant for all three dose levels of AZD8233 versus placebo. The changes from baseline were clearly dose dependent, with the largest decrease from baseline seen in the CCI cohort.

There was a significant decrease in LDL-C levels within 8 days after the first dose for all doses, reflected in both the direct measurement and the calculation using the Friedewald formula. The changes from baseline did not discriminate as sharply as for the PCSK9 inhibition curves and particularly lacked a clear dose dependency initially but were more distinguishable for the CCI cohort. No obvious changes from baseline were observed in the placebo group.

Reductions in plasma LDL-C levels were reflected in a decrease in total cholesterol levels within 48 hours (3 days) after the first dose for all the doses. The changes from baseline were higher with higher doses, but not at all time points. No obvious changes from baseline were noted for HDL-C and triglycerides. No obvious changes from baseline were observed in the placebo group for either of the lipid profile parameters.

Summary of pharmacokinetic/pharmacodynamic relationships

After stop of treatment the plasma PCSK9 levels slowly returned to baseline or close to baseline levels during the follow period up to 16 weeks post last dose. The slow return to baseline levels were in agreement with the long terminal half-life of AZD8233 full length ASOs observed in plasma.

Summary of safety results

Adverse events:

Overall, AEs occurred in a similar frequency in the AZD8233 pooled group compared to the placebo group. Adverse events occurred with increasing frequency with dose, ie, 50.0%, 66.7%, and 87.5% in the CCI cohorts, respectively. No SAEs or deaths were reported in the study. One subject in the AZD8233 CCI group discontinued from the IMP due to an AE (COVID-19).

All AEs reported in the study, except for 1 AE of peripheral swelling in the CCI cohort, were mild or moderate. In total, 6 (24.0%) of subjects receiving active treatment reported AEs that were considered related to the IMP, all of which were mild in intensity.

Injection site reactions were reported for 4 subjects in the CCI cohort, all of which were mild in intensity and considered related to the IMP by the Investigator.

In the AZD8233 treatment groups, 4 AEs of hepatic enzyme increase/liver function test increase were reported.

Laboratory assessments:

One subject in the CCI cohort had an ALT elevation > 2 × upper limit of normal (ULN) and 3 subjects in the CCI cohort had an ALT elevation > 2 × ULN, one of which had and ALT elevation > 3 × ULN (2.467 ukat/L [0.067 – 0.717 ukat/L]).

No individually clinically important hematology abnormalities were observed for any of the subjects in this study. No event of platelet count < 100 × 10^9/L was reported in any subjects receiving AZD8233, and there was no indication of renal impairment/renal toxicity.

A total of 5 subjects in the AZD8233 groups had treatment-emergent ADAs. No ADA-related safety concerns were observed.

Vital signs:

No clinically meaningful trends in vital signs were observed. No AEs based on vital sign measurements were reported in this study.

Electrocardiogram:

No clinically meaningful trends in ECG results were identified. One (11.1%) subject in the placebo group had an AE of ECG QTcF prolonged. The QTcF increased with > 30 msec during treatment.

Conclusions

- The reported AEs and other safety results did not raise any new safety concerns.

- Mild transient increase in ALT in 4 subjects in the AZD8233 groups was observed (of which 3 subjects were in the CCI cohort). Injection site reactions were reported for 4 subjects in the CCI cohort. No event of platelet count < 100 × 10^9/L was reported for any of the subjects receiving AZD8233, nor was there any indication of renal toxicity.
- AZD8233 and AZD8233 full length ASOs appeared rapidly in plasma with median tmax values of 1.25 to 2.52 hours across both analytes and study days.
- At early time points the AZD8233 full length ASOs concentration versus time profiles mirrored that of the parent AZD8233, but at slightly higher concentrations. At later time points AZD8233 full length ASOs were the only quantifiable species in plasma.
- The full length ASOs had a biphasic plasma concentration versus time profile with a geometric mean terminal t½λz ranging between approximately 18 and 27 days.
- Clearance of AZD8233 full length ASOs was predominantly non-renal with less than 0.7% of the dose excreted as full length ASOs in urine during the first 24h.
- Exposure based upon $AUC_{(0-48)}$, AUCτ and C_{max} for AZD8233 and full length ASOs increased in a supra-proportional manner with dose but approximately proportional with dose between the CCI dose.
- There was minimal accumulation with no evidence of substantial time-dependent changes in AZD8233 and full length ASOs exposure following multiple AZD8233 doses.
- Intersubject variability in AZD8233 and AZD8233 full length ASO exposures AUC, $AUC_{(0-48)}$, AUClast and AUCτ was generally low (< 25%) to moderate (25% to 40%) but high (> 40%) at the CCI dose level.
- AZD8233 dose dependently decreased the concentrations of plasma PCSK9.
- AZD8233 dose dependently reduced LDL-C with sustained effects over the 4 weeks dosing interval.
- AZD8233 decreased the total cholesterol levels but had no discernible effect on HDL-C and triglycerides.