

## ARTICLE

# Initial Clinical Experience with AZD5718, a Novel Once Daily Oral 5-Lipoxygenase Activating Protein Inhibitor

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We evaluated safety, tolerability, pharmacokinetics, and pharmacodynamics of AZD5718, a novel 5-lipoxygenase activating protein (FLAP) inhibitor, in a randomized, single-blind, placebo-controlled, first-in-human (FIH) study consisting of single and multiple ascending dosing (SAD and MAD) for 10 days in healthy subjects. Target engagement was measured by *ex vivo* calcium ionophore stimulated leukotriene B (LTB<sub>4</sub>) production in whole blood and endogenous leukotriene E (LTE<sub>4</sub>) in urine. No clinically relevant safety and tolerability findings were observed. The AZD5718 was rapidly absorbed and plasma concentrations declined biphasically with a mean terminal half-life of 10–12 h. Steady-state levels were achieved after ~3 days. After both SADs and MADs, a dose/concentration-effect relationship between both LTB<sub>4</sub> and LTE<sub>4</sub> vs. AZD5718 exposure was observed with concentration of half inhibition (IC<sub>50</sub>) values in the lower nM range. Based on obtained result, AZD5718 is considered as a suitable drug candidate for future evaluation in patients with coronary artery disease (CAD).

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## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ FLAP is essential for the production of leukotrienes by the 5-LO pathway and it is hypothesized that inhibition of the pathway will reduce mortality, morbidity, and cardiovascular hospitalization in patients with CAD by attenuation of pro-inflammatory and vasoactive leukotriene production in the coronary circulation. AZD5718 is a novel FLAP inhibitor currently investigated in patients with CAD.

### WHAT QUESTION DID THIS STUDY ADDRESS?

✓ This was the FIH study consisting of single and multiple ascending once daily dosing to explore safety, tolerability, PKs, and PDs in healthy subjects.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

✓ No clinically relevant safety and tolerability findings were observed. AZD5718 was rapidly absorbed and a half-life consistent with once daily dosing.

### HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

✓ Target engagement was established and a dose/concentration-effect relationship between both LTB<sub>4</sub> and LTE<sub>4</sub> vs. AZD5718 exposure was observed with IC<sub>50</sub> values in the lower nM range. The results showed that AZD5718 is a suitable drug candidate for evaluation in patients with CAD.

Coronary artery disease (CAD) is currently treated with drugs that control key risk factors driving disease progression, including low density lipoprotein (LDL) cholesterol-lowering, antihypertensive, and antiplatelet drugs. Despite the availability of such drugs, patients remain at risk of experiencing an acute coronary syndrome (ACS) event, such as myocardial infarction or unstable angina. The pathophysiology underlying this remaining risk is not fully understood. However, growing preclinical and clinical evidence has highlighted the role of inflammation and microvascular dysfunction as important drivers.<sup>1</sup> Recent trial results, most notably the FOURIER trial with the proprotein convertase subtilisin/kexin type 9 neutralizing antibody evolocumab, indicate the existence of a non-LDL-dependent residual risk in the CAD population.<sup>2</sup> Further, interventional evidence from the CANTOS trial demonstrated that this residual non-LDL-

dependent inflammatory risk can be modified resulting in a cardiovascular benefit.<sup>3</sup>

Although the CANTOS trial specifically addressed the role of interleukin-1 $\beta$  inhibition, several inflammatory pathways could be of relevance for cardiovascular disease, including those mediated by leukotrienes.<sup>4</sup>

Leukotrienes are lipid mediators derived from arachidonic acid via the action of the 5-lipoxygenase (5-LO) pathway that have potent inflammatory and vasoactive properties.<sup>5</sup> The initial step in the biosynthesis of leukotrienes, formation of the unstable precursor leukotriene A<sub>4</sub>, is mediated by 5-LO in combination with 5-lipoxygenase activating protein (FLAP). FLAP is an integral membrane protein that facilitates the docking of 5-LO in the nuclear membrane and mediates the transfer of arachidonic acid released from membrane-derived phospholipids to the active site of 5-LO.<sup>5</sup>

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Leukotrienes include leukotriene B<sub>4</sub> (LTB<sub>4</sub>), a potent leukocyte activator and chemokine, and the cysteinyl leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>, and leukotriene E (LTE<sub>4</sub>), potent vasoactive, and inflammatory mediators. Leukotrienes are recognized in the pathophysiology of respiratory diseases but there is growing evidence that they could also play a role in the progression of CAD, in particular, the impact on inflammation and microvascular function.

A possible link between leukotrienes and cardiovascular disease was initially proposed following the observation that enhanced vasoconstrictive responses to LTC<sub>4</sub> and LTD<sub>4</sub> occur in coronary arteries that have developed atherosclerotic plaques.<sup>6</sup> This observation has been extended by additional studies that have elucidated an important role for leukotrienes in cardiovascular disease, including atherosclerotic plaque progression and symptoms of myocardial ischemia in patients with ACS.<sup>7–10</sup> In addition, genetic haplotypes in the FLAP gene (ALOX5AP) have been associated with the risk of myocardial infarction.<sup>11,12</sup> Interventional clinical evidence also supports a rationale for 5-LO pathway inhibition in cardiovascular disease. Tardif *et al.*<sup>13</sup> showed that the 5-LO inhibitor VIA-2291 reduced *ex vivo* stimulated LTB<sub>4</sub> production, noncalcified plaque volume, and appearance of new coronary lesions in patients with ACS.<sup>13</sup> Additionally, VIA-2291 reduced plaque progression and dose dependency improved the left ventricular ejection fraction in patients with ACS.<sup>13,14</sup> Moreover, the 5-LO inhibitor zileuton inhibited leukotriene production in blood and improved flow-mediated dilation in the brachial artery in at-risk patients with CAD.<sup>15</sup>

It is hypothesized that inhibition of the 5-LO pathway will reduce mortality, morbidity, and cardiovascular hospitalization in patients with CAD by attenuation of pro-inflammatory and vasoactive leukotriene production in the coronary circulation.

AZD5718 is a novel, potent, and selective FLAP inhibitor that inhibits the LTB<sub>4</sub> production in human whole blood *in vitro* with a concentration of half inhibition (IC<sub>50</sub>) of 39 nM. The compound is neutral with a logD of 2.9 at pH 7.4. The human plasma protein binding is high, 95.7%, and *in vitro* metabolism studies have indicated that cytochrome P450 (CYP)3A4 and CYP3A5 are the CYP-isoforms involved in the metabolism of AZD5718 but uridine 5'-diphosphate glucuronosyltransferase-conjugation contributes to the overall metabolism. The permeability of AZD5718 in Caco-2 is high, P<sub>app</sub> of  $24 \times 10^{-6}$  cm/s and the efflux ratio is 7.4 in the same system. The compound has low solubility, thus both amorphous (solubility 20 μM) and crystalline (solubility 240 μM) suspension formulation was evaluated in the present study. Here, we describe the results of a First in Human (FIH) study with AZD5718 with the objective to evaluate safety, tolerability, pharmacokinetics and pharmacodynamics in healthy subjects after single and multiple ascending doses (SADs and MADs).

## MATERIALS AND METHODS

### Study design

This study was a first-in-human (FIH) study consisting of two parts, SADs and MADs in different healthy subjects performed at a single study center (NCT02632526). Eight

subjects were included in each cohort and within each cohort six subjects received AZD5718 and two subjects received placebo. The SAD part of the study was a randomized, single-blind, placebo-controlled, sequential group design. Dosing for each cohort was proceeded with two subjects in a sentinel cohort, such that one subject was randomized to receive AZD5718 and one subject was randomized to receive placebo. The safety data from the sentinel subjects up to 24 h postdose were reviewed before the remaining subjects in the cohort were dosed. Primarily, gradual escalation of the dose was conducted with amorphous suspension and safety, tolerability, and pharmacokinetic/pharmacodynamic (PK/PD) data were evaluated. In addition, two cohorts were dosed with a crystalline suspension. Dosing with crystalline suspension was evaluated due to the limited solubility of AZD5718 and performed at dose/exposure levels below the previously established safe and tolerable doses with the amorphous suspension. The MAD part of the study was a randomized, single-blind, placebo-controlled, MAD sequential group design with no sentinel dosing applied. A total of eight SAD cohorts were conducted of which two were dosed with crystalline suspension. Only amorphous suspension was dosed in the four MAD cohorts. In both the SAD and the MAD parts, study personnel, including the principal investigator and the sponsor, were blinded to the results until the safety review meeting of the entire cohort. The decision to proceed with the dose escalation was based on unblinded data.

Eligibility criteria for the SAD and MAD included healthy male subjects aged 18–50 years, a body mass index between 18 and 30 kg/m<sup>2</sup>, and a body weight between 50 kg and 100 kg. Exclusion criteria were any clinically significant disease, disorder, or any clinically important abnormalities in hematology, clinical chemistry, or urinalysis results at screening or check-in. The study was approved by an independent ethics committee at the study site (South-Central Berkshire B Research Ethics Committee) and all subjects gave written informed consent before participating in the study.

### Clinical safety

The tolerability and safety assessments involved evaluation of vital signs, electrocardiogram (ECG), telemetry, routine clinical laboratory examinations, and adverse events. These assessments were taken before and at scheduled times after dosing as well as at the follow-up visit 7–10 days post final dose administration.

### Starting dose, escalation, and dosing

In the SAD part, each subject received a single dose of 25–1,200 mg AZD5718 or placebo and in the MAD part of the study, each subject received once daily dosing of 60–600 mg AZD5718 or placebo for 10 days. The starting dose in the SAD study was derived based on the human equivalent dose at the no observed adverse effect level in the most sensitive species, in accordance with the US Food and Drug Administration guidance for industry with a safety factor of 100. For the dose-escalation, pharmacokinetic (PK) and PK/PD-based maximum exposure limits based on no observed adverse effect level and an exposure of five times the concentration predicted to result in a 90% inhibition of

LTB<sub>4</sub> at C<sub>trough</sub> were used. The initial doses in the SAD were 25, 100, 300, 600, and 1,200 mg. However, based on the emerging results, an additional cohort was included at 50 mg in order to obtain more PK and pharmacodynamic (PD) measurements to cover the complete dose/concentration-LTB<sub>4</sub> inhibition curve. The starting dose in the MAD part was set to 60 mg, based on the emerging PK and PD data obtained in the SAD part and subsequent doses to be below the PK and PK/PD stopping criteria used in the SAD part. Before frequent PK sampling of the study (day 1 in the SAD and day 1 and day 10 in the MAD) subjects fasted for 10 h overnight before the morning dose. Up to 150 mL water was allowed up to 1 h prior to each morning dose and could be resumed 1 h after dosing. A meal was served 4 h after the morning dose. On other days during the repeated dosing phase of the study, subjects fasted for 10 h overnight prior to the morning dose and breakfast was delayed until 2 h after dosing. Water was allowed up to 1 h before and from 1 h after the morning dose. Due to the limited solubility of AZD5718, both an amorphous and a crystalline oral suspension of AZD5718 were evaluated in the SAD part of the study. The relative bioavailability of the crystalline formulation (results not presented) was much lower compared with the amorphous formulation, thus only amorphous suspension was used in the subsequent MAD part of the study.

## PHARMACOKINETIC AND PHARMACODYNAMIC ASSESSMENTS

### Blood and urine sampling for quantification of AZD5718

Blood was collected into tubes containing K<sub>2</sub> EDTA as anti-coagulant and plasma was separated and stored at nominal -80°C until analysis. Urine was collected in a pre-weighted, transparent polyethylene urine collection container. During the collection interval, the filled urine collection container was stored at nominal +4°C. At the end of each collection interval, an aliquot of the urine collected was transferred to a sample tube and stored at nominal -80°C until analysis. Samples for determination of AZD5718 in plasma and urine were analyzed by Covance Laboratories (Harrogate, UK). AZD5718 and the stable labeled carbon-13 internal standard (<sup>13</sup>C<sub>6</sub>) AZD5718 were extracted from plasma by protein precipitation and from urine by sample dilution.

The extracts were injected and analyzed by ultraperformance liquid-chromatography tandem mass spectrometry (LC-MS/MS) using an Acquity, BEH C18, 50 × 2.1 mm, particle size 1.7 μm column (Waters, Milford, MA) maintained at 40°C using a gradient of 20–98% acetonitrile in 10 mM ammonium formate (aq, 0.2% formic acid) as the mobile phase. The extracts were nebulized using heated nitrogen in electrospray positive ionization mode. The ionized compounds were detected by a Sciex API4000 (AB Sciex, San Mateo, CA) with the transitions monitored m/z 429.2 > 249.2 for AZD5718 and m/z 435.3 > 255.3 for [<sup>13</sup>C<sub>6</sub>]AZD5718. The methods were validated prior to sample analysis in the range of 1–1,000 nmol/L in plasma and 20–20,000 nmol/L in urine, using a 40 μL sample aliquot. The accuracy and precision of quality control (QC) samples of AZD5718 were determined at concentrations 1, 3, 50, 800, and 5,000 (dilution QC) nmol/L in plasma and at concentrations of 20, 60, 1,000, 16,000, and 100,000 (dilution QC) nmol/L in urine.

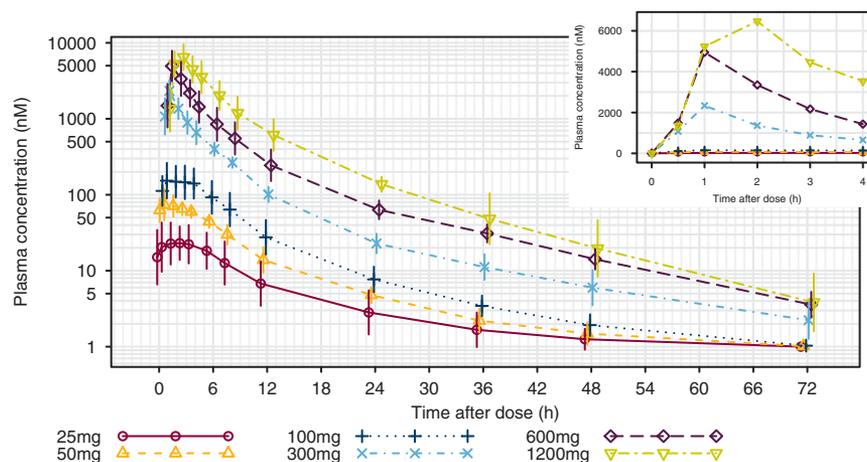
Inter-run accuracy and precision were in the ranges of 97.0–100.0% and 2.6–7.0% and 91.5–98.1% and 3.4–9.0% for plasma and urine, respectively. At a minimum, each analytical run included a calibration curve, a matrix blank, a control zero sample (matrix blank containing internal standard), a reagent blank, and duplicate QC samples at three concentrations within the calibration range. To demonstrate acceptable in-study performance, incurred sample reproducibility analyses were performed during the study. For plasma, 130 of the 140 samples (92.9%) tested were within 20% of the mean of the two values. For urine, 37 of the 40 samples (92.5%) tested were within 20% of the mean of the two values. Both assessments were within the acceptance criteria. All study samples were analyzed within the known stability period of 391 days (plasma) and 374 days (urine).

### Blood and urine sampling for LTB<sub>4</sub> and LTE<sub>4</sub> and whole blood assay for LTB<sub>4</sub>

In both SAD and MAD, 10 mL whole blood samples were collected in lithium-heparin tubes (BD Vacutainer; BD Biosciences, San Jose, CA) 24 h prior to the first dose, immediately prior to dosing, and at specified time intervals thereafter (30 min, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h post dosing in the SAD study; 12 h post first dose, predose on days 2 to 10, and at 2, 6, 12, 24, and 48 h post last dose in the MAD study). We then took 2 mL of blood that was subsequently stimulated in duplicate with 30 μM calcium ionophore A23187 (Sigma Chemical, St Louis, MO) or dimethylsulfoxide vehicle and incubated at 37°C with gentle agitation (100 rpm) for 30 min. Samples were placed on ice and assays stopped by addition of 250 μl 100 mM EGTA (Sigma Chemical) followed by inversion two times. Following centrifugation at 2,000 rcf, 4°C, for 15 min, plasma was carefully transferred to a fresh tube and stored at -80°C until analysis of LTB<sub>4</sub>. Quantitative analysis of LTB<sub>4</sub> was performed by supported liquid extraction and LC-MS/MS using a Waters Xevo TQ-S mass spectrometer. The sensitivity of the method was 20 pg/mL within the validated range of 20–20,000 pg/mL, and had a within donor precision (percentage of coefficient of variation of <15%. To calculate fresh LTB<sub>4</sub> production, LTB<sub>4</sub> levels in the dimethylsulfoxide-stimulated control assays were subtracted from the corresponding ionophore-stimulated assays. For each subject, the mean of the day 1 and predose samples was set as 100% baseline.

### LTE<sub>4</sub> quantification in urine

Spot urine was collected 24 h prior to dosing and immediately prior to dosing for both SAD and MAD studies to provide baseline samples. In the SAD study, pooled urine was subsequently collected in the intervals 0–3, 3–6, and 9–12 h postdose followed by spot urine at 24, 36, and 48 h postdose. In the MAD study, pooled urine was collected on days 1 and 10 in the intervals 0–3, 3–6, and 9–12 h postdose, whereas spot urine was collected predose on days 2 to 9 and at 24 and 48 h following the last dose. Urine was stored at -80°C until analysis of LTE<sub>4</sub> and creatinine. Urine LTE<sub>4</sub> levels were determined using a solid phase extraction and LC-MS/MS method. The method had a lower limit of quantification (LLOQ) of 5 pM. Creatinine levels were quantified using an enzymatic colorimetric method (Horiba ABX, France) on



**Figure 1** Geometric mean  $\pm$ SD for plasma concentration-time profiles by treatment following single ascending doses of AZD5718,  $n = 6$  per dose. Insert: first 4 h after dose.

an ABX Pentra 4000 instrument (Horiba ABX). The urine LTE<sub>4</sub> level was corrected to the creatinine level for each sample (pM uLTE<sub>4</sub> per mM creatinine) and normalized to the mean of the day 1 and predose samples.

### Pharmacokinetic analysis

Actual blood sampling times were used in all analyses and plasma concentration vs. time data of AZD5718 were analyzed by noncompartmental analysis with Phoenix WinNonlin version 6.2. The maximum plasma concentrations ( $C_{max}$ ) and the time to reach this concentration ( $T_{max}$ ), elimination half-life ( $t_{1/2}$ ), and the total area under the plasma concentration time-curve (AUC) were estimated. The AUC was calculated by the log-linear trapezoidal rule from time zero to the time for the last measurable concentration ( $t_{last}$ ) plus the extrapolated residual area to infinity. The residual area after  $t_{last}$  was calculated as  $C_{last,pred}/\lambda_z$ , where  $C_{last,pred}$  was the predicted concentration at  $t_{last}$  and  $\lambda_z$  was the terminal rate constant determined by linear regression analysis of  $\ln$  plasma concentration vs. time, using the last plasma concentrations from each subject. The  $t_{1/2}$  was calculated as  $\ln 2/\lambda_z$ . The apparent oral plasma clearance (CL/F) was estimated as dose/AUC and renal clearance as the amount excreted unchanged/AUC. Dose linearity was explored by eyeballing and linear regression exploration.

### Statistical analysis

The sample size was chosen to obtain reasonable evidence of safety and tolerability without exposing undue numbers of healthy subjects to the compound at this stage of clinical drug development. Previous experience from similar studies has shown that the sample size used was reasonable to accomplish the objectives of this study.

## RESULTS

### Subjects

Mean age ( $\pm$ SD) of the subjects was  $34.3 \pm 9$  years and mean body mass index ( $\pm$ SD)  $24.7 \pm 3$  kg/m<sup>2</sup>. Subjects were

judged to be in good health based on the results of medical history, physical examination, clinical laboratory evaluations, and ECG obtained within 28 days of the initial study drug administration.

### Safety and tolerability

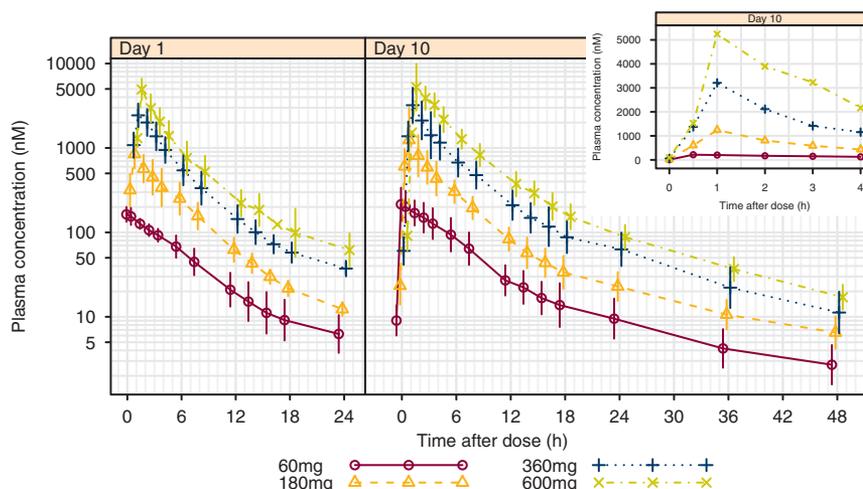
A total of 96 healthy male subjects between the age of 18 and 50 years were included. All randomized subjects completed the study and no subject was withdrawn for any reason. There were no serious adverse events and a total of 63 adverse events (AEs), all of which were considered mild or moderate in intensity, as judged by the investigator. Of the 48 subjects randomized in the SAD study, 17 experienced an AE on active drug and 3 on placebo. The most commonly reported AE was headache, reported by three subjects who received AZD5718. None of the subjects who received placebo reported having a headache. Of the 32 randomized subjects in the MAD study, 10 experienced an AE on active drug and 4 on placebo. The most commonly reported AE was headache, reported by one subject who received placebo and five subjects who received AZD5718. No deaths or other serious adverse events were reported during this study and the maximal tolerated dose was not established.

There were no apparent clinically significant trends observed in vital signs assessments, ECG measurements, telemetry, or any laboratory results.

### Pharmacokinetics

Geometric mean plasma concentration-time profiles for AZD5718 following single and once daily multiple dose administration are shown in **Figures 1** and **2**, respectively. All PK parameters are summarized in **Table 1**.

Following single and multiple dose administration of AZD5718 amorphous suspension once daily for 10 days to healthy subjects under fasted conditions, the rate of AZD5718 absorption was rapid with median  $T_{max}$  occurring at  $\sim 1$  h postdose (**Figure 3**). In **Figure 4**, the analysis with regard to dose-linearity indicated a more than dose proportional increase in exposure following both single and multiple

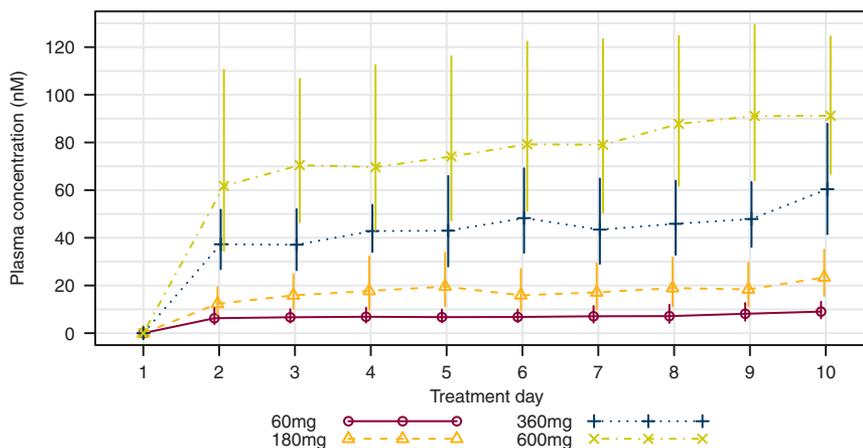


**Figure 2** Geometric mean  $\pm$  SD for plasma concentration-time profiles by treatment following multiple ascending doses of AZD5718 (after first and last dose),  $n = 6$  per dose. Insert; first 4 h after dose.

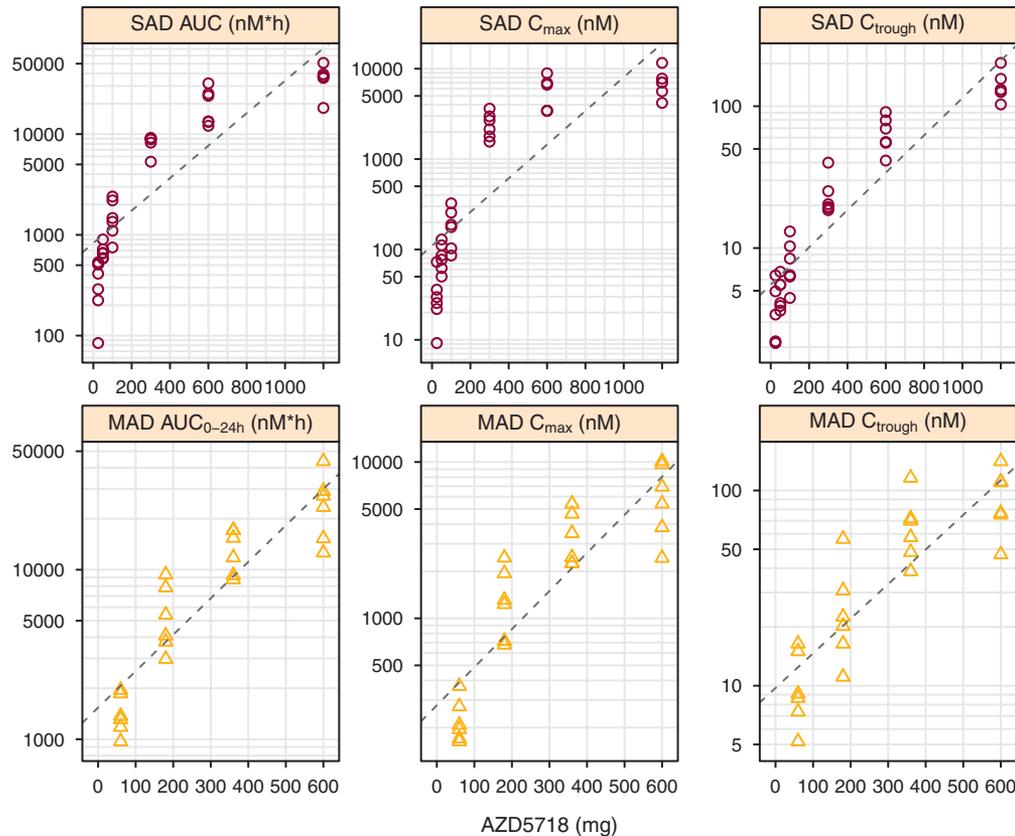
**Table 1** Summary of the pharmacokinetic parameters (geometric mean percentage of coefficient of variation), except for  $T_{max}$  median (range) following single and repeated once daily dosing of AZD5718 in healthy volunteers,  $N = 6$

Study part	Dose dosing regimen	$C_{max}$ nmol/L	$T_{max}$ h	AUC nmol*h/L	AUC $\tau$ nmol*h/L	CL/F L/h	$t_{1/2}$ h	CL $_R$ L/h
SAD	25 mg single dose	27.1 (86.0)	3.0 (1.0–6.0)	376.3 (38.1)	NE	148.8 (38.1)	10.3 (51.0)	0.68 (39.4)
	50 mg single dose	81.6 (36.4)	1.0 (1.0–3.0)	727.0 (15.4)	NE	153.9 (15.4)	12.5 (33.3)	0.62 (14.7)
	100 mg single dose	170.3 (54.9)	1.0 (0.5–3.0)	1,441 (45.3)	NE	155.6 (45.3)	12.7 (35.7)	0.66 (41.0)
	300 mg single dose	2,358 (32.9)	1.0 (0.5–1.0)	8,462 (20.1)	NE	79.4 (20.1)	13.7 (43.9)	0.67 (23.7)
	600 mg single dose	5,052 (45.8)	1.0 (1.0–2.0)	18,810 (42.1)	NE	71.5 (42.1)	11.4 (18.0)	0.70 (15.4)
	1,200 mg single dose	6,875 (34.9)	1.5 (1.0–2.0)	35,840 (33.8)	NE	75.0 (33.8)	10.3 (19.0)	0.62 (12.1)
MAD	60 mg OD, day 1	167.3 (19.8)	0.8 (0.5–2.0)	NE	1,018 (22.4)	NE	NE	NE
	60 mg OD, day 10	219.8 (31.9)	0.5 (0.5–1.0)	NE	1,386 (27.4)	96.9 (27.4)	11.6 (6.7)	0.64 (16.0)
	180 mg OD, day 1	846.9 (48.0)	1.0 (1.0–1.0)	NE	3,732 (41.2)	NE	NE	NE
	180 mg OD, day 10	1,243 (55.2)	1.0 (1.0–1.0)	NE	5,128 (46.9)	78.6 (46.9)	11.2 (13.0)	0.75 (16.0)
	360 mg OD, day 1	2,561 (31.7)	1.0 (1.0–2.0)	NE	10,350 (28.8)	NE	NE	NE
	360 mg OD, day 10	3,209 (40.0)	1.0 (1.0–1.0)	NE	12,760 (31.0)	63.1 (31.0)	9.1 (10.7)	0.99 (5.2)
	600 mg OD, day 1	4,933 (47.6)	1.0 (1.0–1.0)	NE	16,290 (48.6)	NE	NE	NE
	600 mg OD, day 10	5,693 (60.0)	1.5 (1.0–3.0)	NE	23,170 (47.4)	58.0 (47.4)	9.9 (29.2)	0.72 (23.3)

AUC, area under the concentration-time curve; CL/F, oral plasma clearance; CL $_R$ , renal clearance;  $C_{max}$ , peak plasma concentration; %CV, percentage of coefficient of variation; MAD, multiple ascending dose; NE, not estimated; OD, once daily; SAD, single ascending dose;  $t_{1/2}$ , terminal half-life;  $T_{max}$ , time of maximum plasma concentration.



**Figure 3** Geometric mean  $\pm$  SD  $C_{trough}$  concentration-time profiles by treatment following multiple ascending doses of AZD5718,  $n = 6$  per dose.



**Figure 4** Evaluation of dose proportionality: area under the concentration-time curve (AUC), peak plasma concentration ( $C_{max}$ ), and  $C_{trough}$  in single ascending dose (SAD) and  $AUC_{0-24h}$ ,  $C_{max}$ , and  $C_{trough}$  after last dose in multiple ascending dose (MAD) vs. dose of AZD5718,  $n = 6$  per cohort. Dashed line linear regression.

administration of AZD5718, except  $C_{trough}$  in the MAD. However, at the highest doses studied,  $C_{max}$  increased less than dose proportional, likely explained by the limited solubility of AZD5718. Following attainment of  $C_{max}$ , after both single dose administration and at steady state, plasma concentrations declined in a biphasic manner with a mean terminal half-life of 10–12 h. **Table 1** shows that the mean apparent oral clearance (CL/F) of AZD5718 ranged between 63 and 157 L/h with the highest oral clearances at doses <100 mg AZD5718. The amount of unchanged AZD5718 excreted in urine was small and the renal clearance accounted only for ~1% of the total oral clearance of AZD5718.

The  $C_{trough}$  concentrations are shown in **Figure 3** and based on visual inspections, there was a small accumulation, and steady-state conditions were achieved after ~3 days of repeated dosing, which is in agreement with the half-life of AZD5718. At all dose level and following attainment of steady-state conditions,  $C_{trough}$  remained constant, and no indication of time dependency in the PKs of AZD5718 was observed.

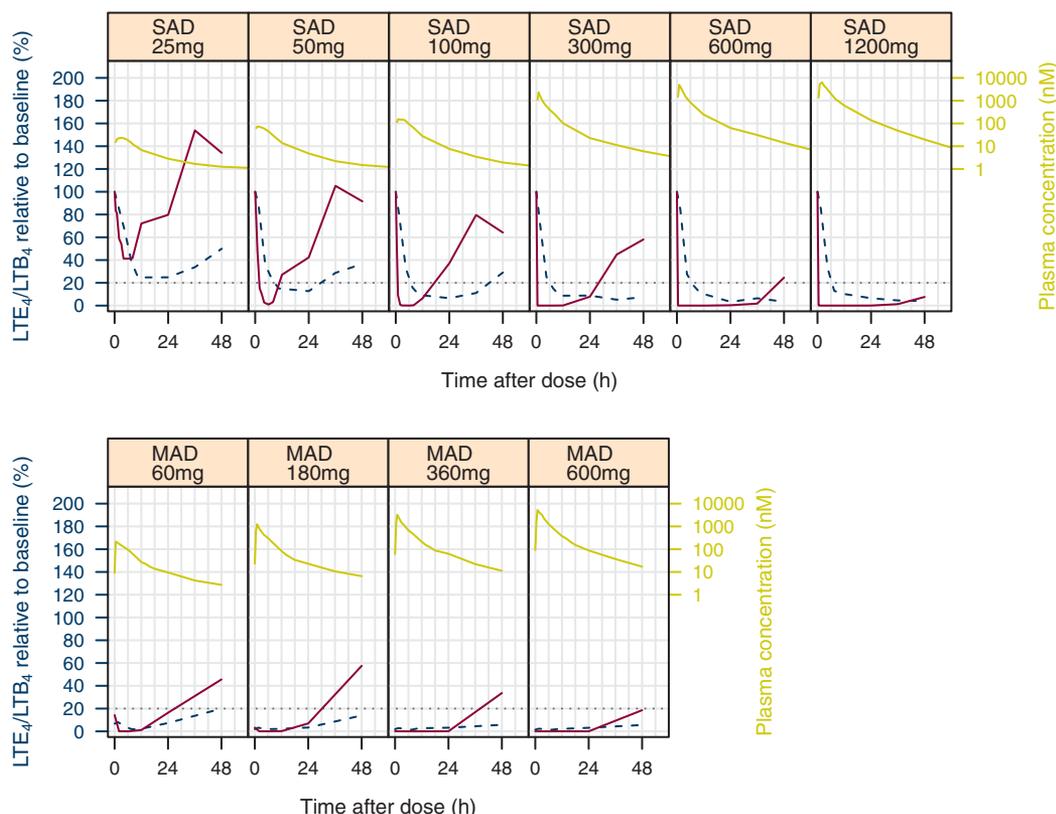
The between subject variability in AZD5718 exposure was generally moderate to high across the dose range evaluated following both single and multiple daily dosing (**Table 1**).

### Pharmacodynamics

Dose-dependent target engagement, as assessed by measuring *ex vivo* calcium ionophore stimulated  $LTB_4$  production in fresh blood and endogenous  $LTE_4$  inhibition in urine, was observed after both single and repeated dosing of AZD5718 suspension (**Figure 5**). In the SAD part, single doses of 300–1,200 mg AZD5718 produced a sustained effect on the mean  $LTB_4$  inhibition throughout the whole day (>80% inhibition), whereas the 180, 360, and 600 mg doses in the MAD part inhibited  $LTB_4$  production throughout the day (>80% inhibition) at the last day of repeated dosing. The  $LTE_4$  inhibition was seen at lower doses/exposures compared with the  $LTB_4$  inhibition but the effect on both biomarkers appeared rapidly, within hours, already after the first dose. After the last dose, the effect duration of  $LTE_4$  inhibition was longer compared with the  $LTB_4$  inhibition.

### PK/PD relationship

The exposure-dependent relationship between the inhibition of *ex vivo* calcium ionophore stimulated  $LTB_4$  production in fresh blood and inhibition of endogenous  $LTE_4$  in urine was explored graphically and by fitting  $LTE_4$ , and  $LTB_4$ , as percent of baseline vs. concentration at trough to a sigmoid maximum effect ( $E_{max}$ ) model (**Figure 6**). The model fitting did not



**Figure 5** Geometric mean concentration-time profiles (light green) and geometric mean leukotriene E<sub>4</sub> (LTE<sub>4</sub>) (dashed blue) and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) (solid purple) inhibition-time profiles, following single ascending doses (SADs) and multiple ascending doses (MADs) of AZD5718,  $n = 6$  per dose.

take into account the potential time delay in the effect on the biomarkers of the plasma concentration of AZD5718. In addition, the analysis did not take into account the effect of different individuals for the multiple observations in the MAD study, and also ignored whether the underlying observations were true observations or if they were below the LLOQ, as described in the **Supplementary Information**. The relative value at concentration 0 was set to a maximal inhibition of 100%. The  $E_{max}$  models described above predicted an  $IC_{50}$  of 5.3 [4.8–5.8] nM and 0.8 [0.7–1.0] nM for LTB<sub>4</sub> and LTE<sub>4</sub> inhibition, respectively (95% confidence intervals based on parameter uncertainty in brackets).

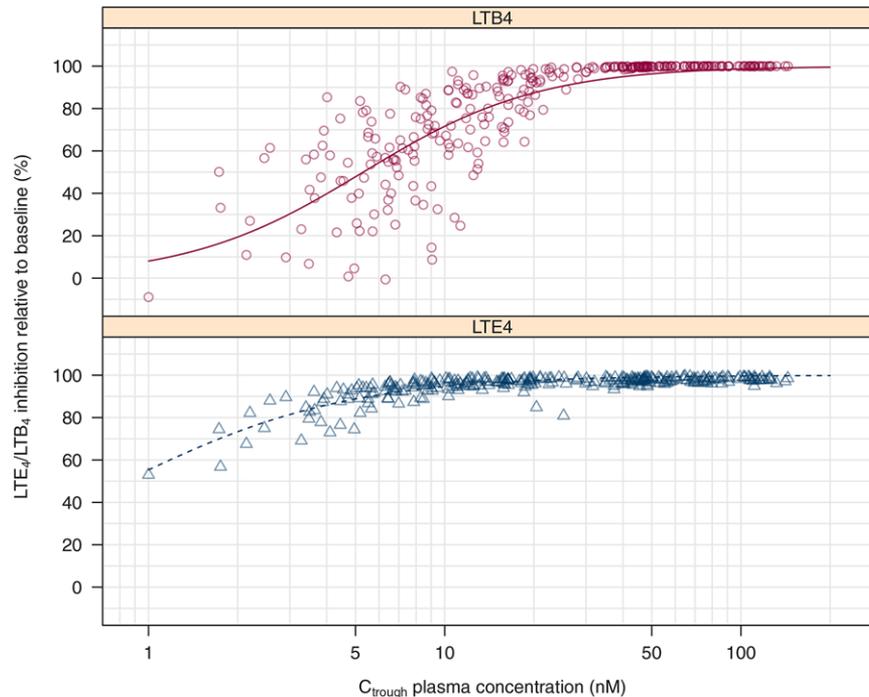
## DISCUSSION

This study was a FIH study of AZD5718, a novel oral FLAP inhibitor. Several 5-LO inhibitors acting on the same pathway have been evaluated in the clinic but, to our knowledge, only the second FLAP inhibitor intended to be developed for treatment of patients with CAD.<sup>16–19</sup> In healthy subjects, AZD5718 was well tolerated following both single and multiple doses in a dose/concentration range predicted to be relevant for future clinical development and no safety concerns were raised during the study.

Considering the early clinical formulation used in the present study and the wide dose range explored, the PK analysis suggested a more than dose proportional increase

in exposure. The reasons for this observation is not fully understood but the more than dose proportional increase in AUC and  $C_{max}$  are likely explained by a combination of saturated P-glycoprotein efflux and metabolism during the first passage. This hypothesis is based on *in vitro* transporter kinetic and metabolism data derived from MDCK-MDR1 cells and human intestinal Ussing chamber experiments, suggesting AZD5718 to be a substrate for P-glycoprotein and to display saturable metabolism (data not shown). Except for the observation mentioned above, the PKs, including half-life and renal clearance, were predictive over the dose range explored.

Provided similar PK and PK/PD relationship in patients, the generated data suggest that a once daily dosing regimen may well be possible in order to yield  $C_{trough}$  concentrations in the lower nM range and to achieve an almost complete inhibition of both LTB<sub>4</sub> and LTE<sub>4</sub> throughout 24 h. Interestingly, the effect on LTB<sub>4</sub> inhibition was in a similar magnitude as reported for the 5-LO inhibitor VIA2291, whereas inhibition on LTE<sub>4</sub> production was much more pronounced and seen at lower concentrations compared with the VIA2291 compound.<sup>16</sup> The finding is in agreement with a report for another FLAP inhibitor, AM103, which showed a more pronounced effect on endogenous LTE<sub>4</sub> production at lower concentrations compared with the effect seen on LTB<sub>4</sub>, although the concentrations needed were much higher compared with AZD5718.<sup>17</sup> The



**Figure 6** Leukotriene E4 (LTE<sub>4</sub>) (blue triangles) and leukotriene B4 (LTB<sub>4</sub>) (purple circles) inhibition vs. concentration at trough for single ascending doses and multiple ascending doses. Included lines are based on maximum effect (E<sub>max</sub>) model estimates.

AZD5718 IC<sub>50</sub> value for the effect on *ex vivo* stimulated LTB<sub>4</sub> inhibition is almost 100-fold lower compared with both AM103 and VIA2291. It is noteworthy that 6 months of treatment with once daily VIA2291, resulting in similar LTB<sub>4</sub> inhibition, as seen for AZD5718 in the present study, led to a beneficial effect on plaque progression and dose-dependent improvement in left ventricular ejection fraction in patients with ACS.<sup>13,14,16</sup> The clinical relevance of the higher potency combined with a more pronounced effect on the LTE<sub>4</sub> pathway needs to be evaluated in clinical studies.

To conclude, AZD5718 was generally well tolerated by healthy subjects following both single doses of 25–1,200 mg and multiple doses of 60–600 mg. No safety concerns were raised during the study. The PK parameters of AZD5718, in combination with the sustained effect on the target engagement biomarkers LTB<sub>4</sub> and LTE<sub>4</sub> throughout 24 h at concentrations in the lower nM range, support future once-daily dosing. Furthermore, the low renal clearance suggests that renal excretion of AZD5718 is not likely to be a major elimination pathway. Taken together, the generated data support continued clinical development of AZD5718 and ongoing study in patients with CAD (NCT03317002).

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**Conflict of Interest.** H.E., K.N., M.L.-F., C.B., M.B., L.-M.G., M.H., M.K., E.-L.L., G.-B.F., C.W., and S.S., are all employees of AstraZeneca and may own company stocks. L.C. and J.L. are employees of PAREXEL and may own company stocks.

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