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Design and rationale of FLAVOUR: A phase IIa efficacy study of the 5-lipoxygenase activating protein antagonist AZD5718 in patients with recent myocardial infarction

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ABSTRACT

Patients with coronary artery disease remain at increased risk of recurrent life-threatening cardiovascular events even after adequate guideline-based treatment of conventional risk factors, including blood lipid levels. Inflammation is a critical pathway in the pathogenesis of atherosclerosis and is independently associated with risk of recurrent cardiovascular events. Leukotrienes are potent pro-inflammatory and vasoactive mediators synthesized by leukocytes in atherosclerotic lesions. AZD5718 is a novel antagonist of 5-lipoxygenase activating protein that suppresses leukotriene biosynthesis.

FLAVOUR is a phase IIa efficacy and safety study of AZD5718 in patients with myocardial infarction 1–4 weeks before randomization. Stenosis of the left anterior descending coronary artery after percutaneous intervention must be $<50\%$, and Thrombolysis In Myocardial Infarction flow grade must be ≥ 2. Enrolled participants receive standard care plus oral AZD5718 200 mg, 50 mg, or placebo once daily for up to 12 weeks (extended from 4 weeks by protocol amendment). The planned sample size is 100 participants randomized to 12 weeks' treatment. Change in urine leukotriene E_4 levels is the primary efficacy outcome. FLAVOUR also aims to evaluate whether AZD5718 can improve coronary microvascular function, as measured by transthoracic colour Doppler-assisted coronary flow velocity reserve. Centrally pretrained study sonographers use standardized protocols and equipment. Additional outcomes include assessment of comprehensive echocardiographic parameters (including coronary flow, global strain, early diastolic strain rate and left ventricular ejection fraction), arterial stiffness, biomarkers, health-related quality of life, and safety.

Specific anti-inflammatory therapies may represent novel promising treatments to reduce residual risk in patients with coronary artery disease. By combining primary pharmacodynamic and secondary cardiovascular surrogate efficacy outcomes, FLAVOUR aims to investigate the mechanistic basis and potential benefits of AZD5718 treatment in patients with coronary artery disease.

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1. Introduction

Inflammation plays a causative role in driving the progression of atherosclerosis, independent of blood lipid levels [1]. Survivors of acute coronary syndrome remain at risk of recurrent cardiovascular events, and humoral and cellular biomarkers of inflammation predict myocardial infarction and cardiac death in this population [2]. Despite the effectiveness of standard secondary prevention measures, including antiplatelet therapy, treatment of hypertension, hyperglycaemia and hypercholesterolaemia, and modification of lifestyle risk factors [3], cardiovascular disease remains the leading cause of death worldwide [4,5].

Anti-inflammatory therapies may reduce the residual risk of recurrent cardiovascular events in patients with previous myocardial infarction, independent of the risk reduction achieved by lowering low-density lipoprotein cholesterol levels with statins or other therapies [6]. Results from recent phase 3 cardiovascular outcome trials of canakinumab [7] colchicine [8] and methotrexate [9] in patients with myocardial infarction suggest that anti-inflammatory therapies should target signalling pathways that are mechanistically involved in atherosclerosis [10]. Current evidence highlights the central interleukin-1β, interleukin-6, and C-reactive protein pathway, but many other inflammatory mechanisms influence the development of both large-vessel occlusive disease and coronary microvascular dysfunction associated with atherosclerosis [11].

Leukotrienes are potent pro-inflammatory and vasoconstrictive lipid mediators that are produced in atherosclerotic lesions and ischaemic tissue, and are implicated in the pathogenesis of atherosclerotic cardiovascular disease [12]. Leukocytes initiate leukotriene biosynthesis by converting arachidonic acid to leukotriene A_4 via 5-lipoxygenase in conjunction with 5-lipoxygenase activating protein (FLAP) (Fig. 1a) [13]. Leukotriene A_4 is the precursor for synthesis of leukotriene B_4 and the cysteinyl leukotrienes (C_4, D_4 and E_4) by leukocytes and neighbouring cells. Leukotriene B_4 stimulates leukocyte recruitment and activation in coronary blood vessels, with involvement in both atherosclerotic plaque initiation and rupture [14]. Leukotriene B_4 and the cysteinyl leukotrienes promote vasoconstriction, vessel permeability, and smooth muscle cell proliferation in the coronary microvasculature, leading to endothelial dysfunction (Fig. 1b) [14].

Evidence suggests that inhibiting leukotriene biosynthesis may have beneficial effects in patients with coronary artery disease [15]. Single-nucleotide polymorphisms in *ALOX5AP* (encoding FLAP) were significantly associated with increased risks of spontaneous myocardial infarction and a composite of spontaneous myocardial infarction, stroke or cardiovascular death in a genetic sub-study in patients with acute coronary syndrome in the Platelet Inhibition and Patient Outcomes (PLATO) trial [16]. The 5-lipoxygenase inhibitor atreleuton (VIA-2291) slowed atherosclerotic plaque progression, prevented formation of new lesions, and improved left ventricular ejection fraction in patients with recent acute coronary syndrome in a randomized, double-blind, placebo-controlled, phase II trial [17,18]. Another 5-lipoxygenase inhibitor, zileuton, is approved for treatment of patients with asthma [19,20]. Zileuton improved endothelial function in 10 patients undergoing coronary angiography who had high-risk haplotypes of *ALOX5AP* and *LTA4H* (encoding leukotriene A_4 hydrolase) [21]. No inhibitor of leukotriene biosynthesis has yet progressed beyond phase II clinical trials in patients with cardiovascular disease, however, and hepatotoxicity may limit development of 5-lipoxygenase inhibitors [15].

AZD5718 is a novel FLAP antagonist that acts at the first step of biosynthesis to block production of all leukotrienes (Fig. 1a), with a half-maximal inhibitory concentration of 39 nM for *ex vivo* leukotriene B_4 production in whole human blood [22]. Multiple oral doses of AZD5718 60–600 mg were well tolerated, and potently reduced both leukotriene E_4 levels in urine and leukotriene B_4 synthesis by leukocytes *ex vivo* in a phase I clinical trial in healthy volunteers [22]. AZD5718 inhibited leukotriene B_4 production by 90% throughout the day following once-daily administration of 200 mg oral tablets in pharmacodynamic models in healthy volunteers [23]. Oral co-administration of AZD5718 with rosuvastatin had no clinically meaningful effect on the pharmacokinetic profiles of either drug in a phase I drug–drug interaction study, indicating that AZD5718 is a compatible adjunct to standard care in patients with coronary artery disease [23].

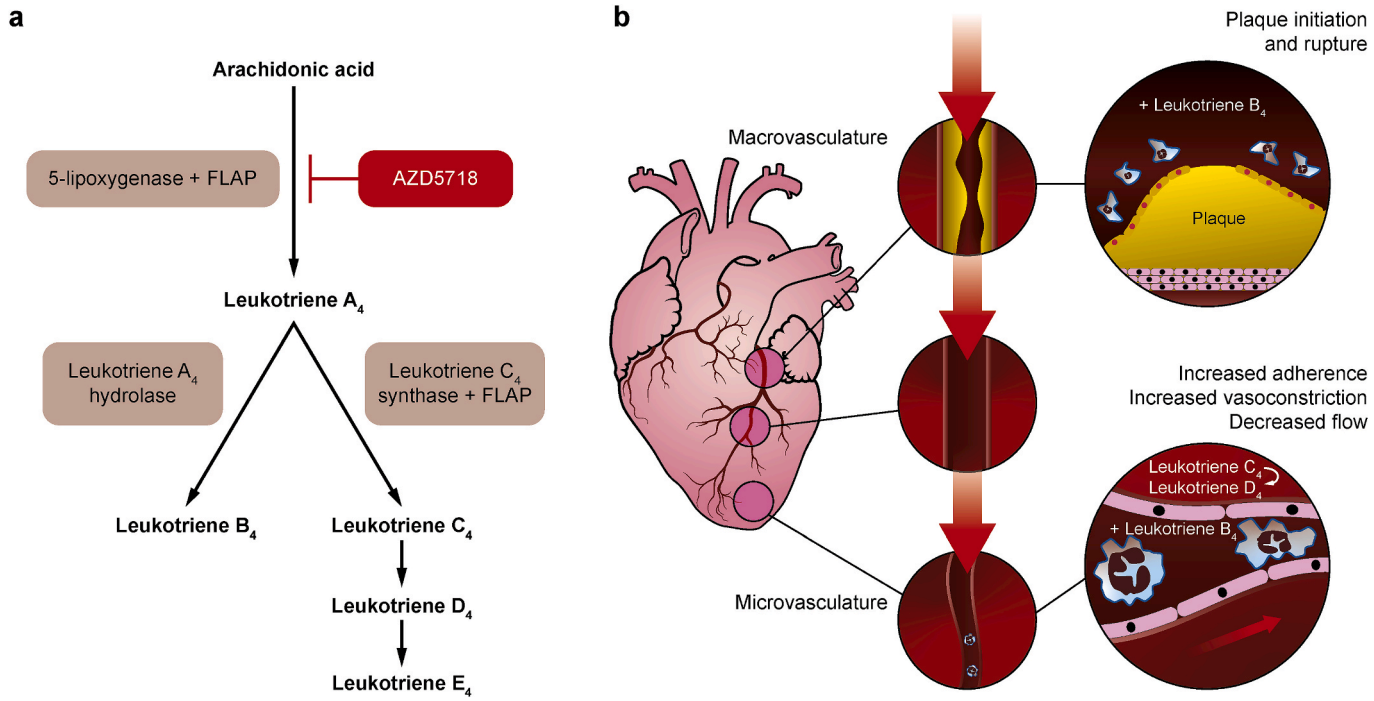

Fig. 1. a Leukotriene biosynthesis pathway and inhibition by AZD5718. **b** Role of leukotrienes in cardiovascular disease. FLAP, 5-lipoxygenase activating protein.

By inhibiting leukotriene biosynthesis, AZD5718 may have the potential not only to slow the progression of atherosclerosis, but also to enhance coronary microvascular function and to improve ventricular contractility following myocardial infarction [15]. Based on this rationale, we designed a phase IIa proof-of-principle study to investigate the efficacy and safety of AZD5718 in patients with recent myocardial infarction (FLAVOUR). The study aims primarily to assess inhibition of leukotriene biosynthesis and secondarily to assess the efficacy of AZD5718 compared with placebo in this high-risk patient population, using surrogate outcomes. The comprehensive panel of transthoracic echocardiography efficacy outcomes includes left ventricular ejection fraction as a measure of myocardial contractility and coronary flow velocity reserve (CFVR) as a measure of coronary microvascular function.

2. Methods and analysis

2.1. Overview

FLAVOUR is an ongoing 12-week, randomized, placebo-controlled, multicentre, parallel-group, phase IIa clinical trial of the efficacy, safety and tolerability of AZD5718 in patients with coronary artery disease who have recently had a myocardial infarction. The study started in October 2017.

2.2. Protocol amendment to extend treatment duration

Enrolment into the study began with a 4-week treatment period as specified in the original protocol. Following completion of long-term animal toxicity studies, a protocol amendment in April 2018 extended the treatment period to 12 weeks for subsequently enrolled patients, to enable longer-term assessment of efficacy and safety. The amendment introduced two extra study visits and new 12-week secondary endpoints (Table 1). The planned sample size was increased from 100 to approximately 138, because the 38 patients already enrolled under the original protocol had a 4-week treatment period. This ensured that the same statistical power as originally planned is available for the new 12-week primary and key secondary endpoints (Table 1).

2.3. Objectives

The primary objective is to assess the effect of AZD5718 treatment on urine leukotriene E_4 levels in patients with recent myocardial infarction. Assessing the effect of AZD5718 on echocardiographic CFVR is one of the key secondary objectives (Table 1). The primary and key secondary endpoints are ranked for hierarchical testing (see Statistical methods). Other secondary objectives and exploratory objectives are listed in Table 1, and include assessing the safety and pharmacokinetics of AZD5718, as well as additional efficacy parameters.

2.4. Participants

Eligible patients are men and women 18–75 years of age with a body mass index of 18–35 kg/m^2, who have had a myocardial infarction with or without ST-segment elevation from 7 to 28 days before randomization. Diagnosis of myocardial infarction is based on the fourth universal definition [24], with mandatory coronary angiography (analysed at the study site). Residual stenosis of the left anterior descending coronary artery must be below 50% and the Thrombolysis In Myocardial Infarction (TIMI) flow grade [25] must be 2 or above after percutaneous coronary intervention (if performed). Bypass graft of the left anterior descending coronary artery is an exclusion criterion. To be included, women must be surgically sterile or postmenopausal (amenorrhea for ≥12 months) and men must be surgically sterile or be using barrier contraception to prevent pregnancy in partners. The complete list of exclusion criteria is provided in Table 2.

Table 1
Objectives.

Objective	Assessment
Primary[a]	• Effect of AZD5718 on urine leukotriene E_4 levels at 4 weeks
Key secondary (ranked)[a]	• Effect of AZD5718 on: **1.** Urine leukotriene E_4 levels at 12 weeks[b] **2.** Coronary flow velocity reserve at 12 weeks[b] **3.** Coronary flow velocity reserve at 4 weeks
Other secondary[c]	• Pharmacokinetics of AZD5718 at 4 and 12 weeks[b] • Effect of AZD5718 on: ◦ resting and hyperaemic coronary blood flow at 4 weeks ◦ left ventricular functional parameters at 4 weeks(including ejection fraction) • Safety and tolerability of AZD5718
Exploratory[c]	• Effect of AZD5718 on: ◦ resting and hyperaemic coronary blood flow at 12weeks[b] ◦ left ventricular functional parameters at 12 weeks(including ejection fraction)[b] ◦ arterial stiffness at 4 and 12 weeks[b] ◦ cardiovascular disease biomarker levels ◦ leukotriene B_4 production in blood *ex vivo* ◦ patient-reported HRQoL, functioning, dyspnoea andfatigue • Effect of baseline variables on outcomes related to AZD5718treatment • Relationship between AZD5718 exposure and efficacyoutcomes

HRQoL, health-related quality of life.
[a] Primary and key secondary outcomes are hierarchically ranked for inferential statistical analysis in sequence to control the overall false positive rate; the study was powered for these outcomes only (see Statistical methods).
[b] Outcomes at 12 weeks were introduced in a protocol amendment that extended the study treatment duration from 4 to 12 weeks.
[c] Other secondary and exploratory outcomes were not included in the power calculation or the hierarchical testing procedure.

2.5. Randomization and blinding

Enrolled patients are randomized 2:1:2 to receive AZD5718 200 mg, AZD5718 50 mg or matching placebo as once-daily oral tablets (Fig. 2). Efficacy assessments will be based on comparisons of the AZD5718 200 mg group versus the placebo group; the smaller 50 mg group serves only to guide dose selection in future studies. Randomization is stratified by type of myocardial infarction (with or without ST-segment elevation). Randomization block sequences are generated by the contract research organization (PAREXEL) using an algorithm provided by the sponsor (AstraZeneca).

Participants, study site staff and the sponsor study team will remain blinded to treatment assignment throughout the study. Investigators will remain blinded unless they need to know a patient's assigned treatment in a medical emergency. Placebo matches either AZD5718 50 mg or 200 mg tablets, so the identity of the treatment cannot be discerned. To reduce tablet burden, the tablets do not match between doses. The study is therefore double-blind for AZD5718 versus placebo, but single-blind for assignment to placebo/AZD5718 50 mg or placebo/AZD5718 200 mg.

Table 2
Exclusion criteria.

1	Alanine aminotransferase levels >2 × ULN at visit 2; cirrhosis; recent hepatitis; or positive test for hepatitis B or C virus
2	Uncontrolled type 1 or 2 diabetes, defined as haemoglobin A1c level >9% or >74.9 mmol/mol
3	Atrial fibrillation; history of ventricular tachycardia requiring therapy; symptomatic sustained ventricular tachycardia; sick sinus syndrome; or atrioventricular block (degree 2–3)
4	Presence of a pacemaker
5	Previous coronary artery bypass grafting to the left anterior descending coronary artery
6	Left ventricular ejection fraction <30%
7	Unacceptable level of angina despite maximal medical therapy or unstable angina at study entry[a]
8	Stroke within 6 months of myocardial infarction or ongoing treatment with dipyridamole or dipyridamole/aspirin
9	Planned treatment with zileuton, leukotriene receptor antagonists (e.g., montelukast), coumadin, <!--Soft-enter Run-on-- > or steroids during the study
10	Planned statin dose or regimen changes during the study
11	Use of anticoagulants at a therapeutic dose (not including thrombosis prophylaxis)
12	Planned cardiac surgery or percutaneous coronary intervention during the study
13	NYHA class III–IV heart failure; decompensated heart failure at discharge; or hospitalization for exacerbation of chronic heart failure within 3 months of myocardial infarction
14	Previous severe kidney disease (chronic kidney disease stage 4 or 5) or previous creatinine clearance <30 mL/min[b]
15	Aortic or mitral valvular disease classified as severe
16	Allergy to adenosine or mannitol; or previous adverse effects of adenosine stress testing
17	Elevated intracranial pressure
18	Heart rate <40 bpm
19	Systolic blood pressure <90 mmHg
20	Asthma or chronic obstructive pulmonary disease with a strong reactive component
21	Treatment with dipyridamole, theophylline, fluvoxamine, rifampicin, phenytoin, or carbamazepine
22	Inability to comply with the study protocol
23	Severe allergy or hypersensitivity to drugs with a chemical structure or class similar to that of AZD5718
24	Vulnerability[c] or inability to give consent or communicate reliably with the investigator
25	Involvement in the planning or conduct of the study
26	Participation in another interventional clinical study of an investigational drug in the last 3 months
27	Previous randomization in the study
28	Previous or current abuse of drugs or alcohol

NYHA, New York Heart Association; ULN, upper limit of normal.
[a] Defined as Canadian Cardiovascular Society grade ≥3 at study visit 1 or 2.
[b] Estimated using the Cockcroft and Gault equation.
[c] Including in detention, under guardianship, under trusteeship, and committed to an institution.

2.6. Procedures

Potentially eligible patients are screened in hospital within 5 days after their myocardial infarction (Fig. 2). Participants are enrolled and randomized within 7–28 days of their myocardial infarction to receive AZD5718 or matching placebo once daily for 12 weeks (or 4 weeks for those enrolled under the original protocol). AZD5718 and placebo are provided as matching film-coated tablets to be taken every morning with 2 dL of water. Participants' adherence to the regimen is monitored using diary cards. Participants are scheduled to attend study visits at randomization (baseline), at weeks 2, 4, 8 and 12 of treatment, and at follow-up (4 weeks after the last dose). Safety and efficacy are assessed throughout the study (Fig. 2).

2.7. Efficacy outcomes

2.7.1. Urine leukotriene E_4 levels

The percentage change in creatinine-normalized urine leukotriene E_4 levels from baseline to week 4 is the primary outcome and change from baseline to week 12 is the first of three key secondary outcomes (Table 1). The primary efficacy measure was based on the change from baseline to week 4, reflecting the original duration of the study and the rapid decrease in urine leukotriene E_4 levels observed in phase I studies in healthy volunteers [22,23].

2.7.2. Echocardiography

CFVR is measured as the ratio of hyperaemic to resting diastolic blood flow velocity in the left anterior descending coronary artery. Hyperaemia is induced by adenosine infusion (140 μg/kg/min for up to 5 min). Changes in CFVR from baseline to week 12 and week 4 are the second and third of the three key secondary outcomes, respectively (Table 1). Ranking change to week 12 above change to week 4 in the key secondary efficacy outcomes reflects the extension of the study to allow detection of potential longer-term improvement in myocardial microvascular function.

Other secondary echocardiographic efficacy parameters include the change from baseline in measures of coronary flow (diastolic flow velocity in the left anterior descending coronary artery) and measures of left ventricular functional reserve (global longitudinal strain, global circumferential strain, early diastolic strain rate and left ventricular ejection fraction). Exploratory echocardiographic parameters include 15 strain measures and 11 3D functional measures assessed at rest and during hyperaemia. Standard cardiac and Doppler-assisted imaging parameters, such as the ratio between early mitral inflow velocity and early diastolic mitral annular velocity (E/e′), are also assessed.

Study sonographers were trained and certified in person at Sahlgrenska University Hospital (Gothenburg, Sweden) prior to the study. The sponsor provided a detailed protocol for comprehensive transthoracic echocardiographic examinations, together with dedicated pre-configured equipment (Siemens Acuson SC2000 PRIME Ultrasound System, Siemens Healthineers, Mountain View, CA, USA). Imaging includes B-mode, colour Doppler and tissue Doppler modalities in 2D and 3D. High-frame-rate cine loops and still images are captured at the study site. All echocardiographic data are analysed offline by the core laboratory at Sahlgrenska University Hospital.

2.7.3. Arterial stiffness

Changes in arterial stiffness from baseline to 4 and 12 weeks are exploratory efficacy outcomes. Carotid–femoral pulse wave velocity is measured using a femoral cuff and carotid tonometer. Brachial pulse wave analysis is conducted using a brachial cuff.

2.7.4. Biomarkers

Whole blood and plasma are collected for exploratory analyses of cardiovascular disease and inflammation biomarkers. Samples are collected during screening (days 1, 2, 3 and 5 after myocardial infarction, if feasible) and throughout the study (Fig. 2). Inflammatory biomarkers include interleukin-1β, interleukin-6, high-sensitivity C-reactive protein and growth differentiation factor 15.

2.8. Safety outcomes

Safety is assessed throughout the study (Fig. 2). Safety assessments include clinical laboratory tests (chemistry, haematology, coagulation, serology and urinalysis), physical examinations, monitoring of vital signs (pulse and blood pressure) and body weight, electrocardiography and recording of adverse events.

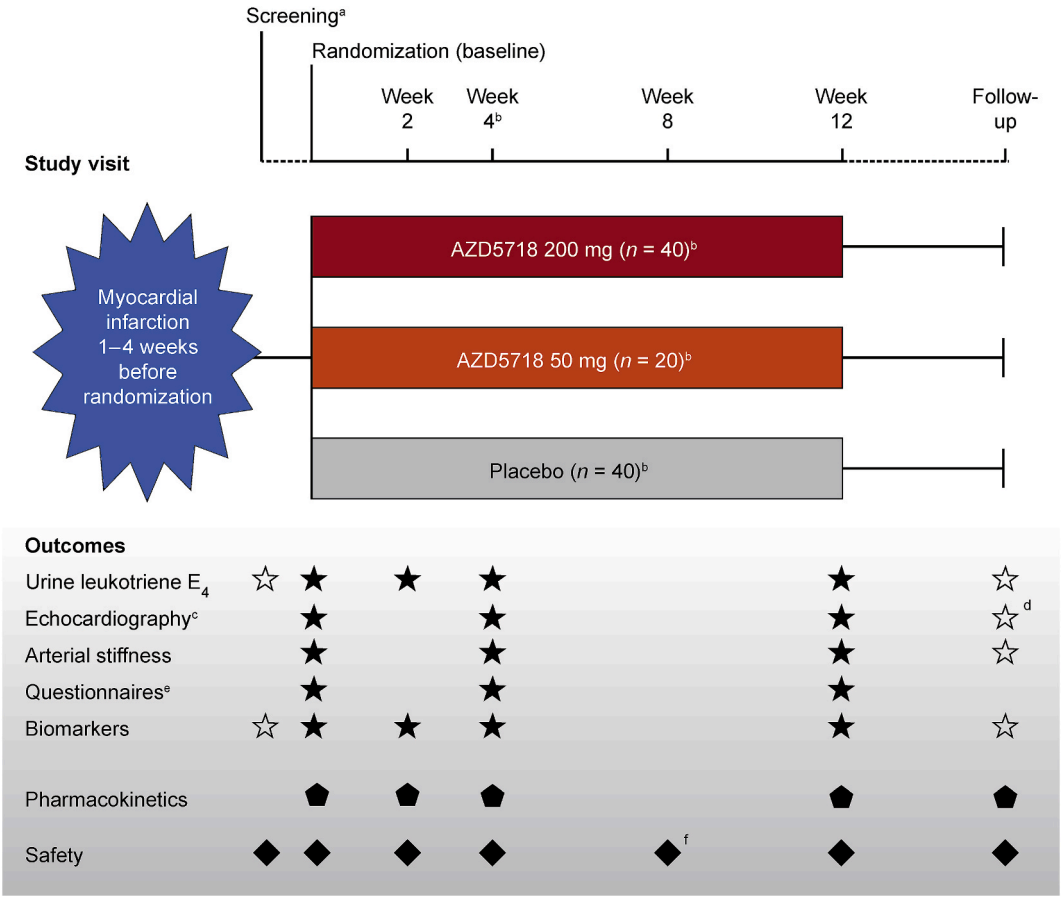

Fig. 2. Study design. Stars indicate efficacy assessments, pentagons indicate pharmacokinetic assessments, and diamonds indicate safety assessments. Empty (white) stars indicate efficacy assessments that were not used in statistical analyses of AZD5718 versus placebo. [a] Screening assessments take place during days 1–5 after myocardial infarction and include TIMI coronary flow grade, GRACE risk score, and plasma sampling for biomarkers on days 1, 2, 3, and 5 (if feasible). [b] Study duration originally 4 weeks (n = 38) before amendment to 12 weeks (n = 100; total N = 138). [c] Includes CFVR measurement (key secondary efficacy outcome). [d] Without adenosine stress. [e] Patient-reported HRQoL, functioning, and symptoms of dyspnoea and fatigue. [f] Adverse events only (other tests optional). CFVR, coronary flow velocity reserve; GRACE, Global Registry of Acute Coronary Events; HRQoL, health-related quality of life; TIMI, Thrombolysis In Myocardial Infarction.

2.9. Questionnaires

Participants complete questionnaires assessing their health-related quality of life (HRQoL), functioning, and symptoms of dyspnoea and fatigue as exploratory efficacy outcomes. HRQoL is assessed with the standardized 5-dimension EuroQol questionnaire (EQ-5D) [26]; functional health and well-being are assessed in eight domains with the 36-item Short Form Health Survey (SF-36) version 2 [27]; dyspnoea is assessed using the Rose Dyspnea Scale (4 items) [28]; and fatigue during usual daily activities is assessed using the Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System (13 items) [29].

2.10. Statistical methods

2.10.1. Sample size calculation

The study is powered to compare AZD5718 200 mg with placebo for the primary and the three key secondary efficacy outcomes of urine leukotriene E_4 levels and CFVR (Table 1). Other endpoints were not included in the power calculation, including other echocardiographic outcomes. A sample size of 33 evaluable patients per group will provide more than 99% power to detect an 80% decrease in urine leukotriene E_4 levels, and 80% power to detect a 20% increase in CFVR in the AZD5718 200 mg group versus the placebo group (both with one-sided $\alpha = 0.05$). Approximately 40 patients per group are being recruited to retain at least 33 evaluable patients per group at week 12, assuming an approximate 18% discontinuation rate. The AZD5718 50 mg group will include approximately 20 patients and serves only to guide dose selection in future studies. Power calculations used a log-normal distribution of leukotriene E_4 levels with expected inhibition of 96% for AZD5718 and 0% for placebo (both with a standard deviation of $<4\%$), based on a phase I multiple ascending dose study in healthy volunteers [22]. A 30% coefficient of variation was assumed for change in CFVR power calculations, based on data from an observational study in patients with heart failure [30].

2.10.2. Control for multiple comparisons

A hierarchical testing procedure was pre-specified to preserve the overall false positive rate at 0.05 or below when performing multiple comparisons of AZD5718 200 mg with placebo for the primary and key secondary endpoints only (Table 1). These will be tested in the following sequence: first, change in urine leukotriene E_4 levels to week 4; secondly, change in urine leukotriene E_4 levels to week 12; thirdly, change in CFVR at 12 weeks; and finally, change in CFVR at 4 weeks. This hierarchy reflects the original 4-week design of the study and anticipated rapid change in the primary efficacy outcome (leukotriene E_4 levels) but prioritizes the 12-week extension for CFVR outcomes, in which more gradual changes are anticipated. Testing will stop as soon as a non-significant result is observed ($p > 0.05$) and all subsequent comparisons will be declared non-significant. Compar-

isons of AZD5718 50 mg with placebo will be performed in parallel, but no adjustment for multiplicity is required because the results will not be used to judge efficacy. Other secondary and exploratory endpoints are not included in hierarchical testing procedure, including other echocardiographic outcomes.

2.10.3. Inferential analyses

A mixed model repeated measures analysis will be used to test the change from baseline in urine leukotriene E_4 levels in each AZD5718 group versus placebo (one-sided $\alpha = 0.05$), with treatment, visit, and treatment–visit interaction as fixed factors and type of myocardial infarction and baseline leukotriene E_4 value as covariates. The model will use an unequally spaced variance–covariance matrix, if possible. An analysis of covariance will be used to test the change in CFVR from baseline in each AZD5718 group versus placebo (one-sided $\alpha = 0.05$), with treatment as a fixed factor and type of myocardial infarction and baseline CFVR value as covariates. Similar analyses will be used for other secondary and exploratory efficacy outcomes (two-sided $\alpha = 0.05$), with no corrections for multiplicity.

2.10.4. Subgroup analyses

Descriptive efficacy results will be stratified by type of myocardial infarction (with or without ST-segment elevation) and protocol cohort (4-week or 12-week treatment). The study is not powered for comparisons in subgroups. No statistical analyses are pre-specified in these or other subgroups of patients (e.g., baseline level of inflammatory biomarkers, baseline CFVR impairment or site of enrolment). Any *post hoc* subgroup analysis will be non-inferential.

2.11. Ethics and dissemination

FLAVOUR is taking place at three sites in Denmark, two sites in Finland, and four sites in Sweden. The study is registered on ClinicalTrials.gov (identifier: NCT03317002) and EudraCT (2017-001582-25), and conforms to the principles of the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice, and all applicable regulatory requirements. An ethical review board in each country reviewed and approved the study protocol and its amendments. All participants give their written informed consent before study enrolment.

2.12. Current status

As of November 2019, we have enrolled 150 participants and randomized 120 participants in FLAVOUR (those not randomized did not pass screening). A total of 100 participants are planned for randomization to 12 weeks of treatment with AZD5718 or placebo. An additional 38 patients were enrolled under the original 4-week protocol. The first patient was enrolled in October 2017 and the first dose was taken in November 2017. We expect to enrol the last patient in December 2019 and complete the last follow-up visit in April 2020.

2.13. Patient and public involvement

Neither patients nor the public were involved in this research, except as participants in the study. Participants are recruited at the study centres and advertising is approved by the ethics committee.

3. Discussion

FLAVOUR is the first study to assess the effect of a FLAP antagonist in patients with coronary artery disease. The primary efficacy outcome is pharmacodynamic and is expected to confirm the rapid and sustained 90% or greater inhibition of leukotriene biosynthesis observed in healthy volunteers receiving AZD5718 [22,23]. We also designed the study to encompass multiple cardiovascular surrogate efficacy measures, including assessment of coronary microvascular function in pre-specified, multiplicity-controlled analyses of echocardiographic CFVR.

Leukotriene E_4 levels were assessed non-invasively in the urine for the primary outcome measure. Blood leukotriene levels are typically very low, and assays involve technically challenging *ex vivo* stimulation of lymphocyte leukotriene B_4 secretion. AZD5718 reduced urine leukotriene E_4 and blood leukotriene B_4 levels in the phase I study in human volunteers, demonstrating target engagement using both assays [22]. Leukotriene levels were therefore assessed only in urine in the present study. Atherosclerosis involves both large-vessel occlusive disease and microvascular dysfunction, and leukotrienes are involved in both of these pathologies [11]. FLAVOUR includes a comprehensive panel of echocardiographic parameters assessed at rest and under stress as secondary or exploratory outcomes, together with CFVR as the key secondary outcome. These will provide a thorough assessment of the participants' cardiac function before and during treatment with AZD5718. We hypothesize that not only myocardial contractility (e.g., left ventricular ejection fraction), but also coronary flow and cardiac reserve may be improved with AZD5718 treatment. The extended 12-week duration of FLAVOUR will enable potential enhancements in CFVR and other surrogate efficacy parameters to be assessed over a suitably extended period of treatment with AZD5718. Any potential effects of AZD5718 will need to be detected in addition to spontaneous improvement in CFVR in the weeks and months following myocardial infarction, and in addition to improvements resulting from standard care. Improvements in CFVR over an 8-week period have been reported in patients with myocardial ischaemia but without occlusive coronary artery disease in a small double-blind placebo-controlled study of ranolazine ($N = 58$) [31].

CFVR is an appealing surrogate outcome measure for detecting potentially beneficial therapeutic effects of AZD5718 in a phase II study for several reasons. First, coronary microvascular dysfunction is common in patients with obstructive coronary artery disease [32]. CFVR provides an integrated measure of blood flow through the large epicardial arteries and the coronary microcirculation [33]. When stenosis of the coronary arteries is below 50%, as in the present study, CFVR is a marker of coronary microvascular function [34,35]. Second, in echocardiographic studies, CFVR is prognostic of maximal exercise performance and represents a composite of systolic and diastolic function in patients with chest pain and patients with coronary artery disease [34,35]. Third, independent of other risk factors, impaired CFVR predicts mortality in patients with heart failure [36]; and known or suspected coronary artery disease [37]; predicts cardiovascular death, myocardial infarction, or acute revascularization in patients with suspected myocardial ischaemia [38]; and predicts death, myocardial infarction, or hospitalization for unstable angina in patients with known or suspected coronary artery disease [39]. In risk modelling studies, CFVR was a significant independent predictor of cardiovascular mortality in patients with diabetes and patients with chronic kidney disease [40,41]. Fourth, CFVR also reflects basal myocardial oxygen demand [42,43], suggesting that improved or unchanged CFVR might indicate that any enhancement in resting ventricular contractility is energy neutral and therefore potentially beneficial. Finally, echocardiographic measurement of CFVR is highly reproducible and has low variability among trained operators at a single experienced study site [44]. The design of the present study reduces variability between sites by using standardized cardiac sonography protocols and equipment, centralized image reading, and a hands-on sonographer training and certification procedure (S Svedlund et al., manuscript in preparation).

We specified an interval of 1–4 weeks from patients having a myocardial infarction to starting treatment with AZD5718 or placebo in FLAVOUR. This time interval offers the best therapeutic window between feasibility in a clinical trial and early intervention, and eligible

patients represent the most likely potential target population for AZD5718. Rates of recurrent myocardial infarction are highest soon after an initial myocardial infarction and decline thereafter [45,46]. Inflammatory responses to necrotic cells in a myocardial infarction begin immediately with leukocyte infiltration and sustained upregulation of pro-inflammatory cytokines [47]. This is followed by proliferation of reparative immune cells in a remodelling and reperfusion phase lasting for a few weeks [47]. CFVR may improve spontaneously in all patients during the remodelling phase following myocardial infarction. Nevertheless, interventions able to improve microvascular function may be more beneficial in this remodelling stage than in the subsequent resolution and scar maturation stage, when the majority of reparative immune cells undergo apoptosis [47].

A potential limitation of the study design is that we did not require participants to have high levels of leukotrienes or other inflammatory biomarkers, or to have impaired CFVR at study entry. In CANTOS, eligible patients had high C-reactive protein levels indicative of high residual inflammatory risk [7]. Furthermore, in a subgroup analysis, participants whose high-sensitivity C-reactive protein levels remained below 2 mg/L after 3 months of anti-interleukin-1β treatment with canakinumab had a lower risk of major adverse cardiovascular events than those with higher levels (≥2 mg/L) [48]. Exploratory outcomes in the present study include comprehensive inflammation and cardiovascular biomarker panels.

In conclusion, FLAVOUR will provide information on potentially clinically meaningful effects of FLAP inhibition with AZD5718 in patients with recent myocardial infarction receiving standard secondary preventive care. Any potentially beneficial effects of AZD5718 on surrogate efficacy outcomes would require further investigation, for example in a phase 3 cardiovascular outcomes trial.

Author contributions

Conceptualization: ML-F, L-MG; Data curation and formal analysis: MK; Investigation: AA, OA, DE, ELG, MH, LOJ, EP, JP, AS, SS; Methodology: ML-F, L-MG, ELG, MK, EP, SS; Reviewing, editing and approving manuscript: all authors.

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Declaration of competing interest

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