CXCR2 inhibition: a novel approach to treating coronary heart disease

Protocol Short Title/Acronym:		CXCR2 inhibition and coronary disease / CICADA
Version:		v 5.0 (5th July 2019)
Trial Identifiers		
EudraCT Number	-	2016-000775-24
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1. Study Synopsis

Title of clinical trial	CXCR2 inhibition: a novel approach to treating coronary heart disease
Protocol Short Title/Acronym	CXCR2 inhibition and coronary disease / CICADA
Trial Phase if not mentioned in title	Phase 2A
Sponsor name	King's College London
Chief Investigator	Professor Albert Ferro
Eudract number	2016-000775-24
IRAS number	192947
Medical condition or disease under investigation	Coronary heart disease
Purpose of clinical trial	To assess whether CXCR2 inhibition improves vascular function and vascular plaque morphology in patients with atherosclerosis affecting the coronary arteries following percutaneous coronary intervention (PCI)
Primary objective	To measure effect of CXCR2 inhibition on coronary flow reserve
Secondary objective (s)	To determine the effects of CXCR2 inhibition on coronary plaque composition, the coronary wave profile and on restenosis post-PCI
Trial Design	Randomised double-blind parallel group placebo-controlled trial
Endpoints	Primary endpoint: • change in coronary flow reserve from baseline (by invasive ComboWire assessment) Secondary endpoints: (1) change in plaque composition from baseline (2) degree of instent restenosis (3) change in the backward expansion wave from baseline
Sample Size	102 (68 in active arm and 34 in placebo arm)

Summary of eligibility criteria	Inclusion criteria: 1) men and women ≥ 18 years of age; 2) angiographically proven coronary heart disease undergoing native-vessel PCI; 3) persistent neutrophil count ≥ $3.0x10^9$/L; 4) otherwise receiving standard of care for ischaemic heart disease (including appropriate anti-platelet therapy, statin, and/or antihypertensives as clinically indicated). Exclusion criteria: 1) known active or recent infection; 2) known immunocompromised state or history of organ transplantation; 3) known major organ dysfunction or other significant co-morbidity; 4) use of a CYP3A4 inhibitor and inducers; 5) pregnancy or breast feeding; 6) women of child-bearing potential not using a highly effective method of contraception; 7) unwilling, or unable, to give informed consent.
IMP, dosage and route of administration	AZD5069 will be provided as tablets for oral administration in 40mg per tablet. Dose will be 40mg twice daily. Matching placebo will also be employed.
Active comparator product(s)	No active comparator (placebo).
Maximum duration of treatment of a subject	6 months
Version and date of protocol amendments	CXCR2 inhibition: a novel approach to treating coronary artery disease (version 5.0, 05/07/2019)

2. Contents

3. Background & Rationale

Atherosclerosis, the principal cause of myocardial infarction and stroke, is a progressive inflammatory disease characterised by the accumulation of lipids and fibrous elements in the large arteries (1). Being an inflammatory disease, atherosclerosis involves recruitment of leucocytes (predominantly neutrophils and monocytes) to the sites of vascular injury; this is triggered by the accumulation of oxidized low-density lipoprotein (LDL) within the intima, which stimulates endothelial cells to express an atherosclerotic phenotype and leads to the adherence of leucocytes on their surface. They then transmigrate into the intima, where monocytes proliferate and differentiate into macrophages which take up oxidized LDL, forming foam cells (1).

Atherosclerotic plaques exhibit two major phenotypes: stable plaques, characterized by a thick fibrous cap separating a relatively small lipid core from the lumen, which are associated with a low risk of thromboembolic complications; and unstable plaques, characterized by a large lipid core covered by a thin fibrous cap prone to rupture and thrombus formation and associated with a higher risk of thromboembolic complications (2,3). Leucocytes – and in particular neutrophils and monocytes – are involved both in atherogenesis and in plaque destabilization, the latter especially so for neutrophils (4,5). Specifically, neutrophils are over-represented in the vasculature of patients with unstable atherosclerotic plaque (4) and are associated with histologic features of plaque vulnerability (5,6); also in human studies, a mild neutrophilia along with endothelial dysfunction are elicited by a high-fat meal (7). Notably, colchicine, a known neutrophil chemotaxis inhibitor, has been recently shown to reduce vascular events in patients with coronary heart disease (8).

CXCR2 is a chemokine receptor which binds interleukin 8 (IL8) with high affinity and also exhibits binding to chemokine (C-X-C motif) ligands 1, 2, 3 and 5 (CXCL1, CXCL2, CXCL3 and CXCL5). It is a G-protein coupled receptor, signal transduction following ligand binding being mediated through G_o and G_i proteins. It is expressed at a high level in neutrophils, and plays a crucial role in mediating neutrophil migration such that its inhibition greatly decreases neutrophil recruitment to sites of inflammation (9,10). In studies conducted by AstraZeneca, AZD5069, a specific CXCR2 antagonist, was found to be potent in inhibiting calcium flux, chemotaxis and CD11b expression on human neutrophils in vitro in response to the CXCR2 ligands IL-8 and GROalpha. A structurally related CXCR2 inhibitor, AZ13381758, was also able to inhibit CD11b expression in vivo in mice administered the CXCR2 ligand MIP-2. In a rodent lipopolysaccharide (LPS) challenge model of pulmonary inflammation, AZD5069 reduced LPS-induced neutrophilia in a dose-dependent manner in both bronchial fluid and serum.

Since neutrophil recruitment into atherosclerotic plaques plays a crucial role in plaque destabilisation and vascular inflammation, manifest through neutrophil-endothelial interaction as endothelial dysfunction, we hypothesise that CXCR2 inhibition with AZD5069 will give rise to improved coronary endothelial function, which will be reflected in an improvement in coronary flow reserve (CFR). We will study this in a cohort of patients undergoing percutaneous coronary intervention (PCI). CFR is an integrated measure of coronary artery status encompassing plaque burden / vulnerability and both macro- and microvascular function, and has prognostic value over and above other markers of cardiovascular risk (11). Therefore, improvement in CFR with CXCR2 inhibition would be expected to translate into prognostic benefits clinically on long-term follow-up.

Although CFR is the primary outcome measure of this study, we expect decreased neutrophil recruitment to also translate into a reduction in the high-risk morphologic features of coronary plaque (visualised as a diminution in size of the necrotic core of lesions on virtual histology). Additionally, there is evidence that neutrophil infiltration is involved in the pathophysiology of restenosis post-PCI (12, 13). Therefore, two of our secondary outcome measures will be lesion size / composition and restenosis rate as measured by intravascular ultrasound (IVUS). We have chosen 6 months (24 weeks) of treatment and follow up since, although effects on CFR would be expected to occur more rapidly than this, effects on lesion composition and restenosis may take up to 6 months.

Sequential patients who fulfil the inclusion criteria and have none of the exclusion criteria, who are undergoing PCI following admission to St Thomas' Hospital or King's College Hospital, will be

recruited into the study. At the time of PCI, they will also undergo IVUS using the Volcano system to obtain virtual histology of the lesions. Patients will undergo ComboWire assessment of coronary flow indices at the time of angiography (see below). Four weeks post-PCI, eligible patients will be allocated 2:1, in a randomised double-blind fashion, to receive either AZD5069 or matched placebo, orally, for the succeeding 24 weeks (pending safety review – see below). At the end of this time, repeat angiography for ComboWire assessment and IVUS-based virtual histologic assessment (change in plaque composition) and assessment of degree of in-stent restenosis will be performed.

ComboWire assessment enables simultaneous measurements of coronary perfusion pressure and flow velocity the provides more specific coronary physiological data. These measurements are commonly used to determine the functional significance of epicardial coronary artery disease to facilitate clinical decision-making in the catheter lab. At the same time, utilising these dual pressure and flow sensor wires, we can determine coronary flow, the components of the coronary wave profile and indices of microvascular function. To understand the coronary haemodynamic effects of CXCR2 inhibition we will measure:

1. *Coronary flow* - estimated using average peak velocity (APV), the average of 3-5 instantaneous peak coronary flow velocities. This is a doppler-derived measurement and provides useful information of coronary flow beyond the pressure-derived fractional flow reserve (FFR). The ratio of coronary flow velocity at baseline and during adenosine induced hyperaemia will provide a reliable assessment of coronary flow reserve, even in patients with acute myocardial infarction. (14,15)
2. *Wave Intensity Analysis (WIA)* - a time-domain method of depicting a waveform in terms of a succession of multiple small wavefronts. WIA is invaluable in understanding the forces driving coronary flow and a key element of this, the backward expansion wave, has been implicated in a number of disease processes (16).
3. *Microvascular resistance* – the ratio between distal coronary arterial pressure and coronary venous pressure. The hyperaemic microvascular resistance (hMR) is a velocity-based index of microvascular resistance and is the ratio of distal coronary arterial pressure to APV during maximal hyperaemia.

The dose of AZD5069 chosen for this study is 40mg by mouth twice daily. This dose is pharmacologically equivalent to the highest dose utilized in a recently completed PhIIa study of AZD5069 in bronchial asthma (NIMBUS, NCT01704495). In that study, AZD5069 showed clear pharmacodynamic effect, specifically a reduction of serum neutrophil count, and was well tolerated. The most salient risk, that of infection due to inhibition of neutrophil chemotaxis and function, and the risk of an absolute neutropenia itself, was minimal at this dose. In the context of the study proposed, an inclusion requirement of elevated neutrophil count at the time of screening and randomization, combined with the projected percent reduction in circulating neutrophils at the dose proposed, reduces the risk that patients in the proposed study will achieve frank neutropenia. Please see sections 5.2-5.3 for full details.

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4. Trial Objectives and Design

4.1. Trial Objectives

The primary objective of this trial is to determine whether CXCR2 inhibition, in patients following PCI for atherosclerotic coronary disease, will give rise to improvement in coronary endothelial function. The secondary objectives are to ascertain whether CXCR2 inhibition in this context gives rise to a change in plaque composition towards a more stable form and to a decrease in in-stent restenosis.

4.1.1 Primary endpoint

- Change in mean CFR from baseline, as measured by ComboWire assessment at week 24 vs baseline

4.1.2 Secondary endpoints

1) Change in plaque composition, as measured by IVUS at week 24 vs baseline
2) Degree of in-stent restenosis, as measured by IVUS at week 24 vs baseline

3) Change in the magnitude of the backward expansion wave, as measured by ComboWire assessment at week 24 vs baseline

4) Change in vessel-specific CFR, as measured by ComboWire assessment at week 24 vs baseline

4.2 Trial Design

The trial will be a randomised double-blind placebo-controlled parallel group study. Sequential patients (n=102) undergoing PCI either for stable coronary disease or following admission to hospital with ACS, who fulfil the inclusion criteria and with none of the exclusion criteria (see below), will be recruited from the Cardiac Catheterisation laboratories at St Thomas' Hospitals and King's College Hospital. Once written informed consent has been obtained, patients will undergo coronary angiography with PCI as per normal clinical care, and following completion of the planned PCI will also undergo IVUS and ComboWire assessment of all major coronary vessels (culprit and non-culprit, intervened and non-intervened) using the Volcano system to provide virtual histology and to provide evaluation of coronary and microvascular physiology, respectively. Four weeks later (+/- 5 days), subjects will re-attend the Cardiology department at St Thomas' Hospital, at which time a full history and physical examination will be performed and study entry criteria confirmed. Assessments will include 12-lead electrocardiogram, and blood tests (fasted) for blood count and biochemistry (renal, liver, lipid, glycaemic and thyroid profiles), high sensitivity C-reactive protein (hs-CRP, a well validated marker of cardiovascular risk) and homocysteine level. Screening blood test for disorders which may exclude patients from the study will be drawn as indicated (e.g. serum hepatitis B surface antigen, hepatitis C antibody, and HIV testing if prompted by history). Additionally, an aliquot of blood will be taken for measurement of circulating monocyte-platelet aggregates (MPA, a highly sensitive and reproducible index of degree of platelet activation – by flow cytometry, using a method in routine use in our laboratory). In the case of women of child-bearing potential (see section 6.1 for definition), pregnancy will be excluded by performing a pregnancy test on a spot urine.

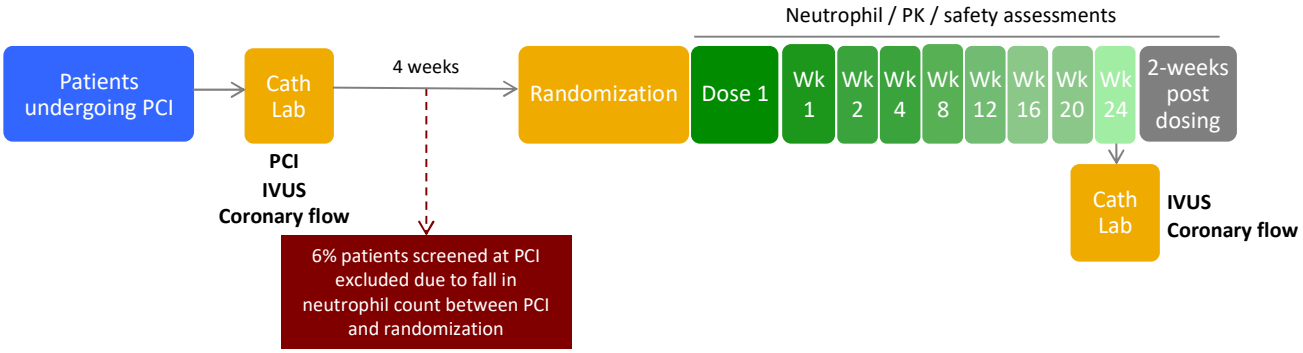
Neutrophil count data from the St Thomas' Hospital cardiac catheterization laboratory patient records suggest that 83% of patients with acute coronary syndrome undergoing emergent PCI, who meet the other inclusion criteria and none of the exclusion criteria, have neutrophil count $\geq 3.0 \times 10^9$/L; in comparison, 78% of patients with stable coronary heart disease undergoing non-emergent PCI have neutrophil count $\geq 3.0 \times 10^9$/L. Therefore it is expected that maximally 5% of patients who satisfy neutrophil count entry criteria at the time of PCI and undergo IVUS assessment will fail to meet neutrophil count inclusion criteria at the time of randomization 4 weeks post-PCI. Based on this, it is projected that approximately 129 patients will be screened at PCI to enrol 107 patients (83%) who meet entry criteria at time of PCI. Loss of a further 5% (78% of screened) will yield 102 patients meeting entry criteria at time of randomization.

After randomization, subjects will be allocated 2:1 to receive either AZD5069 or matched placebo, orally, for the succeeding 24 weeks (pending safety review). At different time points (weeks 1, 2, 4, 8, 12, 16, 20 and 24 [each time point +/- 5 days]), patients will undergo consultations to monitor for compliance and screen for adverse events. Visits 2, 7 and 10 will involve attendance for face-to-face clinical review, at which time participants will undergo physical examination and have blood samples taken for the purpose of determining safety and efficacy biomarkers; including plasma drug levels. Accepting the safety profile of the drug demonstrated in the trial to date, visits 3-6, 8 and 9 will be telephone consultations in the first instance, with recourse for a face to face consultation in the event of concerns being identified. We will, however, arrange for full blood count to be performed locally, usually by the subject's general practitioner, 4 weeks post-randomisation, to exclude drug-induced neutropenia (which, if it occurs, will be within this time period): if absolute neutrophil count at this point is below 1.0×10^9/L, we will arrange for the subject to return to us within 48 hours to repeat the blood count and decide whether or not they should be withdrawn from the study. At visits 2, 7 and 10, spot urines will be obtained from women of child-bearing potential in order to exclude pregnancy; if positive at any point, they will be immediately withdrawn from the study. After week 24, a repeat coronary angiography together with IVUS and Combowire

assessment will be performed, to assess change in CFR as well as in plaque composition and degree of restenosis.

At visit 2, we will take pre-dose samples for full blood count and biochemistry (renal, liver, glycaemic and lipid profiles, homocysteine and thyroid function), and additional biomarkers (hs-CRP, circulating MPA) to establish baseline before the study start. We will also bank 10 mL blood at -80C to allow for the possibility of future analyses in future studies. At visits 7 and 8, in all subjects we will repeat all of the pre-dose measurements from day 1; including pre-dose (trough) and peak (2 h post-dose) AZD5069 plasma concentration Following the final dose at week 24, a 2-week (+/- 5 days) post-dosing follow-up safety telephone consultation (visit 11) will be conducted.

4.3 Trial Flowchart



4.4 Trial Visit Table

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit9	Visit 10	Visit 11
	At the time of PCI	4 weeks post-PCI/ Day 1 of dosing	Week 1 of dosing	Week 2 of dosing	Week 4 of dosing	Week 8 of dosing	Week 12 of dosing	Week 16 of dosing	Week 20 of dosing	Week 24 of dosing	2-weeks post dosing
Patient information and informed consent	X										
ECG	X										
Full History		X									
Telephone based consultation for adverse event monitoring (+/- face to face visit)			X	X	X	X		X	X		X
Physical examination (including adverse event monitoring and pill counts)		X					X			X	
Urine for pregnancy test (women of child-bearing potential)		X					X			X	
Blood for haematology, biochemistry, TSH, free T3 and T4		X			X[#]		X			X	
Blood for measurement of hs-CRP and monocyte-platelet aggregates		X					X			X	
Pre-dose (trough) drug assay		X					X			X	
2 h post-dose (peak) drug assay		X					X			X	
Coronary angiography, IVUS & ComboWire	X									X	

Patients will have a full blood count measured through their local general practitioner. The results will be provided to the study team through the research nurse, and reviewed by Professor Ferro or Dr Allen.

5. Trial Medication

5.1 Investigational Medicinal Product

The drug, AZD5069, is manufactured by AstraZeneca. The drug product is presented as plain, round, beige, film-coated tablets packaged in bottles. The tablets contain mannitol, microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulphate, sodium stearyl fumarate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, iron oxide yellow, iron oxide red and black iron oxide.

Matching placebo tablets will also be provided by AstraZeneca.

All investigational products are manufactured in accordance with Good Manufacturing Practice (GMP).

The drug products should be stored in accordance with the labelling.

5.2 Dosing Regimen

The dose of AZD5069 for use in the proposed study is 40mg administered as tablets by mouth twice daily. Patients will be screened at the time of PCI, with eligibility confirmed at a 4-week post-PCI visit (visit 2). If eligbility is confirmed, the patient will be randomised and the compound will then be dosed, to continue for 6 months continuously until cessation of dosing, at which time a 2-week off-IMP observation period will be completed.

The dose chosen is pharmacologically equivalent to the maximal dose used in the recently completed human study of AZD5069 in uncontrolled persistent asthma (NIMBUS Trial, NCT01704495), and below the doses (50 and 80mg twice daily) used in a human study of COPD (Cirrus Trial, NCT01233232).

The dose was initially chosen based on its ability to inhibit GROalpha-stimulated CD11b expression on human neutrophils ex vivo. In the CIRRUS study, 3 patients (from a total of 30 patients) and 1 patient (from a total of 28 patients) were withdrawn due to low blood neutrophil counts at 50 mg twice daily and 80 mg twice daily, respectively.

In the proposed study, the inclusion of patients with persistent neutrophilia (defined as a neutrophil count $\geq 3.0 \times 10^9$ at the time of PCI as well as 4 weeks thereafter) is designed not only to enrich the study population for those patients who are hypothesized to have a neutrophil-predominant aetiology for their endothelial dysfunction, but also to provide a sufficient "window" within which neutrophil counts may fall by the expected 20-25% and still remain in an acceptable range ($> 1.5 \times 10^9$/L) and above the accepted clinical definition for neutropenia (0.5×10^9/L). Specifically, with an inclusion criteria proposed of neutrophil count $\geq 3.0 \times 10^9$/L, projecting a maximal reduction of 40%, all patients are expected to remain above the stopping criteria of 1.0×10^9/L and the clinical definition of neutropenia ($<0.5 \times 10^9$/L).

The systemic exposure at 40mg twice daily is expected to be well below the exposure limits, as defined by the lowest no observed adverse effect level (NOAEL) in the toxicology species, rat and Cynomolgus monkey (a predicted area under the plasma concentration-time curve [AUC] of 27 h*µM [NOAEL=100 h*µM] and the maximum plasma concentration (Cmax) of 2 µM [NOAEL=25.7 µM]).

Please see section 5.3 for an expanded discussion of the IMP risks.

AZD5069 should be administered either 2 hours before or 2 hours after food. Dose should be taken on an empty stomach at approximately the same time each day. During the study, patients are to abstain from eating or drinking Seville orange marmalade, grapefruit, grapefruit juice, grapefruit marmalade, or other Seville orange or grapefruit products.

Lifestyle and other restrictions are detailed in section 6.2.1

5.3 IMP Risks

To date, AZD5069 has been studied in 8 completed Phase I clinical studies in a total of 255 healthy volunteers, of whom 202 received AZD5069 (single doses up to 200 mg; multiple doses up to 80 mg BD for up to 7 days and 100 mg BD for up to 6.5 days). Two 4-week studies have been conducted in patients: 87 patients with chronic obstructive pulmonary disease (COPD; GOLD stage II-III) (50 mg and 80 mg BD/placebo), and 52 patients with bronchiectasis (80 mg BD/placebo). A Phase II efficacy study in 640 patients with persistent uncontrolled asthma has also been completed (478 patients received AZD5069 at doses of 5 mg, 15 mg BD, or 45 mg BD, while 162 patients received placebo for 6 months). In addition, approximately 70% of patients enrolled during this first portion of the trial completed a planned optional 6 month safety extension, even though that portion of the study was terminated early due to a lack of efficacy.

Safety profile of AZD5069 is inferred primarily from the PhII NIMBUS trial of 640 asthmatic patients treated with a range of doses 0 (placebo) to 45mg twice daily for 6 months. In that study, the following were noted:

- Highest incidence of AEs were in the System Organ Classes (SOCs) of infections and infestations, nervous system disorders, and gastrointestinal disorders. In the SOC gastrointestinal disorders, more patients reported AEs on AZD5069 45 mg compared to AZD5069 5 mg, AZD5069 15 mg, and placebo.
- The proportion of patients with infections was evenly spread across the 4 dose groups. More patients on placebo reported upper respiratory tract infections
- More patients reported pneumonia and lower respiratory tract infections in the AZD5069 45 mg and placebo groups (4 and 3 patients respectively for each AE) compared to the other treatment groups
- The number of patients with severe infections (defined as requiring IV antibiotics or SAE) was low in general (<2.5% of patients), with the highest number observed in the AZD5069 45 mg group. Pneumonia was the most common severe infection, with 6 patients overall (3 patients in the AZD5069 45 mg group)
- 4 patients in the AZD5069 45 mg group discontinued due to low neutrophil counts which was a predefined stopping criterion (3 patients reported AEs of decreased neutrophil counts, 1 patient reported neutropenia)
- No clinically significant changes in any haematology parameters, except expected dose dependent reductions in neutrophils and white blood cell counts
- Dose-dependent decreases in neutrophils appeared rapidly after treatment started but reversed back to baseline values after treatment discontinuation during follow-up. All discontinuations from treatment due to low blood neutrophil counts (4 patients) occurred in the AZD5069 45 mg group.

In terms of neutrophils, specifically, the maximum reduction in circulating neutrophils is shown in the following table for the NIMBUS (Asthma) and CIRRUS (COPD) trials:

Circulating neutrophils (maximum change from baseline)

	NIMBUS (Asthma; 12 months)			CIRRUS (COPD; 28 days)	
AZD5069	5 mg	15 mg	45 mg	50 mg	80 mg
Max reduction(±SD)	0.68 (0.28)	0.9 (0.24)	1.45 (0.24)	Not reported	Not reported
Max % reduction (time to max)	6 (2d)	15 (8m)	28 (1m)	40(2d)	35(2d)
%reduction at end of treatment	4	12	20	18	25

Based on an expected reduction in absolute neutrophil count of 20-25%, and the stated inclusion criteria of a neutrophil count ≥ $3.0x10^9$/L at both the time of PCI and 4 weeks later at randomization, it is projected that all patients will remain non-neutropenic (defined as an absolute neutrophil count < $0.5x10^9$/L) throughout the study. Furthermore, all patients are projected to remain above the stopping criteria of $1.0x10^9$/L and the clinical definition of neutropenia (<$0.5x10^9$/L).

Given maximal neutrophil reductions were generally seen within the 1st week of dosing, a 1-week visit for haematologic assessment is included in the study design. We will monitor neutrophil count at frequent intervals during the course of the study, and will withdraw subjects if absolute neutrophil count is below $1.0x10^9$/L in 2 consecutive samples (a further sample arranged at an additional visit within 48 hours if a neutrophil count <$1.0x10^9$/L is measured).

As food effect has not been formally assessed, the investigational product is to be taken on an empty stomach (please see section 5.2).

From the non-clinical data (embryo-fetal development studies in rat and rabbit), it is predicted that there is a low likelihood of causing fetal harm based on expected therapeutic exposures. However, as embryo-fetal toxicity has not been comprehensively assessed in full pre- and post-natal development toxicology studies, women of childbearing potential and men who are sexually active with a female partner of childbearing potential must be surgically sterilised, practicing abstinence, or agree to use highly effective methods of birth control from the time of screening until 1 week (i.e. 5 elimination half-lives rounded up to the nearest week) after final dose of study drug. Women of childbearing potential are defined as those who are not surgically sterile (ie, bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or postmenopausal (defined in section 6.1). Please see section 6.2.1 for the defined list of accepted methods of contraception considered to be highly effective. Periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of birth control.

Animal studies to date have shown no evidence of any effect of AZD5069 on spermatogenesis, and data from a 6-month toxicology study in rat showed no adverse effects on male mating and fertility at any dose level. Male study participants who are sexually active should avoid procreation for the duration of the trial and for a washout period equivalent to 5 elimination half-lives after the trial rounded up to the nearest week. In the case of AZD5069 this precaution should be exercised for 1 week after final dose of study drug.

Given that AZD5069 is not mutagenic, there is no mandatory requirement for condom use, either for avoidance of procreation or in the case of treated males with a pregnant partner; avoidance of procreation can be through use of a highly effective contraceptive method by the study participant or by the partner. Highly effective contraceptive methods are defined in section 6.2.1.

5.4 Drug Accountability

At each visit, pill counts will be conducted. Any investigational medicinal product, dispensed and unused or not dispensed, will be disposed of by the Pharmacy department at Guy's and St Thomas' NHS Foundation Trust.

5.5 Storage of IMP

The drug products should be stored in accordance with the labelling which will accompany the investigational product.

5.6 Subject Compliance.

Compliance will be assessed at visits 3-11. At visits 7 and 10, pill counts will be performed and pre-dose blood samples taken for drug assay.

5.7 Concomitant Medication

All patients will receive usual care medications for ischaemic heart disease (including appropriate anti-platelet therapy, statin, antihypertensive as clinically indicated). Medications which may affect the primary or secondary endpoint (specifically lipid lowering, antihypertensive or immunomodulatory therapy) may not be initiated between PCI and randomization (this is not part of routine care), however dose adjustments of chronic therapies initiated prior to PCI is permissible.

Concomitant medications may not violate the following AZD5069 restrictions: (Restrictions apply starting 14 days prior to the first dose of AZD5069 and last for as long as the patient is treated with AZD5069 and until 24 hours after the last dose of AZD5069)

- Potent and moderate cytochrome P450 (CYP) 3A4 inhibitors, including, but not limited to: amprenavir, aprepitant, atazanavir, boceprevir ciprofloxacin, clarithromycin, conivaptan, crizotinib, darunavir/ritonavir, diltiazem, erythromycin, fluconazole, fosamprenavir, grapefruit juice, imatinib, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, verapamil, and voriconazole.
- Potent and moderate CYP3A inducers, including, but not limited to avasimibe, bosentan, carbamazepine, efavirenz, etravirine, modafinil, nafcillin, phenytoin, rifampin and St. John's wort
- Use of sensitive CYP2B6 substrates, including, but not limited to: bupropion and efavirenz.
- P-glycoprotein (P-gp) substrates: digoxin and dabigatran.
- Warfarin and other coumarin derivatives**: acenocoumarol, phenprocoumon, warfarin.**
- Any herbal medications, but are not limited to dehydroepiandrosterone, ephedra (ma huang), Gingko biloba, ginseng, kava, saw palmetto, St. John's wort, yohimbe, seville orange and grapefruit products, any medications that are known to depress blood neutrophil counts or general bone marrow function.
- Introduction of a new medication after PCI which may affect trial endpoints (see exclusion criteria).

6. Selection and Withdrawal of Subjects

6.1 Inclusion Criteria

- Men and women ≥ 18 years of age.
- Angiographically proven coronary heart disease undergoing native-vessel PCI for myocardial infarction or unstable or stable coronary disease.
- Absolute blood neutrophil count at the time of PCI as well as 4 weeks after PCI of ≥ 3.0 x 10^9/L.
- Otherwise receiving standard of care for ischaemic heart disease (including appropriate anti-platelet therapy, statin, antihypertensive as clinically indicated).
- Women of childbearing potential may be included in the study provided they are established on, and continue to use highly effective contraceptive methods (as defined in section 6.2.1) from the time of screening until 1 week after the final dose of study drug. Women will be considered post-menopausal if they are:
    - under 50 years of age and have been amenorrhoeic for 12 months or more (following cessation of exogenous hormonal treatments – if these have been

previously taken) and with luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels in the post-menopausal range, or

- ≥ 50 years old and have been amenorrhoeic for 12 months or more following cessation of all exogenous hormonal treatments

6.2 Exclusion Criteria

- At any time after initial screening (i.e. at the time of PCI + IVUS), requirement or anticipated requirement, by clinical care guideline or in the opinion of the treating physician, for a new medication which may affect trial primary and / or secondary endpoints (i.e. specifically new lipid lowering therapy, anti-hypertensive therapy, or immunomodulatory therapy). Note that dose titration of chronic therapy initiated prior to PCI is permissible during the trial.
- History of inability or, in the opinion of the investigator, anticipated inability to tolerate pharmacologic stress testing (e.g. second or third degree AV block without a cardiac pacemaker, resting systolic blood pressure <90mmHg, unstable coronary disease, use of medications which may interfere with the test).
- Significant vascular anatomic abnormality which in the opinion of the investigator portends unacceptable risk to serial coronary IVUS and ComboWire examination.
- Scheduled inpatient surgery or planned hospitalisation during the study period.
- Any clinically significant disease or disorder (e.g., cardiovascular, gastrointestinal, liver, renal, neurological, musculoskeletal, endocrine, metabolic, psychiatric, major physical impairment) which, as judged by the investigator, might put the patient at risk because of participation in the study.
- Recurrent, latent, or chronic infections, as judged by the investigator, or with a history of osteomyelitis, or at risk of infection (surgery, trauma, or significant infection within 30 days before enrolment), or with a hist ory of skin abscesses or a soft tissue infection within 60 days before enrolment.
- Evidence of active tuberculosis, either treated or untreated, or latent tuberculosis without completion of an appropriate course of treatment or appropriate ongoing prophylactic treatment.
- Positive test for serum hepatitis B surface antigen, hepatitis C antibody, or HIV.
- Patients vaccinated with a live or live-attenuated vaccine in the 2 weeks prior to enrolment.
- Use of any immunosuppressive treatment (eg, methotrexate, troleandomycin oral gold, cyclosporine, azathioprine, intramuscular long-acting corticosteroid [except for asthma exacerbations]) within 60 days prior to enrolment.
- Prior solid organ or bone marrow transplantation.
- History of any primary immunodeficiency disorder excluding asymptomatic selective IgA or IgG subclass deficiency.
- Active malignancy or neoplastic disease in the previous 5 years other than superficial basal cell carcinoma.
- Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level ≥2.5 times the upper limit of normal (ULN) 4 weeks after PCI.
- QTc of >450 ms for males and >470 ms for females 4-weeks after PCI.
- Current evidence of drug abuse or significant history of drug abuse, as judged by the investigator.

- Current evidence of alcohol abuse or a significant history of alcohol abuse, as judged by the investigator.
- Pregnancy or breast feeding during the study.
- Contraindication to any of the study treatments or known or suspected hypersensitivity to the investigational product, compounds of the same class, other study treatments or any excipients as specified in Section 5.7.
- Unwilling, or unable, to give informed consent.

6.2.1 Restrictions During the Study

- Patients are to be instructed not to take any medications, including over-the-counter products (with the exception of paracetamol), without first consulting the investigator.
- Patients are to abstain from eating or drinking Seville orange marmalade, grapefruit, grapefruit juice, grapefruit marmalade, or other Seville orange or grapefruit products.
- Patients are to abstain from taking any prohibited medications.
- Patients are to abstain from abuse of alcohol or use of illicit drugs of abuse.
- Patients are to abstain from donating blood and plasma during the study.
- Women of childbearing potential may be included in the study provided they are established on, and continue to use highly effective contraceptive methods from the time of screening until 1 week after the final dose of study drug. Highly effective methods of contraception are defined as combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (either oral, intravaginal or transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (either oral [specifically Cerazette], injectable or implantable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner or sexual abstinence.
- Male study participants who are sexually active with a female partner of childbearing potential must be surgically sterilised, practicing abstinence, or agree to use highly effective methods of birth control, and not rely on barrier methods and spermicide alone, from the time of screening until 1 week after final dose of study drug. Male study participants must also not donate sperm from the time of screening until 1 week after final dose of study drug. Given that AZD5069 is not mutagenic, there is no mandatory requirement for condom use, either for avoidance of procreation or in the case of treated males with a pregnant partner. Avoidance of procreation can be through use of a highly effective contraceptive method by the study participant or by the partner, as detailed above.

6.3 Selection of Participants

Subjects will be recruited sequentially from patients due to attend the cardiac catheterisation labs (either on the waiting list for outpatient procedures, or on the cardiology ward prior to inpatient procedures) at St Thomas' Hospital or King's College Hospital, for PCI, who fulfil the inclusion criteria and have none of the exclusion criteria.

6.4 Randomisation Procedure / Code Break

6.4.1 Randomisation

Randomisation service will be provided by the King's Clinical Trials Unit. Subjects will be randomised 2:1 to AZD5069 vs placebo, using block randomization with randomly selected block

sizes. Randomisation will be stratified based on neutrophil count above or below $4.0x10^9$/L and separately by the presence or absence of a history of prior cardiac surgery and clinical presentation.

6.4.2 Emergency Code Break

24hr Emergency Code Break and Medical Information will be provided by Emergency Scientific and Medical Services (ESMS). Each randomised subject will be provided with a card detailing code break telephone numbers and emergency contact details. Subjects will be requested to carry this card with them at all times whilst participating in the trial.

6.5 Withdrawal of Subjects

Study drug must be discontinued if:

- An exclusion criteria is incident during the course of the study
- Absolute neutrophil count is noted below $1x10^9$/L in 2 consecutive samples within 48 hours
- the participant misses > 20% of their doses
- the participant decides they no longer wish to continue
- recommended by the investigator

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study drug in the event of inter-current illness, AEs, SAE's, SUSAR's, protocol violations, cure, administrative or other reasons. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible. Should a patient withdraw from study drug only, efforts will be made to continue to obtain follow-up data, with the permission of the patient.

Participants who wish to withdraw from trial medication (IMP) will be asked to confirm whether they are still willing to provide the following.

- trial specific data at subsequent scheduled visits
- clinical follow-up data collected as per routine clinical practice

6.6 Expected Duration of Trial.

The full study duration (first patient first visit to last patient last visit) is expected to be 5 years. The end of the trial will be defined as last patient last visit - each patient being in the trial until two weeks post-dosing (i.e. Visit 12).

7. Trial Procedures

7.1 By Visit

Visit 1 (at time of PCI): patient information and informed consent confirmed, the patient will undergo coronary angiography and PCI as per standard of care. In addition, they will have IVUS, and in a subset, ComboWire assessment performed. Wherever possible, where PCI is being performed in a non-emergency setting, subjects will receive the information at least 24 hours (and ideally 1 week) prior to PCI, to allow them time to decide, discuss with relatives, ask questions etc. In patients in whom PCI is dependant on angiographic findings, an initial screening consent form will be used. The study includes recruiting patients undergoing PCI following non-ST-elevation myocardial infarction. These patients have their index procedure urgently following presentation to medical care (often within 24 hours). Wherever possible, these patients will receive the information at least

2 hours prior to PCI, to allow them time to decide, discuss with relatives, ask questions etc without providing any delay to their clinical care.

Visit 2 (4 weeks +/- 5 days post-PCI): full history and examination, blood sampling, pregnancy test (if woman of child-bearing potential); followed by randomisation to active drug or placebo and pre-dose and 2 h post-dose blood samplings.

Visit 3 (day 1 +/- 5 days of dosing): telephone consultation to monitor compliance and screen for adverse events +/- face to face consultation if concerns identified.

Visit 4 (week 2 +/- 5 days of dosing): telephone consultation to monitor compliance and screen for adverse events +/- face to face consultation if concerns identified.

Visit 5 (week 4 +/- 5 days of dosing): telephone consultation to monitor compliance and screen for adverse events +/- face to face consultation if concerns identified. Supplemented by review of full blood count result performed locally, usually by the subject's general practitioner.

Visit 6 (week 8 +/- 5 days of dosing): telephone consultation to monitor compliance and screen for adverse events +/- face to face consultation if concerns identified.

Visit 7 (week 12 +/- 5 days of dosing): physical examination, and pre-dose and 2 h post-dose blood samplings. Pregnancy test (if woman of child-bearing potential).

Visit 8 (week 16 +/- 5 days of dosing): telephone consultation to monitor compliance and screen for adverse events +/- face to face consultation if concerns identified.

Visit 9 (week 20 +/- 5 days of dosing): telephone consultation to monitor compliance and screen for adverse events +/- face to face consultation if concerns identified.

Visit 10 (week 24 +/- 5 days of dosing): physical examination, and pre-dose and 2 h post-dose blood samplings. Pregnancy test (if woman of child-bearing potential). Repeat coronary angiography, IVUS and ComboWire.

Visit 11 (week 2 post-dosing +/- 5 days): telephone consultation to screen for adverse events +/- face to face consultation if concerns identified.

7.2 Laboratory Tests

Full blood count will be performed by the clinical haematology laboratory at St Thomas' Hospital. Blood (2 ml) will be sent in an EDTA vacutainer tube to the laboratory within 30 min of venesection. (Visit 2.)

Blood biochemistry (renal, liver, lipid, glycaemic and thyroid profiles), hs-CRP and homocysteine assays will be performed by the clinical biochemistry laboratory at St Thomas' Hospital. Blood (10 ml) will be sent in a clotted vacutainer tube to the laboratory within 30 min of venesection. (Visits 2-8.)

Circulating MPA measurement will be performed in our research labs by flow cytometry analysis on whole blood (4 ml) collected in sodium citrate (0.3% final concentration). Immediately after venepuncture, blood will be immunostained with different combinations of peridinin chlorophyll protein complex (PerCP)-conjugated anti-human CD14, FITC-conjugated anti-human CD16 and APC-conjugated anti-human CD42b or CD62P. Isotype control antibodies will be used as negative control. After red cell lysis using FACS lysing solution, samples will be fixed in 1% paraformaldehyde and kept at 4°C until analysed within 48 h maximum by flow cytometry. (Visits 2-8.)

Pharmacokinetic assays of AZD5069 and its major human metabolite AZ13587715 will be conducted via a contracted vendor (Covance) who have created and validated the LC/MS-based analytical method for AstraZeneca for assessment of AZD5069 and AZ135587715 in human

plasma preserved in EDTA and stored between -10 and -30C. The analytical method will be identical to that used in prior human study of AZD5069.

8. Assessment of Efficacy

8.1.1 Primary Efficacy Parameters

Change in coronary endothelial function from baseline, assessed by change in mean coronary flow reserve (CFR) which will be measured by invasive ComboWire assessment.

8.1.2 Secondary Efficacy Parameters

Change in plaque composition from baseline, as measured by IVUS. Specifically, we will assess the change in the volume of plaque which is deemed to be high risk (i.e. necrotic core volume)

Degree of restenosis, as measured by IVUS. Specifically, we will assess the change in minimum luminal cross-sectional area of the stented segment.

Change in backward expansion wave component of the coronary wave profile using ComboWire assessment. Specifically, we will assess the percentage change in the backward expansion wave from baseline with adenosine induced hyperaemia.

Change in vessel-specific coronary flow reserve from baseline, which will be measured by invasive ComboWire assessment.

8.2 Procedures for Assessing Efficacy Parameters

Coronary endothelial function will be assessed at baseline and after 24 weeks of therapy, by mean CFR using invasive ComboWire Assessment (basal and in response to pharmacological stress) as outlined in section 4.2.

Plaque composition will be assessed at baseline and after 24 weeks of therapy, by IVUS using the Volcano system to obtain virtual histology in native coronary arteries. Each native coronary artery will have the total plaque volume recorded and percentage volumes of fibrous, fibro-fatty, necrotic core and calcific tissue. We will and assess the change in necrotic core volume between baseline and after 24 weeks of therapy. The volume of necrotic core is thought to decrease following CXCR2 inhibition.

Degree of restenosis will be assessed at 24 weeks by IVUS to measure minimum luminal cross-sectional area. This will be measured during the baseline PCI and again following 24 weeks of therapy. The degree of restenosis at six months is expected to reduced with treatment with CXCR2 inhibition.

Change in the magnitude of the backward expansion wave will be assessed by ComboWire at the time of coronary angiography. In each of the three main native coronary arteries (left anterior descending, left circumflex and right coronary artery) we will measure the the backward expansion wave part of the coronary flow profile at baseline and following hyperaemia. The percentage increase in the BEW from baseline to adenosine is expected to improve following treatment with CXCR2 inhibition.

Both IVUS and ComboWire will be performed at the same time as angiography (at baseline and following 24 weeks therapy), so will not involve any extra invasive procedures in addition to the angiogram.

8.2.1 Risks of investigational procedures

The trial protocol involves additional diagnostic procedures (Coronary angiogram with IVUS and ComboWire assessment) to be performed at the time of the standard of care percutaneous coronary intervention procedure.

The initial PCI will be part of usual care, but will take up to 30 min longer than normal due to the addition of IVUS (of both culprit and non-culprit arterial territories) and ComboWire assessment. These extra procedures are not envisaged to pose any additional risk to subjects.

After 24 weeks therapy, the repeat coronary angiogram with associated IVUS and ComboWire assessment, will not be part of usual care and are purely for the purposes of the trial. The risk associated with this extra procedure is very low, with an estimated incidence of serious complications (vascular damage at the site of arterial insertion, myocardial infarction, stroke, renal damage or death) < 1:1,000.

9. Assessment of Safety

9.1 Specification, Timing and Recording of Safety Parameters.

Following the start of treatment, subjects will be assessed at weeks 1, 2, 4, 8, 12, 16, 20 and 24 (when treatment will cease) to screen for potential adverse events. Visits 2 (week 0), seven (week 12) and ten (week 24) will be in person at the main trial site and will include physical examination, safety blood tests (renal, hepatic, thyroid, lipid and glycaemic profiles). Visits at 3 (week 1 of dosing), 4 (week 2), 5 (week 4) 6 (week 8), 8 (week 16), 9 (week 20) and 11 (2 weeks post-dosing) will be telephone consultation based in the first instance, with recourse for face-to-face review should concerns be identified. We will also arrange for full blood count to be performed locally, usually by the subject's general practitioner, 4 weeks post-randomisation, to exclude drug-induced neutropenia. All results will be recorded and reported as detailed below.

9.2 Procedures for Recording and Reporting Adverse Events

The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 gives the following definitions:

Adverse Event (AE): Any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction (AR): Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

Unexpected Adverse Reaction (UAR): An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the Investigator's Brochure (IB).

Serious adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR): Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that:

- Results in death;
- Is life-threatening;
- Required hospitalisation or prolongation of existing hospitalisation;
- Results in persistent or significant disability or incapacity;
- Consists of a congenital anomaly or birth defect.

Important Medical Events (IME) & Pregnancy

Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

Although not a serious adverse event, any unplanned pregnancy will also be reported via the SAE reporting system.

Reporting Responsibilities

King's College London and Guy's and St Thomas' NHS Foundation trust have delegated the delivery of the Sponsor's responsibility for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004) to the King's Health Partners Clinical Trials Office (KHP-CTO).

All SAEs, SARs and SUSARs will be reported immediately by the Chief Investigator (and certainly no later than 24hrs) to the KHP-CTO in accordance with the current Pharmacovigilance Policy.

Death as a result of disease progression and other events that are primary or secondary outcome measures are not considered to be SAEs and should be reported in the normal way, on the appropriate CRF.

The KHP-CTO will report SUSARs to the regulatory authorities (MHRA, competent authorities of other EEA (European Economic Area) states in which the trial is taking place.

The Chief Investigator will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.
- The Chief Investigator and KHP-CTO (on behalf of the co-sponsors), will submit a Development Safety Update Report (DSUR) relating to this trial IMP, to the MHRA and REC annually.

9.2.1 Adverse events that do not require reporting

Serious adverse events that are expected to occur during this study, which will not require formal reporting*:*

- Bleeding: Vascular trauma secondary to invasive cardiac procedures is a recognised complication. Formal reporting will not be required unless surgical intervention is required or a blood transfusion is required.

9.2.2 Roles for Trial Steering and Data Monitoring Committees

Steering Committee

A detailed description of the role of the Trial Steering Committee is found the Trial Steering Committee Charter. In summary, the role of the Steering Committee is to provide oversight of the conduct of the trial. This includes oversight of the practical aspects of the study as well as ensuring that the study continues to be run in a way which is both safe for the patients and provides appropriate safety and efficacy data to the sponsors and investigators. In discharging its safety role, the Steering Committee will work in conjunction with the Data Monitoring Committee that will also be established for the trial (see below).

Specific responsibilities of the Steering Committee include, but are not limited to, the following:

- to provide overall supervision of the trial
- to take steps to reduce deviations from the protocol to a minimum
- periodic review of the progress of the study
- to review safety data; this review is typically done blinded to treatment allocation (in case of a major safety concern, the Steering Committee can request unblinding and the review then can be done in an unblinded manner)
- to resolve any differences within the research team or between research team and sponsors (King's College London and Guy's & St Thomas' NHS Foundation Trust) on the

data management and monitoring procedures in the trials or any recommendations for modifications to the protocol.

The Steering Committee will have ultimate responsibility for the trial and will assume primacy over the Data Monitoring Committee or principal investigator. The sponsor and principal investigator will agree, in writing prior to the start of the study, to the charter of the Steering Committee. The Steering Committee will meet prior to the start of the study and 6–12 months after the start of the study. The Steering Committee may also meet on an ad hoc basis should the need arise. A set of minutes (drafted by a member of the research team) will be produced for each meeting of the Steering Committee and be filed in the trial master file.

Data Monitoring Committee

A full description of the Data Monitoring Committee role is found in the Data Monitoring Committee Charter. In summary, the role of the Data Monitoring Committee is to safeguard the interests of the trial's participants and to monitor the data collected in the trial. Specific responsibilities of the Data Monitoring Committee include, but are not limited to, the following:

- to agree to and evaluate the data management and monitoring procedures of the trial as proposed by the research team
- to assess recruitment figures and data quality, including completeness
- to assess the extent of protocol deviations (including a comparison of patients enrolled in the trials and other patients in the research database using the same medication)
- to review safety data, including line-listings of case reports of suspected ADRs and of iatrogenic conditions and to request further analyses; this review will be done blinded to treatment allocation; the Data Monitoring Committee will inform the Steering Committee of any major safety concerns and request their unblinded review of safety data
- to implement early stopping rules for the trials
- to perform an interim analysis after 20 patients have completed the study protocol to determine feasibility

The Data Monitoring Committee can recommend modifications to the data management and monitoring procedures in the trials or to the protocol. The Data Monitoring Committee will consist of three members. Two members will constitute a quorum. The Data Monitoring Committee will meet prior to the start of the study and 6–12 months after the start of the study. The Data Monitoring Committee may also meet on an ad hoc basis should the need arise. Meetings may take place by teleconference, by videoconference, or face to face, whichever is agreed to be most appropriate. Urgent issues may be communicated by e-mail. A set of minutes will be produced for each meeting of the Data Monitoring Committee and filed in the trial master file. The Data Monitoring Committee will report to the Trial Steering Committee via the Trial Management Group.

9.3 Treatment Stopping Rules

The trial may be prematurely discontinued by the Sponsor, Chief Investigator or Regulatory Authority on the basis of new safety information or for other reasons given by the ethics committee concerned.

If the trial is prematurely discontinued, active participants will be informed and no further participant data will be collected. The Competent Authority and Research Ethics Committee will be informed within 15 days of the early termination of the trial.

Individual patients may be withdrawn from the trial if they meet the criteria listed in Section 6.5. The trial will continue until the last randomised patient (i.e.102nd trial subject) has completed Visit 12 (Week 2 Post-Dosing).

10. Statistics

Since the trial is randomised and double blind, all blood assays, coronary angiograms and virtual histology will be performed with no knowledge of subjects' treatment allocation (IMP or placebo).

10.1 Sample Size

The primary outcome is change in total CFR. Formal power calculations are problematic, since no data exist to date in humans on the relationship between CXCR2 inhibition and CFR. However, assuming a medium-large effect size (d=0.7), α error probability of 0.05 (two-tailed), power 0.80 and allocation ratio 2:1 (active drug:placebo), power calculation (using G*Power 3.1.3 software) indicates that we would need 68 subjects in the active and 34 subjects in the placebo group.

Furthermore, unpublished observational data from AstraZeneca and the Göteborg Hostpital, Sweden which relate neutrophil counts in stable CAD and after myocardial infarction with echocardiography coronary flow reserve are shown below.

Study	Neutrophil Count (5.8+/-1.0 is the neutrophil count in a CEVENT-like population at King's)	Göteborg Hospital Echo-based CFR (mean ± SE)	% CFR increase	SD of PET-MPR (from CAD patients from from King's College, Morton JACC 2012)	Patient cohort size required (p<0.05, 80% power)
Stable CAD cohort					
	5.8	2.10 ± 0.19		0.36	
	4.6	2.35 ± 0.12	10.6%	0.36	33
	4.2	2.43 ± 0.11	13.5%	0.36	19
Post MI Cohort					
	5.8	2.45 ± 0.07		0.36	
	4.6	2.64 ± 0.05	7.2%	0.36	57
	4.2	2.70 ± 0.05	9.3%	0.36	33

Based on these analyses, we will recruit 68 into the active and 34 into the placebo group, firstly to allow for lesser effect sizes, and secondly to allow us to better stratify responses to CXCR2 inhibition based on plasma IMP levels achieved.

A secondary end point is the change in the magnitude of the backward expansion wave (BEW), a key component of the coronary wave profile, with maximal hyperaemia. To measure this, a different coronary wire (ComboWire) will be used instead of a standard angioplasty wire. From previous work we expect the magnitude of the BEW to increase 40% with adenosine induced maximal hyperaemia in patients with coronary artery disease. Unpublished data from the host centre suggest that in patients without coronary artery disease, the BEW can increase a further 14%. Assuming an α error probability of 0.05 (two-tailed), power 0.80 and allocation ratio 2:1 (active drug:placebo), power calculation (using G*Power 3.1.3 software) indicates that we would need 26 subjects in the active and 13 subjects in the placebo group. The BEW measurement has a standard

deviation of 21%, as such we will need to perform WIA analysis in 57 patients who undergo ComboWire assessment as part of their angiogram procedure.

10.2 Randomisation

Treatment allocation will be performed by block randomization with randomly selected block sizes, to be performed within the King's Clinical Trials Unit. Randomisation will be stratified based on entry neutrophil count (i.e. $\geq 4.0 \times 10^9$/L vs $\geq 3.0 \times 10^9$/L but $< 4.0 \times 10^9$/L), and the presence or absence of prior cardiac surgery.

10.3 Analysis

Data will be summarised by treatment arm and overall using suitable measures of central tendencies for continuous data (means or medians), variability (SD or IQR), and frequencies and proportions (n (%)) for categorical data. The primary analysis – change in mean CFR from baseline to 24 weeks – will be performed according to ITT principle. An analysis of covariance model will be used to obtain an estimate for the mean difference in CFR between the two treatments groups adjusted for baseline CFR. The estimated treatment effect will be reported with 95% confidence intervals and corresponding p value. Secondary outcomes including change in plaque composition, coronary endothelial function and indices of microvascular function will be analyzed using analysis of covariance. A pre-specified analysis will be performed to analyse the data in groups by neutrophil count at time of randomisation assessing the primary and all secondary endpoints. The two subgroup analyses will be participants with a neutrophil count $\geq 4.0 \times 10^9$/L compared to those $\geq 3.0 \times 10^9$/L and with a neutrophil count $\geq 3.5 \times 10^9$/L compared to those $\geq 3.0 \times 10^9$/L. Proportions and rates of adverse events between arms will be calculated with 95 % confidence intervals using exact methods where appropriate. A detailed statistical analysis plan will be developed for approval by the Trial Steering Group and will be finalized before data lock.

11. Direct Access to Source Data and Documents

The Investigators will permit trial-related monitoring, audits, REC review, and regulatory inspections by providing the Sponsors, Regulators and REC direct access to source data and other documents (e.g. patients' case sheets, blood test reports, X-ray reports, histology reports etc.).

12. Ethics & Regulatory Approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents will be submitted for review to a Research Ethics Committee (REC), and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation.

The Chief Investigator will submit a final report at conclusion of the trial to the KHP-CTO (on behalf of the Sponsor), the REC and the MHRA within the timelines defined in the Regulations.

13. Quality Assurance

Monitoring of this trial will be to ensure compliance with Good Clinical Practice and scientific integrity will be managed and oversight retained, by the KHP-CTO Quality Team.

14. Data Handling

The Chief Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

- Patient data will be anonymised.
- All anonymised data will be stored on a password protected computer.
- All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the King's Health Partners Clinical Trials Office Archiving SOP.

15. Data Management

A web-based electronic databasing system will be used, as provided by King's Clinical Trials Unit: see http://www.ctu.co.uk/page/eCRF-database.aspx

16. Publication Policy

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals.

17. Insurance / Indemnity

King's College London will provide insurance and indemnity for the trial. All clinical procedures will be performed by clinical personnel who have NHS contracts with Guy's and St Thomas' NHS Foundation Trust or King's College Hospital Foundation Trust (either substantive or honorary), and therefore are covered by NHS Trust indemnity.

18. Financial Aspects

Funding to conduct the trial is provided by AstraZeneca.

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

19. Signatures

To be signed by Chief Investigator minimum and statistician if applicable.

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Chief Investigator Date

Professor Albert FERRO

Statistician Dr Abdel DOUIRI	Date