Revised Clinical Study Protocol

Drug Substance	AZD5069
Study Code	D3550C00017
Edition Number	1
Date	

A Randomised, Double-blind, Placebo-Controlled, Two-way Cross-over, Single Centre Study in Healthy Subjects to Assess the Effect of Oral Dosing of AZD5069 on Neutrophil Number and Function in Peripheral Blood and the Ability to Recruit Neutrophils into the Circulation after Exercise and Subcutaneous G-CSF

Sponsor: AstraZeneca AB,

AstraZeneca Research and Development site representative

_____ _____

Date

Quintiles Project Manager

The following Amendment(s) and Administrative Changes are included in this revised protocol:

Amendment No.	Date of Amendment	Local Amendment No.	Date of local Amendment
1			
2			

Administrative change No.	Date of Administrative Change	Local Administrative change No.	Date of local Administrative Change

AstraZeneca

PROTOCOL SYNOPSIS

A Randomised, Double-blind, Placebo-Controlled, Two-way Cross-over, Single Centre Study in Healthy Subjects to Assess the Effect of Oral Dosing of AZD5069 on Neutrophil Number and Function in Peripheral Blood and the Ability to Recruit Neutrophils into the Circulation after Exercise and Subcutaneous G-CSF

Principal Investigator

Hammersmith Medicines Research Ltd (HMR)

Study centre(s) and number of subjects planned

The study will be performed at Hammersmith Medicines Research Ltd (HMR), . Approximately 30 healthy male and post-menopausal/surgically sterile female subjects will be randomised in this study.

Study period	Phase of development
Estimated date of first subject enrolled	Clinical Pharmacology (Phase I)
Estimated date of last subject completed	

Objectives

Primary objective

- To investigate the effect of AZD5069 on neutrophil number and function (phagocytosis and oxidative burst) in the circulation of healthy volunteers
- To investigate the effect of AZD5069 and placebo on circulating neutrophil numbers following a burst of strenuous exercise
- To investigate the effect of AZD5069 and placebo on circulating neutrophils following subcutaneous injection of granulocyte-colony stimulating factor (G-CSF)

Secondary objectives

- To evaluate general safety and tolerability of AZD5069

- To evaluate the steady state 24 hour pharmacokinetic profile of the AZD5069 capsule following twice daily (bid) dosing

- To evaluate the steady state 24 hour profile of circulating neutrophils following bid dosing of AZD5069 and its relationship to plasma concentration

Exploratory objectives

- To assess which subpopulation of neutrophils is reduced on AZD5069

- To collect and store DNA samples for possible retrospective exploratory genetic analysis, investigating the influence of genotype on pharmacodynamic and pharmacokinetic responses, safety and tolerability of AZD5069, and associated biomarkers, where appropriate

- To explore potential up-regulation of biomarkers, ie, interleukin (IL)-8, G-CSF, growth-related oncogene-alpha (GRO-α) and epithelial cell-derived neutrophil activating protein (ENA)-78

Results from exploratory analyses, if performed, may be reported separately from the Clinical Study Report.

Study design

This will be an exploratory, single centre, randomised, double-blind, placebo-controlled, cross-over study in healthy subjects. The study will consist of approximately 30 randomised healthy adult subjects, with at least 28 subjects completing the two treatment periods.

All potentially suitable subjects will attend the study centre for an information visit where the study will be explained and informed consent collected (Visit 1) before starting study specific screening (Visit 2). Visits 1 and 2 can occur on the same day as per the study centre requirements. Screening will be conducted within 28 days of randomisation (Visit 3). After screening, subjects who fulfil all the inclusion criteria and none of the exclusion criteria will be randomised in a 1:1 ratio to either AZD5069 100 mg capsules (2 x 50 mg capsule) twice daily (treatment A) or placebo twice daily (treatment B) (8 am and 8 pm). **The time of investigational product administration is very important due to diurnal variation in neutrophils. Investigational product administration should be at the time points stated above ±30 minutes. Each subject should receive the investigational product at the same time point on each occasion with a maximum difference of 15 minutes.** The investigational product will be administered orally, bid, for 6 days during Treatment Period 1. After Treatment Period 1 there will be a washout period for a minimum of 21 days whereafter the subjects will be admitted for another 6 days of treatment (Treatment Period 2).

At three time points during each treatment period (baseline, at steady state [Day 4], and 7 days after end of treatment) fresh neutrophils will be collected to run functional assays with regards to phagocytosis and oxidative burst. At these time points there will also be an evaluation of flow cytometry to evaluate what subpopulation of neutrophils is affected. Blood samples for

explorative biomarkers (G-CSF, GRO-α, ENA-78 and IL-8) will also be taken at these times. The subjects will stay in the study centre during the whole treatment period and circulating neutrophils will be assessed 12 hours after the last administration of investigational product, on Day 7, in the morning before the subjects are discharged. An additional follow-up visit will take place 7 days after the last day of investigational product administration, when neutrophil function and leukocyte differential counts will be assessed at the same time of the day as the other samples were collected. Neutrophil function will be assessed 7 days after the end of treatment to determine the effect of AZD5069 on neutrophils under maturation in the bone marrow during the treatment period.

Target subject population

Healthy non-smoking Caucasian male and/or post-menopausal/surgical sterile female subjects aged 18 to 45 years, inclusive with a body mass index >18 kg/m^2 and ≤30 kg/m^2 and a minimum weight of 50 kg will be enrolled in the study.

Investigational product, dosage and mode of administration

100 mg AZD5069 (2 x 50 mg capsule) orally twice daily

Comparator, dosage and mode of administration

Placebo matching AZD5069 capsule

Duration of treatment

The study duration for each subject will be approximately 11 weeks, with a 28 day screening period, 6 days of treatment (Treatment Period 1), at least a 21-day washout period (including a 7-day follow-up), a second 6 days of treatment (Treatment Period 2) and a 7-day follow-up. All subjects will receive a total of 12 doses of AZD5069 and 12 doses of placebo.

Outcome variable(s):

- Neutrophil function

  Primary variable: Neutrophil function (phagocytosis and oxidative burst) in subjects on AZD5069 and placebo (change in status normal/not normal from baseline to steady state [Day 4] and 7 days after end of treatment.

- Pharmacodynamics

  Primary variable: circulating neutrophils during exercise challenge and during G-CSF challenge. Average neutrophil values over time will be calculated by individual baseline adjusted area under plasma concentration versus time curves, divided by time ($AUC_{(0-4)}$/4 and $AUC_{(0-36)}$/36). Maximum absolute neutrophil cell count (ANC_{max}) will be computed.

  In addition, baseline adjusted individual neutrophil measurements after morning and evening doses on Day 3 will be used to calculate area under the plasma

concentration time curve [$AUC_{(0-12)}$]. These AUCs will be normalised by dividing by time of measurement (ie, 12 hours). Minimum absolute neutrophil cell count (ANC_{min}) will be computed.

- Pharmacokinetics

  For AZD5069 after both morning and evening doses: $AUC_{(0-12),ss}$, maximum plasma concentration after multiple dose administration ($C_{ss,max}$), time to reach maximum plasma concentration ($t_{ss,max}$), minimum plasma concentration after multiple dose administration ($C_{ss,min}$), time to reach minimum plasma concentration ($t_{ss,min}$), plasma concentration 12 hours post-dose (C_{12}).

- Safety and tolerability variables

  – Adverse events (AEs)

  – 12-Lead electrocardiogram (ECG)

  – Physical examination - including signs of infection

  – Haematology, clinical chemistry and urinalysis

  – Neutrophil counts daily

  – Vital signs and body temperature

Statistical methods

All recorded circulating neutrophil count in blood will be listed by treatment group and visit. The absolute value, along with change and percentage change from baseline, will be summarised by treatment group and visit.

Analyses of circulating neutrophils *during exercise challenge*: Analysis will be performed by fitting a mixed effect linear model, using the logarithm of $AUC_{(0-4)}/4$ (and ANC_{max}) as the response variable. Estimates of the geometric means from the fitted model, together with corresponding 95% confidence interval (CI) (2-sided) will be presented. Also the ratio of the geometric means for AZD5069 versus placebo will be presented together with corresponding 95% CI (2-sided).

Analyses of circulating neutrophils *during G-CSF challenge*: Analysis will be performed by fitting a mixed effect linear model, using the logarithm of $AUC_{(0-36)}/36$ (and ANC_{max}) as the response variable. Estimates of the geometric means from the fitted model, together with corresponding 95% CI (2-sided) will be presented. Also the ratio of the geometric means for AZD5069 versus placebo will be presented together with corresponding 95% CI (2-sided).

For Day 3, plasma concentrations and pharmacokinetic parameters for AZD5069 including $C_{ss,max}$, $t_{ss,max}$, $C_{ss,min}$, $t_{ss,min}$, C_{12}, and $AUC_{(0-12),ss}$ after morning and evening doses will be

summarised using descriptive statistics. Pharmacokinetic parameters will be assessed and compared for morning and evening doses. Mean AZD5069 concentrations will be graphically presented.

For Day 3, absolute neutrophil counts (ANC) and derived pharmacodynamic (PD) parameters including $AUC_{(0-12)}$ and ANC_{min} after morning and evening doses will be summarised using descriptive statistics. Pharmacodynamic parameters will be assessed and compared for morning and evening doses. Mean neutrophil counts will be graphically presented.

The relationship between plasma concentrations and circulating neutrophils after the morning and evening doses on Day 3 will be investigated using appropriate graphical methodology. A population PK/PD model may be fitted to the data. The PD parameters $AUC_{(0-12)}$ and ANC_{min} values will be compared to the pharmacokinetic (PK) parameters $AUC_{(0-12),ss}$ and $C_{ss,max}$ values, respectively, for both morning and evening values. The relationships will be depicted graphically and a regression model may be fitted to the data.

Adverse events will be summarised by Preferred Term and System Organ Class using the Medical Dictionary for Regulatory Activities (current version applicable during the study) by dose/treatment group. Furthermore, listings of serious adverse events and adverse events that led to withdrawal will be made and the number of subjects who had any adverse events, serious adverse events, adverse events that led to withdrawal, and adverse events with severe intensity will be summarised.

Tabulations and listings of data for vital signs (blood pressure and pulse), clinical laboratory tests, electrocardiograms, and physical examination findings will be presented. Where applicable, data will be summarised for the absolute value at each scheduled assessment, and for the corresponding change from baseline. For clinical laboratory tests, listings of values for each subject will be presented with abnormal or out-of-range values flagged.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this Clinical Study Protocol.

Abbreviation or special term	Explanation
ADME	Absorption, distribution, metabolism and excretion
AE	Adverse event (see definition in Section 6.3.1)
ALP	Alkaline phophatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil counts
ANC_{max}	Maximum absolute neutrophil cell count
ANC_{min}	Minimum absolute neutrophil cell count
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
bid	Twice daily
BLQ	Below limit of quantification
BMI	Body mass index
C_{12}	Plasma concentration 12 hours post-dose
CD11b	Cluster of differentiation molecule 11B
CI	Confidence interval
C_{max}	Maximum plasma concentration
COPD	Chronic obstructive pulmonary disease
CRF	Case Report Form
CRP	C-reactive protein
CSA	Clinical Study Agreement
CSP	Clinical Study Protocol
CSR	Clinical Study Report
$C_{ss, max}$	Maximum concentration after multiple dosing
$C_{ss, min}$	Minimum concentration after multiple dosing
CXCL8	Chemokine ligand 8
CXCR2	Chemokine receptor 2
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid

Abbreviation or special term	Explanation
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
ELR	Glutamic acid-leucine-arginine positive
ENA	Epithelial cell-derived neutrophil activating protein
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
G-CSF	Granulocyte colony-stimulating factor
GCV%	Geometric mean coefficient of variation
GLP	Good Laboratory Practice
GMAD	Global multiple ascending dose
GRO-α	Growth-related oncogene-alpha
GSAD	Global single ascending dose
HBsAg	Hepatitis B surface antigen
HBsAg	Hepatitis C virus
HMR	Hammersmith Medicines Research Ltd
HPLC-MS/MS	High performance liquid chromatography tandem mass spectrometry
HRT	Hormone replacement therapy
hsCRP	High sensitive C-reactive protein
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IL	Interleukin
International Co-ordinating Investigator	If a study is conducted in several countries the International Co-ordinating Investigator is the Investigator co-ordinating the investigators and/or activities internationally.
IP	Investigational Product
LH	Luteinising hormone
LIMS	Laboratory Information Management System
LLOQ	Lower limit of quantification
LPS	Liposaccharide
LSLV	Last Subject Last Visit
MAD	Multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation or special term	Explanation
NA	Not Applicable
ND	Not Determined
OAE	Other Significant Adverse Event (see definition in Section 11.1.1)
PD	Pharmacodynamic(s)
PGx	Pharmacogenetic research
PI	Principal Investigator
PK	Pharmacokinetic(s)
PT	Preferred Term
qid	4 times daily
SAD	Single ascending dose
SAE	Serious adverse event (see definition in Section 6.3.2).
s.c.	Subcutaneous
SD	Standard deviation
SOC	System Organ Class
SOP	Standard Operation Procedures
SUSAR	Suspected unexpected adverse reaction
TB	Tuberculosis
t_{max}	Time to C_{max}
$t_{ss, max}$	Time to reach maximum plasma concentration, obtained directly from the observed concentration versus time data
$t_{ss, min}$	Time to reach minimum plasma concentration, obtained directly from the observed concentration versus time data
ULN	Upper Limit of Normal
WBC	White blood cell

1. INTRODUCTION

1.1 Background

AZD5069 is a small molecule chemokine receptor 2 (CXCR2) antagonist. CXCR2 is a chemokine receptor with a very restricted expression pattern in normal tissues, being largely confined to neutrophils and myeloid precursor cells. CXCR2 binds with high affinity to the CXC ligand 8 (CXCL8) and to the CXC group of glutamic acid-leucine-arginine positive (ELR) chemokines that includes CXC ligand 1, epithelial cell-derived neutrophil activating protein (ENA)-78 and neutrophil activating peptide. CXCL8, but not the ELR chemokines, also interacts with the CXC chemokine receptor 1, although with a lower affinity compared with the CXCL8-CXCR2 interaction.

A CXCR2 antagonist previously studied by AstraZeneca, AZD8309, has been shown to inhibit growth-related oncogene-alpha (GRO-α), induced the cluster of differentiation molecule 11B (CD11b) expression on human neutrophils and to reduce the number of peripheral blood neutrophils when administered orally to healthy subjects. Oral administration of AZD8309 also reduced the neutrophil counts in induced sputum following lipopolysaccharide (LPS) challenge to healthy subjects. However, circulating neutrophils increased to the same levels in those individuals taking AZD8309 as in subjects on placebo.

There are a number of CXCR2 receptor antagonists currently in development by other companies. As far as is known the most advanced of these is SCH527123, currently in Phase II clinical studies. Single and multiple ascending dose (SAD, MAD) studies in healthy subjects have shown this compound to have an acceptable safety profile (Khalilieh et al, 2007a, Khalilieh et al, 2007b). A dose-related decline in circulating neutrophils was seen, which could be reversed by administration of granulocyte colony-stimulating factor (G-CSF) (Khalilieh et al, 2007a). No evidence of a cumulative effect was seen with multiple doses (Khalilieh et al, 2007b). SCH527123 inhibited ozone-induced airway neutrophilia in a double-blind controlled study (Holz et al, 2010). The compound had no effect on acquired immunity to hepatitis A vaccine (Khalilieh et al, 2007c).

1.2 Summary of relevant pre-clinical/ clinical information to date

AZD5069 is a potent, reversible antagonist to human CXCR2 and a potent inhibitor of CXCR2-mediated calcium mobilisation, adhesion molecule expression and chemotaxis in human neutrophils *in vitro*. Thus, AZD5069 is predicted to work by inhibiting CXCR2, thus reducing neutrophil migration from both the bone marrow and the systemic circulation into other compartments, eg, lung and mucosal compartments. Thus, along with reducing mucosal infiltration in the lungs, AZD5069 has the potential to reduce systemic neutrophil counts, which is what has been observed in the Clinical Pharmacology studies in healthy volunteers to date. Reductions in blood neutrophil counts and the *ex vivo* GRO-α-stimulated CD11b expression on neutrophils in whole blood appear to be related to plasma drug concentrations of AZD5069.

To date, 6 studies with AZD5069 (D3550C00001 [global single ascending dose (GSAD) in non-smokers or ex-smokers], D3550C00007 [global multiple ascending dose (GMAD) in current smokers], D3550C00010 [food effect and elderly pharmacokinetics (PK)] in smokers and non-smokers, D3550C00005, a combined SAD/MAD study in Japanese healthy volunteers, D3550C00002 a safety and tolerability study in patients with chronic obstructive pulmonary disease [COPD]) and D3550C00013, an absorption, distribution, metabolism and excretion (ADME) study have been completed. Additionally, drug administration is ongoing in a patient study, D3550C00014, an efficacy study in patients with bronchiectasis.

During the course of the completed studies, a total of 142 healthy volunteers have been exposed to AZD5069. Five healthy volunteers in the GSAD Study D3550C00001 received a maximum single oral dose of 200 mg AZD5069 and 6 healthy volunteers in the Japanese SAD (JSAD)/MAD study D3550C00005 received a maximum single oral dose of 120 mg. Twenty-five healthy volunteers have been exposed to multiple oral doses in the GMAD Study D3550C00007 (maximum daily dose 100 mg twice daily for 6 days, plus single doses on Days 1 and 8) and 36 healthy Japanese volunteers were exposed to multiple oral doses up to 80 mg bid in the JSAD/MAD study D3550C00005. The maximum doses used in the GSAD and GMAD studies were restricted by pre-defined exposure internal limits and not by emerging safety and tolerability data.

AZD5069 generally displayed dose-proportional, time independent PK, following both single dose and at steady-state, over the dose range tested. Following bid administration, steady-state kinetics appeared to be attained within 2 days of drug administration (ie, 3 to 4 doses). This is consistent with the mean terminal elimination half-life ($t_{½}$) of approximately 11 hours seen over the 17.99 to 200 mg dose range, which started at approximately 12 to 24 hours post-dose. AZD5069 was absorbed relatively quickly; peak plasma concentrations (C_{max}) were observed from 1 to 2 hours post-dose (time to C_{max} [t_{max}], median), under fasting conditions.

In the 142 healthy volunteers exposed to AZD5069 there were reductions in circulating neutrophils at doses of 5.45 mg and above, as expected from previous experience with CXCR2 antagonists. These reductions were rapid in onset (notable by 8 hours post-dose) and also recovered quickly, with all volunteers in the GSAD and GMAD studies having neutrophils within the laboratory reference range by 96 hours after stopping treatment. There were no other adverse effects observed that are considered to be related to the treatment. After analysis of the safety data from completed Phase I studies, the only topic included in the Emerging Safety Profile section of the Investigator's Brochure will be decreases in circulating neutrophils.

In the safety and tolerability study in COPD patients (D3550C00002) 58 subjects received AZD5069, 80 mg (n=28) and 50 mg (n=30) twice daily, for up to 4 weeks. As with the healthy subjects, mean decreases in circulating neutrophils were seen with 4 subjects being withdrawn as a result (due to predefined stopping criteria on blood neutrophils $<1 \times 10^9$/L observed at least twice during 48 hours). There was no evidence of any increase in infections that might have been related to the neutrophil drops.

No serious adverse events (SAEs) have been reported in the completed Phase I studies. The most frequently reported adverse event (AE) in these studies was headache. The other AEs observed in healthy volunteer studies were all minor and similar to those commonly seen in studies of this type. There were 2 SAEs reported in the COPD study, 1 COPD exacerbation (80 mg AZD5069) and 1 atrial fibrillation of mild intensity (50 mg AZD5069). There were no deaths reported in any of the studies.

An in vitro study on human bone marrow progenitor cells has shown no general cytotoxicity in concentrations up to 200 μM and no myeloid cytotoxic effects in concentrations up to 100 μM.

AZD5069 has been found to have an acceptable safety profile based on the non-clinical and clinical findings and there are no findings that would preclude further investigations in humans.

The sponsor will immediately notify the Principal Investigator of important safety data (eg, toxicology; absorption, distribution, metabolism and excretion; teratology) that becomes available during the study.

1.3 Research hypothesis

AZD5069 has been shown to cause a decrease in circulating neutrophil numbers. The purpose of this exploratory study is to understand the mechanism that leads to this reduction and to measure whether, on treatment, the neutrophil remains capable of normal phagocytosis and respiratory bursts as well as entering the peripheral circulation in response to different danger stimuli, including infectious stimuli.

It is suggested that if neutrophil function and stimulus-induced bone marrow release are both normal after AZD5069 administration, the restrictions based on circulating neutrophil numbers can be significantly reduced.

AZD5069 is predicted to work by inhibiting CXCR2, thus reducing neutrophil migration from both the bone marrow and the systemic circulation into other compartments, eg, lungs and mucosal compartments. Along with reducing mucosal infiltration in the lungs, AZD5069 has the potential to reduce systemic neutrophil counts, which is what has been observed in the Clinical Pharmacology studies in healthy volunteers to date.

1.4 Rationale for conducting this study

AZD5069, a CXCR2 antagonist, is being developed for the treatment of asthma, COPD and potentially other diseases that have a significant neutrophilic inflammatory component. As part of its mechanism of action, it has been shown to induce a rapid reduction in circulating neutrophils. In humans the risk of infection is related to the severity of reduction in the neutrophils, particularly when neutrophil numbers decrease below 1 x 10^9/L and the neutropenia has arisen from disorders of production of precursors in the bone marrow (Lichtman et al, 2006).

The rationale underpinning this study is to demonstrate that although AZD5069 has an effect on the number of neutrophils in circulation there is no effect on the neutrophil function (assessed as neutrophil phagocytosis and oxidative burst) and also to demonstrate the effect of AZD5069 on the release of neutrophils from the bone marrow and the marginal pool after subcutaneous G-CSF (recruitment mainly from the bone marrow) and an exercise challenge (recruitment from the marginal pool). As such, the drug-induced fall in peripheral neutrophil count is not associated with the subject's susceptibility to infection and if neutrophil function is maintained and neutrophils can be recruited to the circulation after stimulus, it is judged that the frequent monitoring of peripheral neutrophil numbers in subjects administered CXCR2 antagonists can be significantly reduced.

1.5 Benefit/risk and ethical assessment

Safety Considerations

It is not expected that subjects will derive any clinical benefit from treatment with AZD5069. Potential risks have been identified through review of the clinical studies so far conducted, as well as review of non-clinical animal studies with AZD5069 and of the literature and unpublished information relating to other CXCR2 antagonists. Risks to subjects will be minimised by incorporating relevant exposure margins to animal toxicology findings and by regular monitoring.

The most relevant findings observed in the non-clinical studies with AZD5069 with potential relevance to humans were changes in the level of white blood cells (WBC). In addition, dose-related increases in globulin and C reactive protein (CRP) were noted with increases in CRP being generally highest on Day 2. Increases in both parameters were observed from 24 hours after the first dose. Following the cessation of investigational product administration, both parameters returned to baseline levels during the recovery period.

The major effect seen to date in the GSAD (D3550C00001) and GMAD (D3550C00007) studies has been a reduction in circulating neutrophils. This was dose-related in the GSAD and GMAD studies, with more subjects having reductions and for longer periods at higher doses. A reduction in neutrophils is an expected effect of a CXCR2 antagonist and was also seen with AZD8309 (see AZD8309 Investigator's Brochure [IB]) and with SCH527123 (Khalilieh et al, 2007a, Khalilieh et al, 2007b, Khalilieh et al, 2007c). In all clinical studies with multiple dosing of AZD5069 there has been predefined stopping criteria based on reduction in neutrophils below the specified limit of 1.0×10^9/L lasting for longer than 48 hours. One subject, administered AZD5069 100 mg bid, was withdrawn from the GMAD (D3550C00007) study. In the JSAD/JMAD (D3550C00005) 10 subjects were withdrawn due to reduction in neutrophils and in the COPD study (D3550C00002) 4 subjects were withdrawn. All subjects have had normal neutrophils within 96 hours after the last administration of investigational product and most subjects within 48 hours.

Reductions in neutrophil count may be associated with an increased risk of infections, particularly soft tissue infections. Two subjects in the GSAD study reported acne but no subject in the GMAD and there was no infectious signal in the completed studies in AZD5069

or AZD8309. Subjects will be regularly monitored by the investigators, including body temperature measurements.

In a rat study, increased urinary excretion of sodium and sodium clearance was reported, but did not affect the plasma sodium concentration. In the 1-month rat Good Laboratory Practice (GLP) study proximal tubule cytoplasmic crystals were seen in male rats at high doses only. These changes were resolving during the recovery period. There were no changes in renal function or electrolytes in the healthy volunteer studies. Kidney function will be monitored during the study.

Histopathological changes have been noted in the livers of some dogs treated with multiple doses of 25/20 mg/kg bid and above, related to neutrophil infiltration with foci of inflammatory cells and small granulomata, together with increases in alkaline phosphatase (ALP). Increases in liver weight were seen in rats receiving doses of 100 mg/kg and 350 mg/kg bid for 1 month. These changes had resolved following the 4-week recovery period. No effects on hepatic biochemistry were observed in healthy volunteer studies except in one of the doses in the MAD study, where changes in aspartate aminotransferase (AST) were seen also in subjects receiving placebo and therefore explained as laboratory artifacts. Liver function will be monitored during the study.

For a more detailed risk benefit assessment of developing a CXCR2 inhibitor for COPD, see the AZD5069 IB.

With the exception of a drop in circulating neutrophils, AZD5069 has not been consistently associated with any safety signals in the Phase I and Phase II studies conducted so far and 100 mg bid during 6 days was well tolerated in the MAD study. Low neutrophils during a few days have not increased the risk of infection in the studies conducted so far and although the risks to the study subjects are considered to be minimal, the following risk mitigation activities will be put in place.

- Exclusion criteria to exclude any subject with increased risk of infection
- Close monitoring of circulating neutrophils and high sensitive CRP (hsCRP)
- Close monitoring of any signs of infection
- Regular monitoring of renal and hepatic function

G-CSF is used to recruit neutrophils from the bone marrow (Thomas et al, 2002). G-CSF is a glycoprotein, growth factor and cytokine produced by a number of different tissues to stimulate the bone marrow to produce granulocytes and stem cells. G-CSF then stimulates the bone marrow to release the mature granulocytes into the circulation. A recombinant form of G-CSF is used with certain cancer patients to accelerate recovery allowing higher-intensity treatment regimens. G-CSF is also used to increase the number of hematopoetic stem cells in the blood of a donor before collection for use in hematopoietic stem cell transplantation. A recombinant form of G-CSF was used with certain cancer patients to accelerate recovery

allowing higher-intensity treatment regimens. It may also be administered to the receiver, to compensate for conditioning regimens. G-CSF is widely used and considered safe and well tolerated when administered as a single dose. The most common side effects occurring after multiple dosing of G-CSF sc (very rare after single dosing), are pain (bone pain), blood test abnormalities (temporary elevation in lactate dehydrogenase, and ALP which are routinely checked during the study) and tenderness at the site of the injection. These will return to normal once treatment is discontinued.

Monitoring

Circulating neutrophil counts will be monitored after investigational product administration and daily during administration of AZD5069. If circulating neutrophils drop $<1 \times 10^9$/L, new tests will be run approximately every 12 h until neutrophils are within the reference range, but the investigational product administration will be continued.

If the circulating neutrophil count has been continuously low ($<0.5 \times 10^9$/L) for over 48 hours when the sample is taken on Day 5, 12 h after G-CSF (including after exercise challenge and G-CSF), the investigational product will be discontinued if no increase in the neutrophil count was seen after G-CSF.

Circulating neutrophil counts will be analysed at a central laboratory and not reported to the Investigator in order to maintain the blind. A physician at the clinical site, not engaged in the study will monitor the circulating neutrophil counts and notify the Investigator when discontinuation criteria are fulfilled based on neutrophil counts. Subjects will also be regularly monitored for the presence of new infections, and additional data will be collected for any AEs relating to infection. High sensitive CRP will be regularly monitored and there are discontinuation criteria based on signs of infections in place.

The most important risk to subjects in the planned study is expected to be that relating to reductions in circulating neutrophil count, and hence a potential adverse effect upon the host defence. Neutrophil numbers can be monitored, and based on the healthy volunteer data with AZD5069 and experience with other CXCR2 antagonists, changes in neutrophil numbers are quickly reversible. This quick reversibility suggests a shift in neutrophil compartments rather than a toxic effect on the bone marrow. After treatment with cancer chemo-therapeutic agents that cause severe neutropenia in patients due to bone marrow toxicity, neutrophil numbers take 20 to 30 days to recover. Safety monitoring of neutrophil counts will be in place throughout studies with AZD5069.

The other AEs seen in the healthy volunteer studies were all minor and similar to those commonly seen in studies of this type. The adverse effects seen in nonclinical studies occurred at high doses and are considered unlikely to pose a risk to subjects. Given the available data and the planned study designs, the risks to subjects are considered to be small.

2. STUDY OBJECTIVES

This is an explorative study and all objectives are therefore exploratory but the main objectives are:

2.1 Primary objective

- To investigate the effect of AZD5069 on neutrophil number and function (phagocytosis and oxidative burst) in the circulation of healthy volunteers
- To investigate the effect of AZD5069 and placebo on circulating neutrophil numbers following a burst of strenuous exercise
- To investigate the effect of AZD5069 and placebo on circulating neutrophils following subcutaneous injection of G-CSF

2.2 Secondary objectives

- To evaluate general safety and tolerability of AZD5069
- To evaluate the steady state 24 hour pharmacokinetic (PK) profile of the AZD5069 capsule following bid dosing
- To evaluate the steady state 24 hour profile of circulating neutrophils following bid dosing of AZD5069 and its relationship to plasma concentration

2.3 Exploratory objectives

- To assess which subpopulation of neutrophils is reduced on AZD5069
- To collect and store DNA samples for possible retrospective exploratory genetic analysis, investigating the influence of genotype on pharmacodynamic and pharmacokinetic responses, safety and tolerability of AZD5069, and associated biomarkers, where appropriate
- To explore potential up-regulation of biomarkers, ie, interleukin (IL)-8, G-CSF, GRO-α and ENA-78

Results from exploratory analyses, if performed, may be reported separately from the Clinical Study Report (CSR).

3. STUDY PLAN AND PROCEDURES

This Clinical Study Protocol (CSP) has been subject to a peer review according to AstraZeneca standard procedures.

3.1 Overall study design and flow chart

This will be an exploratory, single centre, randomised, double-blind, placebo-controlled, cross-over study in healthy subjects. The study will consist of approximately 30 randomised healthy adult subjects, with at least 28 subjects completing the two treatment periods. Non-smoking Caucasian male and post-menopausal/surgical sterile female subjects aged 18 to 45 years, inclusive, will be enrolled in the study.

All potentially suitable subjects will attend the study centre for an information visit where the study will be explained and informed consent collected (Visit 1) before starting study specific screening (Visit 2). Visits 1 and 2 can occur on the same day as per the study centre requirements. Screening will be conducted within 28 days of randomisation (Visit 3). After screening, subjects who fulfil all the inclusion criteria and none of the exclusion criteria will be randomised to one of 2 treatment sequences:

- Treatment Sequence 1: AB
- Treatment Sequence 2: BA
  - Treatment A: 100 mg AZD5069 bid
  - Treatment B: matching placebo bid

The time of administration of the investigational product is very important due to the diurnal variation in neutrophils. Investigational product administration should be at the time points stated in the Study plan (Table 2) ±30 minutes. Each subject should receive the investigational product at the same time point on each occasion with a maximum difference of 15 minutes. Investigational product will be administered orally, bid, for 6 days during Treatment Period 1.

After Treatment Period 1 there will be a washout period for a minimum of 21 days whereafter the subjects will be admitted for another 6 days of treatment (Treatment Period 2).

At three time points during each treatment period (baseline and steady state, [Day 4], and 7 days after end of treatment) fresh neutrophils will be collected to run functional assays with regards to phagocytosis and oxidative burst. At these time points there will also be samples collected for flow cytometry to evaluate what subpopulation of neutrophils is affected. Blood samples for explorative biomarkers (G-CSF, GRO-α, ENA-78 and IL-8) will also be taken at these times.

The subjects will stay in the study centre during the whole treatment period and circulating neutrophils will be assessed 12 h after the last administration of investigational product, on Day 7, in the morning before the subjects are discharged.

An additional follow-up visit will take place 7 days after the last day of investigational product administration, when neutrophil function and flow cytometry will be assessed at the same time of the day as the other samples were collected. Neutrophil function will be

assessed 7 days after the end of treatment to determine the effect of AZD5069 on neutrophils under maturation in the bone marrow during the treatment period.

During the study the following will take place during both treatment periods:

- Subjects will be checked for signs and symptoms of infection on a daily basis (including monitoring of vital signs and body temperature)

- On Day 1 to Day 4 blood samples for circulating neutrophil count and PK sampling will be performed pre-dose in the morning and in the evening. On Day 3 (after dose 4) the circulating neutrophil profile over 24 h and a corresponding PK profile to define the steady state kinetics in each subject will be determined.

- On Day 4 there will be an exercise challenge where the subject will perform a standardised 10-minute sub-maximal exercise test on an exercise bicycle to approximately 80% of the subject's maximum ventricular rate according to their age. Blood samples will be collected before and after the exercise challenge to assess the effect on the circulating neutrophil count and leukocyte differential counts. Prior to the morning dose (AZD5069 steady-state) blood samples will also be taken for neutrophil function, flow cytometry and explorative biomarkers (G-CSF, GRO-α, ENA-78, IL-8).

- Day 5 after the morning dose the subjects will be administered 300 µg G-CSF subcutaneously. Blood samples for circulating neutrophil count and leukocyte differential counts will be collected before and after the G-CSF injection. Circulating neutrophils will also be collected prior to each dose as on Days 1 to 4.

- Thereafter the investigational product will be discontinued with the last administration on Day 6 in the evening. Circulating neutrophils will be collected prior to each dose as on Days 1 to 4. Blood samples for circulating neutrophil counts will also be collected in the morning on Day 7 before discharge (at an equivalent time to the pre morning dose circulating neutrophil blood sample taken on Days 1 to 6). The subjects will return 7 days (in the morning) after the last administration of the investigational product for clinical laboratory safety tests (including leukocyte differential and circulating neutrophil count), blood samples for neutrophil function, flow cytometry and explorative markers (G-CSF, GRO-α, ENA-78 and IL-8).

- Blood samples for circulating neutrophil counts will be collected at the same time of the day in order to control for the diurnal variation in the neutrophil count

- Subjects will return to the study centre 1 week after the last administration of the investigational product in Treatment Period 2 for a follow-up visit (Visit 6). This follow-up visit will also have to take place at the same time of the day in the morning. Blood samples will be collected for haematology (including neutrophil

count and leukocyte differential counts), neutrophil function, flow cytometry and explorative markers (G-CSF, GRO-α, ENA-78 and IL-8).

- If a subject experiences an acute infection (as judged by the subject or study centre) they may need to have circulating neutrophils assessed more frequently than stated in the CSP ie, every 12 hours based on the discretion of the investigator

Restrictions as stipulated in Section 5.1 will be applicable for the duration of the study until follow-up (Visit 6). For all dosing days, subjects should eat a light meal, which should be finished 1 h prior to the scheduled dose, and then another meal 2 h after taking the dose. No food should be consumed between these meals.

A summary of the procedures performed at each visit is given in the Visit schedule (Table 1). Details of the timing of visits and assessments is given in the Study plan (Table 2).

Table 1 Visit schedule

Visit No	Type of Visit	Time window
1[a]	Enrolment	Consent must be given prior to any study-specific assessments and time allowed after consent according to local requirements.
2[a]	Screening	Screening will be performed within 28 days of randomisation (Visit 3). At screening, blood samples, (including neutrophil functional assays and flow cytometry) will be performed in the morning.
3 Residential in the study centre	Treatment Period 1	Day-1: Confirmation of eligibility, clinical safety laboratory tests (haematology, clinical chemistry, urinalysis and urine pregnancy tests for female subjects) electrocardiogram (ECG), physical examination and body weight. The blood sample for assessment of circulating neutrophils will be taken at an equivalent time to the pre morning dose sample taken on Days 1 to 6. Randomisation on Day 1. Baseline neutrophil function, flow cytometry and explorative biomarkers prior to first dose. Pre-dose serum hsCRP, vital signs and body temperature. Circulating neutrophil count and PK pre-dose morning and evening. Start of treatment (twice daily dosing). Day 2, circulating neutrophil count and PK pre-dose morning and evening. Brief physical examination and monitoring for signs of infection (vital signs, body temperature) prior to morning dose.

Visit No	Type of Visit	Time window
		Day 3: 24 h circulating neutrophil profile and PK profile. Brief physical examination, vital signs, body temperature, clinical laboratory safety tests prior to morning dose.
	Exercise challenge	Day 4 (AZD5069 steady-state): neutrophil function, flow cytometry and explorative biomarkers (G-CSF, GRO-α, ENA-78, IL-8) prior to morning dose. Brief physical examination, serum hsCRP, vital signs and body temperature prior to morning dose. Circulating neutrophil count and PK pre-dose morning and evening. Exercise challenge. Neutrophil count and leukocyte differential counts pre and post exercise challenge.
	Subcutaneous G-CSF challenge	Day 5: brief physical examination, vital signs and body temperature, clinical laboratory safety tests prior to morning dose. Circulating neutrophil count prior to morning and evening dose. Subcutaneous G-CSF challenge, blood samples pre- and post G-CSF challenge for neutrophil count and leukocyte differential counts.
		Day 6: brief physical examination, vital signs, body temperature, circulating neutrophil count prior to the morning and evening dose and the continuing post G-CSF challenge circulating neutrophil count and leukocyte differential counts (up to 36 h post challenge). Last dose administered in the evening of Day 6.
		Day 7: full physical examination, vital signs, body temperature, body weight, ECG, clinical laboratory safety tests (including circulating neutrophil count) and discharge. The blood sample for assessment of circulating neutrophils will be taken at an equivalent time to the pre morning dose sample taken on Days 1 to 6.
		Wash out of at least 21 days.
4	Follow-up	7 days after end of Treatment Period 1. Brief physical examination, vital signs, body temperature, clinical laboratory safety tests (including circulating neutrophil count), neutrophil function, flow cytometry and explorative biomarkers. The blood sample for assessment of circulating neutrophils will be taken at an equivalent time to the pre morning dose sample taken on Days 1 to 6.

Visit No	Type of Visit	Time window
5 Residential in the study centre	Treatment Period 2	See Treatment Period 1.
6	Follow-up	7 days after end of Treatment Period 2. Brief physical examination, vital signs, body temperature, body weight, ECG, clinical laboratory safety tests (including circulating neutrophil count), neutrophil function, flow cytometry and explorative biomarkers. The blood sample for assessment of circulating neutrophils will be taken at an equivalent time to the pre morning dose sample taken on Days 1 to 6.

a Visits 1 and 2 can occur on the same day as per the study centre requirements.

Table 2 Study Plan

Visit	**1**	**2**	**3 (and 5)**								**4**	**5**	**6**
Window		**-1 to -28 ≤Visit 3**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**7 days after EOT**	**≥21 days after EOT Treatment Period 1**	**7 days after EOT Treatment Period 2**
Visit type	**Enrolment[a]**	**Screening[a]**	**Admission**	**Start of treat-ment**		**24 h neutrophil and PK profile**	**Exercise challenge[r] Pre and post blood samples**	**G-CSF[s]**		**EOT**	**Follow-up**	**Treatment Period 2 - look at Visit 3**	**Follow-up**
Informed consent	X												
Demographics		X											
Medical and surgical history		X											
Respiratory problems history		X											
Smoking history		X											
Urine drug, alcohol breath and Smokerlyser screening		X	X										
Height		X											
Weight		X	X							X		X	X
Concomitant medication		X	X	X	X	X	X	X	X	X	X	X	X
Inclusion/ Exclusion criteria		X[b]	X[b]										
Physical examination[n]		X	X		X[c]	X[c]	X[c]	X[c]	X[c]	X	X[c]	X	X[c]
Vital signs[n]		X		X	X	X	X	X	X	X	X	X	X
Body temperature[n]		X		X	X	X	X	X	X	X	X	X	X

Visit	**1**	**2**	**3 (and 5)**								**4**	**5**	**6**
Window		**-1 to -28 ≤Visit 3**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**7 days after EOT**	**≥21 days after EOT Treatment Period 1**	**7 days after EOT Treatment Period 2**
Visit type	**Enrolment[a]**	**Screening[a]**	**Admission**	**Start of treat-ment**		**24 h neutrophil and PK profile**	**Exercise challenge[r] Pre and post blood samples**	**G-CSF[s]**		**EOT**	**Follow-up**	**Treatment Period 2 - look at Visit 3**	**Follow-up**
Clinical chemistry[n]		X	X	X[o]		X	X[o]	X		X	X	X	X
Urinalysis[d,n]		X	X			X		X		X	X	X	X
Haematology[e,n]:													
Full panel		X	X			X		X		X	X	X	X
Leukocyte differential counts		X	X			X	X[l]	X[m]	X[m]	X	X	X	X
Circulating neutrophil count		X	X[h]	X[h]	X[h]	X[k]	X[h,l]	X[m]	X[m]	X[h]	X[h]	X	X[h]
Endocrinology (LH and FSH, blood)		X[g]											
HBsAg, HCV, and HIV serology		X											
TB screening blood sample		X											
Urine pregnancy test		X[g]	X[g]									X[g]	X[g]
12-Lead resting ECG		X	X							X		X	X
Randomisation				X									
PK blood sampling				X[i]	X[i]	X[j]	X[i]					X	

Visit	**1**	**2**	**3 (and 5)**								**4**	**5**	**6**
Window		**-1 to -28 ≤Visit 3**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**7 days after EOT**	**≥21 days after EOT Treatment Period 1**	**7 days after EOT Treatment Period 2**
Visit type	**Enrolment[a]**	**Screening[a]**	**Admission**	**Start of treat-ment**		**24 h neutrophil and PK profile**	**Exercise challenge[r] Pre and post blood samples**	**G-CSF[s]**		**EOT**	**Follow-up**	**Treatment Period 2 - look at Visit 3**	**Follow-up**
Neutrophil function assays and flow cytometry[n]		X		X			X				X	X	X
Administer dose of Investigational product at study centre at 8am and 8pm[f]				X	X	X	X	X	X			X	
Explorative biomarkers[n,p]) blood sampling (serum)				X			X				X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X
PGx blood sample				X[q]									

a Informed consent must be obtained prior to any study-specific procedures, restrictions or screening assessments. Adequate time must be given between consent and screening according to local requirements. Visit 1 (Enrolment) and Visit 2 (Screening) can occur on the same day as per the study centre requirements

b Inclusion and exclusion criteria to be assessed at applicable visits, Screening (Visit 2) and Day -1 (Visit 3); eligibility will be confirmed at readmission to Period 2 (Visit 5)

c Brief physical examination as described in Section 6.3.6

d The following will be assessed as part of the urinalysis dipstick: glucose, protein, blood. In addition, samples will be sent to the central laboratory for urine microscopy and quantitative measurements of total protein, albumin and creatinine if the local dipstick test for protein and/or blood is positive or if the urine sample appears abnormal on macroscopic examination, eg, if it is cloudy

e The full haematology panel at the central laboratory will include leukocyte differential (absolute count and percentage), red blood cell count, haemoglobin, reticulocytes and platelets. Circulating neutrophil count will be measured every day during the treatment period. Central laboratory leukocyte and neutrophil measurements will only be available at the end of the study so as not to compromise the blind

f Time of investigational product administration (Days 1 to 6) 8 am and 8 pm (±30 minutes and maximum 15 min difference in time of administration of the investigational product within subjects for each day and treatment period)

g All female subjects

h Circulating neutrophil count will be performed at the same time of the day (pre-dose at 8 am and 8 pm as appropriate); on Day -1, Day 7 and Visits 4 and 6 this will be at an equivalent time to the pre morning dose sample taken on Days 1 to 6 (8 am).

i PK samples will be taken pre-dose morning and evening together with blood samples for neutrophils on Day 1 to Day 4

j PK profile on Day 3 with sampling pre-dose and at 1, 2, 3, 5, 8, 12 hours after the respective morning and evening doses. Note that the 12 h post morning dose sample will be the evening pre-dose sample and that the 12 h post evening dose sample will be the pre morning dose sample collected on Day 4

k Circulating neutrophil count for a 24 h profile, pre-dose and 1, 2, 3, 5, 8, 12 hours after the respective morning and evening doses. Note that the 12 h post morning dose sample will be the evening pre-dose sample and that the 12 h post evening dose sample will be the pre morning dose sample collected on Day 4

l Circulating neutrophil count and leukocyte differential counts: pre challenge (≤30 minutes prior to the challenge) and 10 min (directly after exercise challenge completion), 2 and 4 hours post exercise challenge; an additional manual neutrophil count will be performed using the blood sample collected pre-challenge and at challenge completion (10 minutes). Circulating neutrophils will also be collected prior to each dose as described in footnote h

m Circulating neutrophil count and leukocyte differential count: pre challenge (≤ 30 minutes prior to the challenge), 2, 4, 6, 12 (Day 5), 24, 36 hours (Day 6) post subcutaneous G-CSF challenge; an additional manual neutrophil count will be performed using the blood sample collected pre-challenge and at 12 hours post-challenge. Circulating neutrophils will also be collected prior to each dose as described in footnote h

n Unless otherwise stated assessment to be performed prior to the morning dose, or at equivalent time on non-dosing days

o Only hsCRP

p G-CSF, GRO-α, ENA-78, IL-8

q Optional blood sample for retrospective pharmacogenetic exploratory analysis to be taken on Day 1 (Visit 3) or any subsequent visit after randomisation providing the subject has provided separate informed consent for the pharmacogenetic part of the study

r Exercise challenge: to be performed as soon as possible after the morning dose and no later than 4 hours after the morning dose. The exercise challenge must be performed at the same time point in both treatment periods. Pre-challenge circulating neutrophil count must be taken ≤30 minutes prior to the start of the challenge.

s G-CSF challenge: to be performed within 30 minutes after the morning dose. Pre-challenge circulating neutrophil count must be taken ≤30 minutes prior to the challenge.

3.2 Rationale for study design, doses and control groups

The study will be randomised, placebo-controlled and double-blind to ensure a robust design and minimise bias which could compromise the conduct of the study and/or interpretation of the results. Randomisation should minimise selection bias ensuring a similar mix of subjects in each group balanced for both known and unknown prognostic factors.

The study includes placebo in parallel with active investigational product, in order to make it possible to identify effects that are likely drug-related and not related to the study situation.

A two-way cross-over design is chosen due to the inter-individual variation in neutrophil levels and diurnal variability and the variability in response to the different challenges performed.

Five doses of AZD5069 are considered sufficient for the drug to reach steady state based on the GMAD study. A 24 h PK profile is done at steady state before the different stimuli. Exercise challenge and G-CSF should not be done on the same day.

Approximately 30 non-smoking, healthy Caucasian subjects, male and/or post-menopausal/surgically sterile female subjects aged 18 to 45 years inclusive will be randomised into the study. The aim is to evaluate the effect of AZD5069 on the number and function of neutrophils in the peripheral circulation. Including only Caucasians will minimise the risk of including subjects with ethnic differences in neutrophil kinetics. A maximal exercise test and challenge with G-CSF to recruit neutrophils into the circulating pool will be performed, and to minimise the potential symptoms for the subjects, healthy volunteers will be chosen.

This study will comprise of 2 treatment sequences. Each treatment sequence will consist of AZD5069 and placebo. Approximately 30 subjects will be randomised to one of the treatment sequences. The high dose (100 mg bid) of AZD5069, will be administered in this study. This dose of AZD5069 has been used in MAD studies with continuous treatment for 7 days prior to this study without any safety concerns, apart from a drop in the circulating neutrophil count. The dose is chosen to evaluate the effects on neutrophils on a dose that is higher than the predicted lowest efficacious dose to man.

4. SUBJECT SELECTION CRITERIA

Investigator(s) should keep a record, the subject screening log, of subjects who entered pre-study screening.

Each subject should meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be exceptions to this rule.

4.1 Inclusion criteria

For inclusion in the study subjects should fulfil the following criteria:

1. Provision of informed consent prior to any study-specific procedures

2. Healthy Caucasian male or post-menopausal/surgical sterile females, aged 18 to 45 years inclusive at screening (Visit 2)

3. Only women of non-childbearing potential are included in the study, ie, women who are permanently or surgically sterilised or post-menopausal:

   Women will be considered post-menopausal if they are amenorrheic for 12 months without an alternative medical cause. The following requirements apply:

   Women will be considered post-menopausal if they have been amenorrhoeic for 12 months or more following cessation of exogenous hormonal treatments and with luteinising hormone (LH) and follicle-stimulating hormone (FSH) levels in the post-menopausal range.

   Permanent sterilisation includes hysterectomy and/or bilateral oopherectomy and/or bilateral salpingectomy, but excludes bilateral tubal occlusion.

4. Male subjects must use condoms and spermicide from the time of investigational product administration until 3 months after the follow-up visit, both to prevent pregnancy and to protect partners (and the foetus, if the partner is pregnant) from potential exposure to investigational product. Male subjects should inform the medical staff at the study centre if their partner becomes pregnant during the study. In addition to the use of condoms, female partners of male subjects should use additional contraception from the time of investigational product administration until 3 months after the follow-up visit (Visit 6). Acceptable methods to be used by female partners include the oral contraceptive pill, hormone implants, intra-uterine devices (IUDs) or diaphragms with spermicide. Male subjects must not donate sperm for 3 months after the follow-up visit (Visit 6)

5. Non-smokers or ex-smokers with no smoking history the last 12 months prior to Visit 1 and a smoking history of less than 10 pack years (1 pack year = tobacco consumption corresponding to 20 cigarettes smoked per day for 1 year) at screening (Visit 2)

6. Have a normal physical examination, laboratory values, 12-lead electrocardiogram (ECG) and vital signs (blood pressure and pulse rate), unless the Investigator considers an abnormality to be clinically irrelevant

7. Body mass index (BMI) >18 kg/m^2 and ≤30 kg/m^2 and a minimum weight of 50 kg

8. Provision of signed and dated written informed consent for the optional genetic research. If a subject declines to participate in the genetic component of the study, there will be no penalty or loss of benefit to the subject. The subject will not be

excluded from other aspects of the study described in this Clinical Study Protocol (CSP)

4.2 Exclusion criteria

Subjects should not enter the study if any of the following exclusion criteria are fulfilled:

1. A history or presence of conditions known to interfere with the absorption, distribution, metabolism or excretion of drugs eg, haematological, gastrointestinal, hepatic or renal disease etc.

2. A definite or suspected personal or family history of intolerance or hypersensitivity to drugs and/or their recipients, judged to be clinically relevant by the Investigator

3. Surgery or significant trauma within 3 months of Visit 1.

4. Symptoms, signs or laboratory findings suggestive of an ongoing infective illness as judged by the Investigator at the time of enrolment

5. A history of respiratory disease including asthma

6. Subjects with known human immunodeficiency virus (HIV) or who belong to a high-risk group for HIV infection

7. Evidence of serum hepatitis or presence of hepatitis B surface antigen (HBsAg) or hepatitis C antibodies (HCV)

8. Subjects with a history of or active or latent tuberculosis (TB), or close contact with anybody with active TB

9. Subjects with a history of malignancy or neoplastic disease (except successfully treated basal or squamous cell carcinoma of the skin)

10. Disease history suggesting reduced or abnormal immune function

11. Subjects with other latent or chronic infections (eg, recurrent sinusitis, genital or ocular herpes, urine tract infection) or at risk of infection (surgery, trauma or significant infection within previous 90 days, history of skin abscesses within the previous 90 days)

12. Clinically significant lower respiratory tract infection not resolved 4 weeks prior to Visit 2, as judged by the Investigator

13. Subjects who have had a clinically significant illness within 4 weeks before Visit 2 as determined by the Investigator

14. Scheduled inpatient surgery or hospitalisation during the study, including the follow-up period

15. Abstain from smoking from 12 months before Visit 1 up to the end of the study

16. Subjects who have received live or live-attenuated vaccine in the 2 weeks prior to administration of the investigational product (Visit 3)

17. Subjects who are pyrexial with a body temperature of greater than 37.7°C at Visit 2, or as judged by the Investigator

18. Any clinically relevant abnormal findings in physical examination, clinical chemistry, hematology, urinalysis, vital signs or ECG at baseline, which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or may influence the results of the study, or the subject's ability to participate in the study

19. Use of antibiotics, systemic or nebulised, in the 30 days prior to Visit 2 and until completion of the study follow-up visit (Visit 6)

20. Treatment with immunomodulatory agents within 8 weeks prior to Visit 2 and until completion of the study follow-up visit (Visit 6)

21. Use of oral or systemic glucocorticosteroids within 30 days prior to Visit 2, hormone replacement therapy (HRT) or any prescribed or over the counter medication (paracetamol may be allowed up to 48 hours before administration of the investigational product) from 2 weeks prior to the first dose of the investigational product until the end of the study (Visit 6)

22. Intake of herbal medicine (eg, based on St John's Wort, Echinacea) in the 3 weeks prior to Visit 2 and until completion of the follow-up visit (Visit 6)

23. Any other drugs which in the opinion of the Investigator are likely to compromise the subjects' safety or interfere with the objectives of the study

24. Alanine aminotransferase (ALT)/aspartate aminotransferase (AST) level ≥1.5 x upper limit of normal (ULN) at screening (Visit 2)

25. Abnormal dipstick in the urine if considered clinically relevant by the Investigator at screening (Visit 2)

26. Peripheral blood neutrophils above or below the laboratory reference range at screening (Visit 2)

27. hsCRP above the upper limit of the laboratory reference range at Visit 2

28. Known or suspected hypersensitivity to the investigational product or any excipients or a compound of the same class

29. Current evidence of drug abuse or significant history of drug abuse as judged by the Investigator

30. Current evidence of alcohol abuse or significant history of alcohol abuse as judged by the Investigator

31. Participation (defined as administration of at least one dose of investigational product) in another study within 12 weeks preceding screening (Visit 2)

32. Subjects who, in the opinion of the Investigator, should not participate in the study

33. Previous exposure to AZD5069

34. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study centre)

35. Previous enrolment or randomisation in the present study

For the genetic component of the study:

36. Previous allogeneic bone marrow transplant

37. Non-leukocyte depleted whole blood transfusion within 120 days of the date of genetic sample collection

Procedures for withdrawal of incorrectly enrolled subjects see Section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

1. Male subjects must use condoms and spermicide from the time of administration of the investigational product until 3 months after the follow-up visit (Visit 6), both to prevent pregnancy and to protect partners (and the foetus, if the partner is pregnant) from potential exposure to the investigational product. Male subjects should inform the medical staff at the study centre if their partner becomes pregnant during the study. In addition to the use of condoms, female partners of male subjects should use additional contraception from the time of administration of the investigational product until 3 months after the follow-up visit (Visit 6). Acceptable methods to be used by female partners include the oral contraceptive pill, hormone implants, intra-uterine devices (IUDs) or diaphragms with spermicide. Male subjects must not donate sperm for 3 months after the follow-up visit (Visit 6)

2. Subjects should only eat and drink the standardised meals and drinks provided (apart from water) during the residential period in the study centre.

3. The subjects will be asked to:

   – Avoid strenuous exercise

   – Abstain from food and drink intake (with the exception of water) up to 4 hours before screening (Visit 2) and the follow-up visit (Visit 6)

   – Abstain from alcohol during the study (for 72 hours prior to screening and from 72 hours prior to the first admission until the final follow-up)

4. Subjects should not receive a vaccination during the study and for 2 weeks following the final dose of the investigational product

5. Subjects should abstain from drugs of abuse throughout the entire study

6. Subjects should abstain from donating blood during the study and 12 weeks after the completion of the study

7. Subjects must not take part in another study, whilst participating in the current study

5.2 Subject enrolment and randomisation and initiation of investigational product

The Principal Investigator will ensure:

1. Signed informed consent is obtained from each potential subject before any study specific procedures are performed

2. Each potential subject is assigned a unique enrolment number, beginning with ‘E#’

3. The eligibility of each subject is determined. See Sections 4.1 and 4.2

4. Assign each eligible subject a unique randomisation code (subject number) beginning with #

Randomisation will be performed on Day 1 (Visit 3). Randomisation codes will be assigned strictly sequentially as subjects become eligible for randomisation.

If a subject withdraws his participation in the study, then his enrolment/randomisation code cannot be reused. Additional randomisation numbers will be included in the randomisation schedule to use for replacement subjects. Subject numbers and replacement subject numbers will have the same number of digits.

5.2.1 Procedures for randomisation

A randomisation scheme will be initiated by Quintiles Phase 1 using the global randomisation system (GRand) and produced by AstraZeneca R&D. Subjects will be allocated to one of 2 treatment sequences:

- Treatment Sequence 1: AB
- Treatment Sequence 2: BA
  - Treatment A: 100 mg AZD5069 bid
  - Treatment B: matching placebo bid

5.3 Procedures for handling incorrectly randomised subjects

Subjects who fail to meet the inclusion/exclusion criteria should not, under any circumstances, be randomised into the study. There can be no exceptions to this rule.

Where a subject, who does not meet the selection criteria, is randomised in error and this is identified before administration of the investigational product, the subject should be withdrawn from the study. A discussion should occur between the AstraZeneca Clinical Pharmacology Alliance (CPA) Physician and the Investigator regarding whether a replacement may be considered. The AstraZeneca CPA Physician is to ensure all such decisions are appropriately documented.

If a subject, who does not meet the selection criteria, has received the investigational product before the error is identified, the subject should be advised to continue assessments to ensure their safety and the AstraZeneca CPA Physician should be informed of the error.

5.4 Blinding and procedures for unblinding the study

5.4.1 Methods for ensuring blinding

This study has a double-blind design, meaning that neither the study centre nor the subjects will know which treatment (AZD5069 or placebo) are administered in which treatment period. Only the following people will have access to the randomisation list:

- AstraZeneca personnel carrying out the labelling and packaging of the investigational product
- The pharmacy personnel preparing investigational product at the study centre
- The personnel analysing the PK samples

The randomisation list should be kept in a secure location until the end of the study.

The placebo capsules will be matched in appearance, smell, and taste to the AZD5069 capsules.

5.4.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomisation for each randomised subject, will be available to the Investigator or pharmacist at the study centre.

The treatment code should not be broken except in medical emergencies when the appropriate management of the subject requires knowledge of the treatment randomisation. The Investigator documents and reports the action to AstraZeneca, without revealing the treatment given to the subject to the AstraZeneca personnel.

AstraZeneca retains the right to break the code for SAEs that are unexpected and are suspected to be causally related to an investigational product and that potentially require expedited reporting to regulatory authorities. Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual subject have been made and documented.

5.5 Treatments

5.5.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
AZD5069	50 mg capsules	AstraZeneca
Placebo	Capsules to match AZD5069	AstraZeneca

AZD5069 will be provided as 50 mg capsules for oral administration and with matching placebo capsules of the same size, weight and colour. AZD5069 is a powder with a solubility of approximately 0.003 mg/mL at room temperature in water. The AZD5069 capsules are hard gelatine capsules containing AZD5069, microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate and sodium stearyl fumarate.

The placebo capsules contain microcrystalline cellulose and magnesium stearate.

The capsules are packed in bulk bottles and will be prepared by unblinded personnel at HMR before dosing.

5.5.2 Doses and treatment regimens

The investigational product will be taken orally bid, with doses approximately 12 hours apart (2 capsules in the morning [8 am] and 2 capsules in the evening [8 pm]). All doses of the investigational product will be taken at the study centre during Visit 3 and Visit 5. For all dosing days, subjects should eat a light meal, which should be finished 1 hour prior to the scheduled dose, and then another meal 2 hours after administration of the dose. No food should be consumed between these meals.

5.5.3 Labelling

AstraZeneca R&D will perform the labelling of the bulk supply. The dose for each subject will be dispensed according to the randomisation scheme into individual dosing cups by HMR. AstraZeneca R&D will provide HMR with labels for the dosing cups. Each dosing cup will be labelled by HMR.

Labels will be prepared in accordance with Good Manufacturing Practice and local regulatory guidelines. The labels will fulfil Good Manufacturing Practice Annex 13 requirements for labelling.

5.5.4 Storage

All investigational products must be kept in a secure place under appropriate storage conditions. A description of the appropriate storage and shipment conditions are specified on the investigational product label provided by AstraZeneca, Pharmaceutical Development, Sweden.

5.6 Concomitant and post-study treatment(s)

Immunomodulatory agents are not permitted within 8 weeks prior to Visit 2 and until completion of the follow-up visit (Visit 6). Use of antibiotics (systemic or nebulised) or oral or systemic glucocorticosteroids is not permitted within 30 days of Visit 2 and until completion of the follow-up visit (Visit 6). Intake of herbal medicine (eg based on St John's Wort or echinacea) is not permitted within the 3 weeks prior to Visit 2 and until completion of the follow-up visit. No other prescribed or over-the-counter medication (including HRT) is allowed from 2 weeks prior to the first dose of the investigational product until the end of study (Visit 6); however paracetamol may be allowed up to 48 hours before administration of the investigational product.

However, this should not stop necessary acute treatments as judged by the Investigator. If any medication is necessary during the study period it should be prescribed by the Investigator and the AstraZeneca CPA Physician should be informed and recorded in the appropriate sections of the Case Report Form (CRF).

5.7 Treatment compliance

The administration of all medication (including investigational product) should be recorded in the appropriate sections of the CRFs.

Treatment compliance will be assured by supervised administration of the investigational product by the Investigator or delegate. The dose, date and time of administration of the investigational product will be recorded and checked by the monitor at monitoring visits.

5.7.1 Accountability

The investigational product provided for this study will be used only as directed in this CSP.

The study centre personnel will account for all investigational products dispensed to and returned by the subject.

The study centre personnel will account for all investigational products received at the study centre, unused investigational products, and for appropriate destruction. Certificates of delivery and destruction should be signed.

5.8 Discontinuation of investigational product

Neutrophil functional assays at Day 4 (Visit 3 and Visit 5) and 7 days after the end of the treatment should, if possible, be performed in all subjects (even if investigational product is discontinued).

Subjects may be discontinued from investigational product in the following situations:

- Subject decision. The subject is at any time free to discontinue treatment, without prejudice to further treatment
- Adverse Event
- Severe non-compliance to the CSP
- Risk to subjects as judged by the Investigator and/or AstraZeneca
- Incorrectly enrolled subjects
- Subject lost to follow-up
- Pregnancy. In case of a pregnancy of a female subject, the investigational product should be discontinued. The risk for the embryofoetal development in relation to exposure during the pregnancy has not been established
- Treatment code prematurely broken by the Investigator
- Clinically significant infection during the duration of the study, as judged by the Investigator. Adverse events of infection should be followed up and risk factors, signs and symptoms and diagnostic investigations recorded in the appropriate module(s) provided in the CRF
- Severe gastrointestinal event, as judged by the Investigator
- Subject has hepatic toxicity defined as one or more of:
  - Confirmed ALT or AST increase to >3 x ULN. Except for liver tests taken post-exercise challenge. If AST and ALT are increased after the exercise test these tests should be re-evaluated within 48 h to show a decrease

- Confirmed isolated total bilirubin increase to >2 x ULN;
- Confirmed ALT or AST increase to >2 x ULN concurrent with an increase in total bilirubin to >1.5 x ULN;

or any pattern of liver function test abnormalities giving cause for concern in the opinion of the Investigator. Results of abnormal liver function tests should be confirmed with a repeat test (via the central laboratory) as soon as possible, and within 48 hours, and the findings reported in the CRF. Abnormal liver findings should be reported as AE(s) if in the Investigator's judgment they meet the criteria. Abnormal liver function tests and hepatic AEs should be followed up and risk factors, signs and symptoms and diagnostic investigations recorded in the appropriate module provided in the CRF

- Confirmed serum creatinine >1.5 x ULN
- Neutrophil count $<0.5x10^9$/L continuously over 48 hours, when sample is taken on Day 5, 12 h after G-CSF and no increase seen after G-CSF

5.8.1 Procedures for discontinuation of a subject from investigational product

A subject that decides to discontinue investigational product will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by an Investigator. Adverse events will be followed up (See Sections 6.3.3 and 6.3.4).

If a subject is withdrawn from study, see Section 5.9.

5.9 Withdrawal from study

Subjects are at any time free to withdraw from the study (investigational product and assessments), without prejudice to further treatment (withdrawal of consent). Such subjects will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by an Investigator. Adverse events will be followed up (see Sections 6.3.3 and 6.3.4).

Subjects may be withdrawn at any time but once administration of the investigational product has occurred, every attempt should be made to continue assessments to ensure the safety of the subject. Specific reasons for withdrawing a subject may be:

- Subject decision. The subject is at any time free to withdraw his/her participation in the study, without prejudice
- Risk to subject as judged by the Investigator or AstraZeneca
- Eligibility criteria not fulfilled
- Death

- Adverse events
- Severe non-compliance to the CSP as judged by the Investigator and/or AstraZeneca
- Subject lost to follow-up

Withdrawn subjects will not be replaced.

6. COLLECTION OF STUDY VARIABLES

The study assessments are described in the sections below and the timing of these assessments are detailed in the Study Plan (see Table 2).

It is important that PK sampling occurs as close as possible to the scheduled time. In order to achieve this, other assessments scheduled at the same time may be initiated prior to the PK time point. The sequence at a particular time point is:

1. ECG
2. Blood pressure and pulse rate
3. Pharmacokinetic blood sample (at scheduled time point)
4. Neutrophil count

The time of investigational product administration is very important due to the diurnal variation in neutrophils. The subject should take the investigational product at 8 am and 8 pm ±30 minutes. Each subject should receive the investigational product at the same time point on each occasion with a maximum difference of 15 minutes.

6.1 Recording of data

The Investigator will ensure that data are recorded on the CRF as specified in the CSP. The Principal Investigator ensures the accuracy, completeness, and timeliness of the data recorded, for data queries and all required reports according to any instructions provided.

The Principal Investigator will sign the completed CRF. A copy of the completed CRF will be archived at the study centre.

6.2 Data collection at enrolment and follow-up

6.2.1 Enrolment procedures

At enrolment (Visit 1), each potential subject will provide informed consent prior to starting any study specific procedures. Please note that Visit 1 and Visit 2 can be performed on the same day as per the study centre's requirements.

The following assessments will be performed at screening (Visit 2):

Demographic data and other characteristics will be recorded and will include: date of birth, gender, race, and alcohol consumption.

Each subject will undergo screening within 28 days prior to admission to confirm eligibility. This will consist of:

1. A standard medical, medication and surgical history as well as respiratory problems and smoking history with review of the inclusion and exclusion criteria with the subject
2. A complete physical examination
3. Height, weight and calculation of BMI
4. Vital signs (resting supine BP and pulse rate) and body temperature
5. Recording a resting 12-lead paper ECG
6. Screening for hsCRP
7. Neutrophil functional assay and flow cytometry
8. A blood sample for routine clinical chemistry, haematology, endocrinology and screen for hepatitis B surface antigen, antibodies to hepatitis C virus and antibodies to HIV
9. Screening for TB
10. A urine sample for routine urinalysis, drugs of abuse screen and pregnancy in females
11. Alcohol breath test
12. Smokerlyser breath test
13. Concomitant medication use
14. All AEs and any SAEs will be recorded from screening

After admission and before randomisation (Day -1) the Investigator should re-assess each subject to reconfirm eligibility. Continuing eligibility will be confirmed on admission to Treatment Period 2 (Visit 5).

6.2.2 Follow-up procedures

A post-study medical examination will be performed 7 days after the last administration of the investigational product in Treatment Period 2 (Visit 6). This visit will include a complete physical examination, measurement of vital signs, weight and body temperature, a 12-lead paper ECG, blood samples for clinical chemistry, haematology, circulating neutrophils, neutrophil function and flow cytometry, hsCRP and explorative biomarkers (G-CSF, GRO-α, ENA-78 and IL-8), a urine sample for urinalysis and a pregnancy test, and assessment of any AEs, SAEs or required concomitant medication.

6.3 Safety

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

6.3.1 Definition of adverse events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

6.3.2 Definition of serious adverse event

A serious adverse event is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For further guidance on the definition of a SAE, see Appendix B to the Clinical Study Protocol.

6.3.3 Recording of adverse events

Time period for collection of adverse events

Adverse events as well as SAEs will be collected from the time of informed consent and throughout the treatment periods including the follow-up period (Visit 6).

Follow-up of unresolved adverse events

Any AEs that are unresolved at the subject's last visit in the study are followed up by the Investigator for as long as medically indicated, but without further recording in the CRF. AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collected for each AE:

- AE (verbatim)
- Date and time when the AE started and stopped
- Intensity, rated according to the following scale:
  - Mild (awareness of sign or symptom, but easily tolerated)
  - Moderate (discomfort sufficient to cause interference with normal activities)
  - Severe (incapacitating, with inability to perform normal activities)
- Whether the AE is serious or not
- Investigator causality rating against the investigational product (yes or no)
- Action taken with regard to the investigational product
- Whether the AE caused the subject's withdrawal from study (yes or no)
- Outcome

In addition, the following variables will be collected for SAEs:

- Date the AE met the SAE criteria
- Date the Investigator became aware of the SAE
- AE is serious due to
- Date of hospitalisation

- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to the study procedure(s)
- Causality assessment in relation to additional investigational product
- Description of the AE

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.3.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE.

Causality collection

The Investigator will assess a causal relationship between the investigational product and each AE, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'

For SAEs causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is found in Appendix B of this CSP.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: *'Have you had any health problems since you were last asked?'*, or revealed by observation will be collected and recorded in the CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from the CSP mandated laboratory tests, vital signs, ECGs and other safety assessments will be summarised in the CSR. Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs, ECGs and other safety assessments should

therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory value or vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result or vital sign will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

NB. Cases where a subject shows an AST **or** ALT ≥3 x ULN **or** total bilirubin ≥2 x ULN may need to be reported as SAEs, please refer to Appendix D 'Actions required in cases of combined increase of AST and Total Bilirubin – Hy's Law', for further instructions.

6.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF.

If any SAE occurs in the course of the study, then Investigators or other study centre personnel inform appropriate AstraZeneca representatives within 24 hours following knowledge of the SAE.

The designated AstraZeneca representative works with the Investigator to ensure that all the necessary information is provided to the AstraZeneca patient safety data entry site **within one calendar day** of initial receipt for fatal and life threatening events and **within five calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other study centre personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The reference document for definition of expectedness/listedness is the IB for the AstraZeneca drug.

6.3.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, haematology, endocrinology and urinalysis will be taken at the times indicated in the Study Plan (Table 2). The date and time of collection of all laboratory tests will be recorded in the CRF.

Table 3 Laboratory parameters

Haematology	Clinical Chemistry	Urinalysis
B-Erythrocytes	S-Creatinine	U-Haemoglobin (dipstick)
B-Haemoglobin	S-Bilirubin (total)	U-Protein (dipstick)
B-Leukocyte differential count[a]	S-Alkaline phosphatase	U-Glucose (dipstick)
(absolute count and percentage including neutrophils, basophils, lymphocytes, monocytes, eosinophils)	S-Aspartate aminotransferase (AST)	U-Microscopy (if required *) U-Total protein (if required *) U-Creatinine (if required *)
B-Platelet count	S-Alanine aminotransferase	
B-Reticulocytes	(ALT)	
	S-Lactate dehydrogenase (LDH)	
B-Circulating neutrophil count[a]	S-Albumin	
	S-Protein (total)	
	S-Potassium	
Other	S-Calcium (total)	
HIV (at screening only)[b]	S-Sodium	
Hepatitis B and C (at screening only)[b]	S-Glucose (non-fasted)	
LH and FSH (at screening only)[c]	S-High sensitive C-reactive protein (hsCRP)[d]	
TB screen (screening only)	S-Urate	
	S-Urea	

a Central laboratory leukocyte and neutrophil measurements will only be available at the end of the study so as not to compromise the blind
b HIV, hepatitis B and C results will not be entered into the study database
c Female subjects only
d Collected as a separate sample at the time points indicated on Table 2

*Urine will be tested locally using dipsticks. In addition, samples will be sent to the central laboratory for urine microscopy and quantitative measurements of total protein, albumin and creatinine if the local dipstick test for protein and/or blood is positive or if the urine sample appears abnormal on macroscopic examination, eg, if it is cloudy.

At screening all subjects will be tested for HIV, HBsAg and antibodies to HCV. Subjects will also be screened for TB. Female subjects will be screened for LH and FSH at screening.

Urine will be tested for the following drugs of abuse at screening and each admission: amphetamines, barbiturates, tricyclic antidepressants, cocaine, methadone, morphine,

phencyclidine, tetrahydrocannabinol and opiates. A smokerlyser breath test and an alcohol breath test will be performed at screening and each admission.

A urine pregnancy test will be performed for female subjects at the visits indicated in the Study Plan (Table 2). If a subject tests positive to any of these screening tests she will be excluded from the study.

Laboratory values outside the reference limit suspected to be of any clinical significance will be repeated. Subjects in whom suspected clinical significance is confirmed will either not be included or if already randomised will be followed until normalisation or for as long as the Investigator considers necessary. Additional laboratory variables may be performed for safety reasons if judged appropriate by the Investigator.

Samples will be collected in tubes according to standard routines. The analysis of samples for clinical chemistry, haematology, urinalysis, and endocrinology will be performed at the study centre according to local procedures.

For blood volume see Section 7.1.

NB. In case a subject shows an AST **or** ALT ≥3 x ULN **or** total bilirubin ≥2 x ULN please refer to Appendix D 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

Standard safety monitoring (AEs, ECGs, vital signs, lab data, physical examination)

To mitigate against risks identified in the pre-clinical toxicology studies, subjects will be monitored as follows:

- All AEs related to gastrointestinal disturbance should be kept under close surveillance by Patient Safety surveillance and the Study Team Physician
- Full blood counts including neutrophils, erythrocytes, haemoglobin (Hb), platelets, and leukocytes will be monitored at regular intervals. Neutrophils <1 x 10^9/L will be monitored approximately every 12 hours until values are within the reference range
- Standard liver function tests. High sensitive CRP will also be monitored as a marker of inflammation (not liver function). Events will be kept under close surveillance by Patient Safety surveillance and the Study Team Physician.
- Hepatic follow-up CRF modules will be used to collect additional information on liver events and/or elevated liver function tests.
- Subjects will be closely monitored by the Investigator for signs and symptoms that may reflect the onset of infection including regular monitoring of body temperature. Serum hsCRP will be monitored regularly.

- Plasma urea, creatinine and electrolytes, urinary protein, glucose and erythrocytes will be monitored at regular intervals

- Subjects experiencing acute infection, or meeting discontinuation criteria may need to attend the study centre even in between visits for follow-up assessments. Any AEs of infection should be followed up and risk factors, signs and symptoms and diagnostic investigations recorded in the appropriate module(s) provided in the CRF.

6.3.6 Physical examination

The timing of individual examinations is indicated in the Study Plan (Table 2). A complete physical examination will be performed including an assessment of the following: general appearance, skin, head and neck, ears and throat), mouth (mucosal changes), lymph nodes, thyroid, abdomen, musculoskeletal, cardiovascular, respiratory and neurological systems.

A brief physical examination will be performed at the times shown in the Study Plan (Table 2) and will include an assessment of the following: general appearance, lungs, cardiovascular, and neurological evaluations. Results will be recorded as an overall normal or abnormal with a listing of abnormalities.

Height will be measured in centimetres and weight in kilograms. Measurements should be taken without shoes and the same scale used for all measurements. The BMI will be calculated from the height and weight.

6.3.7 ECG

ECG measurements will be performed supine after at least 10 minutes rest on a bed at the time points indicated in Table 2. This will be recorded in the CRF, as normal/abnormal, with any abnormalities specified.

Heart rate, P and QRS durations, PR, QT and QTc intervals will be recorded from the standard lead of the 12-lead ECG.

6.3.8 Vital signs

6.3.8.1 Pulse and blood pressure

Supine blood pressure and pulse rate will be measured using a semi-automatic blood pressure recording device with an appropriate cuff size after at least 10 minutes rest on a bed.

For timings of assessments refer to the Study Plan (Table 2).

6.3.8.2 Body temperature

Body temperature will be measured in degrees Celsius using an automated thermometer at the times indicated in the Study Plan (Table 2).

6.3.9 Other safety assessments

6.3.9.1 Standardised exercise test on exercise bicycle

The objective of the exercise challenge using an exercise bicycle is to stimulate the neutrophil recruitment from the marginal pool into the circulation by using a 10-minute test with at least 4 minutes of sub-maximal effort at 80% of the maximum ventricular rate according to the subject's age.

Maximum ventricular rate: 210 – (0.5 x age) = estimated maximum ventricular rate

The exercise challenge should be performed as soon as possible after the morning dose on Day 4 and no later than 4 hours after the morning dose. The exercise challenge must be performed at the same time point during both treatment periods. The time of the exercise challenge should be noted in the CRF of each subject.

Before test: ventricular rate at rest, blood sampling for neutrophil count, leukocyte differential counts just before the test (≤30 minutes prior to the start of the challenge).

Start the challenge with 50W and slowly increase during the first 5 minutes until the subject reaches sub-maximal effort based on 80% of the maximum ventricular rate at approximately 5 minutes. Continue at this level until 10 minutes. If the subject finds the exercise too strenuous, the work load can be reduced somewhat. Record the ventricular rate at 6 minutes and 9 minutes. Directly after the end of the challenge blood samples for the neutrophil count and differential count will be collected. Further blood samples will be collected after the exercise according to the Study Plan (Table 2).

6.3.9.2 G-CSF challenge

A subcutaneous injection of 300 µg G-CSF (0.5 mL injection volume) will be administered to subjects within 30 minutes after the morning dose on Day 5. The G-CSF challenge should be performed at a similar time in each treatment period.

6.4 Pharmacokinetics

6.4.1 Collection of samples

Blood samples (6 mL) for PK analysis will be collected at the following time points: pre-dose on Days 1 to 4 (twice daily) and at pre-dose and 1, 2, 3, 5, 8, and 12 hours post-morning and evening doses on Day 3 (Visit 3 and Visit 5). The date and exact time will be recorded in the CRF.

Samples will be collected, labelled, stored and shipped as detailed in Laboratory Manual.

For blood volume see Section 7.1.

6.4.2 Determination of drug concentration

Samples for determination of AZD5069 concentrations in plasma will be analysed by the appointed laboratory on behalf AstraZeneca, using a validated method of high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS) after protein precipitation. The lower limit of quantification (LLOQ) of AZD5069 in plasma is 1 nmol/L. Full details of the analytical method used will be described in a separate bioanalytical report.

Plasma samples from subjects who received placebo will not be routinely analysed for AZD5069. The unblinded bioanalyst will be responsible for determining which samples will be analysed for AZD5069.

Samples that are outside of the known stability of AZD5069 will not be reported.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples and reported in a separate bioanalytical report.

Additional analysis of plasma samples related to metabolism or other PK investigations may be conducted and reported separately from the CSR.

6.5 Pharmacodynamics

6.5.1 Collection of pharmacodynamic markers

Circulating neutrophil counts in blood will be used as a PD marker; blood samples will be collected at the times indicated in Table 2. Circulating neutrophil counts (absolute and percentage) will be analysed within 2 hours (maximum 4 hours) of sample collection.

Slides will be prepared for an additional manual neutrophil count using the blood samples collected at the following times: pre exercise challenge and at exercise challenge completion (10 minutes); pre G-CSF challenge and 12 hours post G-CSF challenge. The slides will be prepared at the investigational site and the manual neutrophil count will be performed at the laboratory of Dr

.

Blood samples for assessment of neutrophil function (neutrophil phagocytosis and oxidative burst) will be collected at the times shown in Table 2. Analysis will be performed at the laboratory of Dr ,

.

6.5.2 Explorative biomarkers and flow cytometry

Blood samples will be collected at the times shown in Table 2 to assess explorative biomarkers (G-CSF, GRO-α, ENA-78, IL-8) and to assess neutrophil subpopulations using flow cytometry. Flow cytometry parameters may include (but are not limited to): CD16, CD11b and CD62L.

Blood samples for explorative biomarkers and flow cytometry will be analysed using appropriate methods at the laboratory of Dr ,

Details of laboratory sample handling and methods will be provided in separate handling instructions. For blood volume see Section 7.1.

6.6 Pharmacogenetics

6.6.1 Collection of pharmacogenetic samples

The blood sample for the optional genetic research will be obtained from the subjects at Visit 3, at or after randomisation. Although genotype is a stable parameter, early sample collection is preferred to avoid introducing bias through excluding subjects who may withdraw due to an AE. Such subjects would be important to include in any genetic analysis. If for any reason the sample is not collected at Visit 3, it may be taken at any visit until the last study visit. Only one sample should be collected per subject for genetics during the study. Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The total volume of blood that will be drawn from each subject in this study is as follows:

Table 4 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)[a]	No. of samples	Total volume (mL)
Safety	Clinical chemistry[b]	2.5	15	37.5
	Haematology[c]	2	69	138
	Serology/endocrinology[d]	2.5	1	2.5
	TB screening	3.0	1	3.0
	Explorative markers (G-CSF, GRO-α, ENA-78 and IL-8)	5	6	30
Pharmacokinetics	PK blood sample	6	36	216
Neutrophil function test (Burst-test and Phago-test) and Flow cytometry		3.5	7	24.5
Pharmacogenetic sample[e]		10	1	10
Total				**461.5**

a If a cannula is used, an additional 1 mL of blood will be collected to flush the cannula.
b At the times indicated on Table 2 only hsCRP will be analysed from this sample.
c Full haematology panel includes leukocyte differential counts and circulating neutrophil count; when one or more of these are scheduled to be collected at the same time point they will be analysed from a single 2 mL sample (see Table 2 for details of sample collection times).
d Endocrinology: female subjects only
e Optional

The number of samples taken, as well as the volume required for each analysis, may be changed during the study as new data on AZD5069 become available. However, the maximum volume to be drawn from each subject will not exceed 550 mL, ie, approximately the same volume as would be drawn during a regular blood donation.

7.2 Handling, storage and destruction of biological samples

The samples will be used up, or disposed of after analyses or retained for further use as described here.

Safety blood samples will be disposed of within 7 days of analysis.

7.2.1 Pharmacokinetic and pharmacodynamic samples

Samples will be disposed of after the CSR has been finalised.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples and reported in a separate Bioanalytical report.

Exploratory biomarkers and flow cytometry samples

Biological samples for future research can be retained at AstraZeneca R&D for a maximum of 15 years following the last subject's last visit in the study. The results from the exploratory analysis may not be reported in the CSR.

7.2.2 Pharmacogenetic samples

The exploratory genetic research component of the study is optional.

The DNA sample will be assigned a unique number replacing the information on the sample tube. Thereafter, the DNA sample will be identifiable by the unique DNA number only. The DNA number will be used to identify the sample and corresponding data at the AstraZeneca genetics laboratories or at the designated contract laboratory. No personal details identifying the individual will be available to any person (AstraZeneca employee or contract laboratory personnel working with the DNA).

The samples and data for genetic analysis in this study will be single coded. The link between the subject randomisation code and the DNA number will be maintained and stored in a secure environment, with restricted access within the Clinical Genotyping Group Laboratory Information Management System (LIMS) at AstraZeneca. The link will be used to identify the relevant DNA samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit, and to trace samples for destruction in the case of withdrawal of consent.

Samples will be retained for up to a maximum of 15 years, from the date of last subject's last visit, after which they will be destroyed. Deoxyribonucleic acid is a finite resource that is used up during analyses. Samples may be stored and used until no further analyses are possible or until the maximum storage time has been reached.

7.3 Labelling and shipment of biohazard samples

The Principal Investigator ensures that samples are labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix C of this CSP 'International Airline Transportation Association (IATA) 6.2 Guidance Document'.

Any samples identified as Infectious Category A materials are not shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labelling, shipment and containment provisions are approved.

7.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

The Principal Investigator keeps full traceability of collected biological samples from the subjects while in storage at the centre until shipment and keeps documentation of receipt of arrival.

The sample receiver keeps full traceability of samples while in storage and during use until used, disposed of or until further shipment or disposal (where appropriate) and keeps documentation of receipt of arrival.

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of study centres and auditing of external laboratory providers.

Samples retained for further use are registered in the AstraZeneca bio bank system during the entire life cycle.

7.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, if not already analysed and the action documented. If samples are already analysed, AstraZeneca is not obliged to destroy the results of this research.

As collection of donated biological samples is an integral part of the study then the subject is withdrawn from further study participation.

As collection of the pharmacogenetic and biomarker samples are an optional part of the study, then the subject may continue in the study.

The Principal Investigator:

- Ensures subject's withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca
- Ensures that biological samples from that subject, if stored at the study centre, are immediately identified, disposed of/destroyed and the action documented
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed, the action documented and the signed document returned to the study centre
- Ensure the above procedures are followed for the optional genetics as well as the optional safety biomarker informed consent
- Ensures that the subject and AstraZeneca are informed about the sample disposal

AstraZeneca ensures the central laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed, the action documented and the signed document returned to the study centre.

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP), applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Subject data protection

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

AstraZeneca will not provide individual genotype results to the subjects, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law.

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a subject. For example, in the case of a medical emergency, an AstraZeneca Physician or an Investigator might know a subject's identity and also have access to his or her genetic data. Also Regulatory Authorities may require access to the relevant files, though the subject's medical information and the genetic files would remain physically separate.

8.3 Ethics and regulatory review

The EC should approve the final CSP, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The Principal Investigator will ensure the distribution of these documents to the applicable EC, and to the study centre personnel.

The opinion of the EC should be given in writing. The Principal Investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study.

The EC should approve all advertising used to recruit subjects for the study.

AstraZeneca should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

If required by local regulations, the CSP should be re-approved by the EC annually.

Before enrolment of any subject into the study, the final CSP, including the final version of the Informed Consent Form, is approved by the Regulatory Authority or a notification to the Regulatory Authority is done, according to local regulations.

AstraZeneca will handle the distribution of any of these documents to the Regulatory Authority.

AstraZeneca will provide the Regulatory Authority, EC and Principal Investigator with safety updates/reports according to local requirements, including suspected unexpected adverse reactions (SUSARs), where relevant.

8.4 Informed consent

The Principal Investigator(s) at each centre will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject
- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the informed consent form that is approved by an Ethics Committee.

8.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Principal Investigator and AstraZeneca.

If there are any substantial changes to the CSP, then these changes will be documented in a study protocol amendment and where required in a new version of the CSP (Revised Protocol).

The amendment should be approved by the EC and if applicable, also the national regulatory authority, before implementation. Local requirements should be followed for revised protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the CSP to the Principal Investigator. For distribution to the EC see Section 8.3.

If a protocol amendment requires a change to the Informed Consent Form, AstraZeneca and the EC should approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by the EC.

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an EC may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the CSP, GCP, guidelines of the ICH, and any applicable regulatory requirements. The Investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre.

9. STUDY MANAGEMENT

Quintiles will be managing the study on behalf of AstraZeneca.

9.1 Pre-study activities

Before the first subject is entered into the study, it is necessary for a representative of AstraZeneca to visit the investigational study centre to:

- Determine the adequacy of the facilities
- Determine availability of appropriate subjects for the study
- Discuss with the Investigator(s) (and other personnel involved with the study) their responsibilities with regard to protocol adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a Clinical Study Agreement between AstraZeneca and the Investigator.

9.2 Training of study centre personnel

Before the first subject is entered into the study, an AstraZeneca representative will review and discuss the requirements of the CSP and related documents with the study centre personnel and also train them in any study-specific procedures and system(s) utilised.

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of the study centre personnel, and that any new information relevant to the performance of this study is forwarded to the study centre personnel involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other personnel).

9.3 Monitoring of the study

During the study, an AstraZeneca representative will have regular contacts with the study centre, including visits to:

- Provide information and support to the Investigator
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the CSP, that data are being accurately and timely recorded in the CRFs, that biological samples are handled in accordance with the Laboratory Manual and that the investigational product accountability checks are being performed
- Perform source data verification (a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study) including verification of written informed consent of participating subjects. This will require direct access to all original records for each subject (eg, clinic charts)
- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented and reported to the subject

The AstraZeneca representative will be available between visits if the Principal Investigator or other personnel at the study centre needs information and advice about the study conduct.

9.3.1 Source data

Refer to the Clinical Study Agreement for the location of the source data.

9.4 Study agreements

The Principal Investigator should comply with all the terms, conditions, and obligations of the Clinical Study Agreement, or equivalent, for this study. In the event of any inconsistency between this CSP and the Clinical Study Agreement, the CSP shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, the terms of the Clinical Study Agreement shall prevail.

Agreements between AstraZeneca and the Principal Investigator should be in place before any study-related procedures can take place, or subjects are enrolled.

9.4.1 Archiving of study documents

The Investigator follows the principles outlined in the Clinical Study Agreement.

9.5 Study timetable and end of study

The end of the study is defined as 'the last visit of the last subject undergoing the study'.

The study is expected to start in Q1 2012 and to end by Q3 2012.

The study may be terminated if the study procedures are not being performed according to GCP, or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with AZD5069.

10. DATA MANAGEMENT

Data management will be performed by Quintiles. The Data Management Plan will describe the methods used to collect, check and process clinical data in detail. It will also clarify the roles and responsibilities for the different functions and personnel involved in the data management process. Furthermore the Data Management Plan will describe the data flow and timelines within the study.

Adverse events and medical/surgical history will be classified according to the terminology of the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the AstraZeneca Drug Dictionary. All coding will be performed by Quintiles.

Quality control procedures will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

After completion of the study and when all collected data is validated and coded, the database will be locked.

11. EVALUATION AND CALCULATION OF VARIABLES

11.1 Calculation or derivation of safety variable(s)

Safety and tolerability variables:

- Adverse events (AEs)
- 12-Lead ECG
- Physical examination including signs of infection
- Haematology, clinical chemistry and urinalysis
- Neutrophil counts daily
- Vital signs and body temperature

11.1.1 Other significant adverse events (OAE)

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs or AEs leading to withdrawal from the study. Based on the expert's judgment, significant AEs of particular clinical importance may, after consultation with the Global Safety Physician, be considered OAEs and reported as such in the CSR. A similar review of other data from clinical laboratory safety assessments, vital signs, ECGs and other safety assessments will be performed for identification of OAEs.

Examples of these are marked haematological and other clinical laboratory safety abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment.

11.2 Calculation or derivation of pharmacokinetic variables

The PK analysis of the plasma concentration data for AZD5069 will be the responsibility of the clinical Pharmacokineticist at Quintiles, Overland Park, Kansas, United States. Quintiles Standard Operation Procedures (SOPs) and Work Instructions will be used as the default methodology, unless otherwise specified.

The actual sampling times will be used in the PK calculations. All PK computations will be performed using WinNonlin Professional 5.2, or higher (Pharsight Corp.,); or SAS® Version 9.2, or higher (SAS Institute, Inc.,). Graphics may be prepared with SAS® Version 9.2, or higher; SigmaPlot® 9.0, or higher (Systat Software, Inc.,); or WinNonlin Profesional® 5.2, or higher.

If the data permits, the following PK parameters will be determined by non-compartmental methods for AZD5069:

$AUC_{(0\text{-}12),ss}$	Area under the concentration versus time curve from time 0 to 12 hours after both morning and evening doses on Day 3
$C_{ss,max}$	Maximum observed plasma concentration after both morning and evening doses on Day 3
$t_{ss,max}$	Time to reach $C_{ss,max}$ following both morning and evening doses on Day 3
$C_{ss,min}$	Minimum observed plasma concentration after both morning and evening doses on Day 3
$t_{ss,min}$	Time to reach $C_{ss,min}$ following both morning and evening doses on Day 3
C_{12}	Observed plasma concentration 12 hours post-dose after both morning and evening doses

Additional parameters may be calculated as deemed appropriate. Samples of subjects who received placebo will not be assayed.

11.3 Calculation or derivation of pharmacodynamic variable(s)

Calculations related to the PD variables will be performed by Quintiles, . Quintiles' SOPs and Work Instructions will be used as the default methodology if not otherwise specified.

$AUC_{(0-4)}$ for circulating neutrophils during exercise challenge (Day 4) will be calculated from the linear trapezoidal rule, using actual times rather than protocol times, if possible. Before calculation of the AUC, the individual neutrophil measurements will be baseline adjusted by computing the relative change from baseline (ie, computing the ratio between the measurement at time point *t* post-dose and its corresponding pre-dose measurement). The baseline value to be used for the baseline adjustment is the last measurement observed just prior to the challenge for the treatment period considered. Before $AUC_{(0-4)}$s are used in the analysis, the AUCs should be standardised by dividing the AUC by its length ie, $AUC_{(0-4)}/4$. The maximum absolute neutrophil cell count (ANC_{max}) observed during exercise challenge will be computed and used for analysis.

$AUC_{(0-36)}$ for circulating neutrophils *during G-CSF challenge (Day 5)* will be calculated from the linear trapezoidal rule, using actual times rather than protocol times, if possible. Before calculation of the AUC, the individual neutrophil measurements will be baseline adjusted by computing the relative change from baseline (ie, computing the ratio between the measurement at time point *t* post-dose and its corresponding pre-dose measurement). The baseline value to be used for the baseline adjustment is the last measurement observed just prior to the challenge for the treatment period considered. Before $AUC_{(0-36)}$s are used in the analysis, the AUCs should be standardised by dividing the AUC by its length ie, $AUC_{(0-36)}/36$. Also, the maximum absolute neutrophil cell count (ANC_{max}) observed during G-CSF challenge will be computed and used for analysis.

For both morning and evening doses, $AUC_{(0-12)}$ for circulating neutrophils (Day 3) will be calculated from the linear trapezoidal rule, using actual times rather than protocol times, if possible. Before calculation of the AUC, the individual neutrophil measurements will be baseline adjusted by computing the relative change from baseline (ie, computing the ratio between the measurement at time point *t* post-dose and its corresponding pre-dose measurement). The baseline value to be used for the baseline adjustment is the pre-dose measurement observed for the treatment period considered. Before $AUC_{(0-12)}$s are used in the analysis, the AUCs should be standardised by dividing the AUC by its length ie, $AUC_{(0-12)}/12$. The ANC_{max} will be computed and used for analysis.

Day 3	$AUC_{(0-12)}$	Area under the baseline adjusted circulating neutrophils versus time curve from time 0 to 12 hours after both morning and evening doses on Day 3

	ANC_{min}	Baseline adjusted minimum circulating neutrophils after both morning and evening doses on Day 3
Day 4 (*during exercise challenge*)	$AUC_{(0-4)}$	Area under the baseline adjusted circulating neutrophils versus time curve from time 0 to 4 hours after the start of the exercise challenge on Day 4
	ANC_{max}	Baseline adjusted maximum circulating neutrophils during the exercise challenge on Day 4
Day 5 (*during G-CSF challenge*)	$AUC_{(0-36)}$	Area under the baseline adjusted circulating neutrophils versus time curve from time 0 to 36 hours after the start of the G-CSF challenge on Day 5
	ANC_{max}	Baseline adjusted maximum circulating neutrophils during the G-CSF challenge on Day 5

11.4 Calculation or derivation of pharmacogenetic variables

The number of subjects who will agree to participate in the optional genetic research is unknown. It is therefore not possible to establish whether sufficient data will be collected to allow a formal statistical evaluation or whether only descriptive statistics will be generated.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of analysis sets

12.1.1 General principles

The analysis of data will be based on different subsets according to the purpose of analysis, ie, for safety, PK and PD, respectively.

The as-treated principle will be applied to all evaluations; ie, subjects who received another treatment than the one assigned in the randomisation list will be analysed as belonging to the actual treatment group and not that assigned by randomisation.

12.1.2 Safety analysis set

All subjects who received at least 1 dose of randomised AZD5069 or placebo, and for whom any post-dose data are available will be included in the safety analysis set. Throughout the safety results sections, erroneously treated subjects (eg, those randomised to Treatment A but actually given Treatment B) will be accounted for in the actual treatment group.

12.1.3 Pharmacodynamic analysis set

The PD analysis set will be based on the safety analysis set. The PD analysis set will include all evaluable PD data appropriate for the evaluation of interest (with no important protocol deviations or violations thought to significantly affect the PD of the investigational product) from all subjects who receive investigational product.

12.1.4 Pharmacokinetic analysis set

The PK analysis set will be based on the safety analysis set. The PK analysis set will include all randomised subjects who took at least 1 dose of AZD5069 and have at least 1 post-dose PK measurement without important protocol deviations or violations thought to significantly affect the PK of the investigational product.

Data for subjects during treatment periods in which they receive placebo will not be a part of the PK analysis set.

12.2 Methods of statistical analyses

12.2.1 General principles

Data will be presented by treatment group for the purposes of summarising the safety, PK (only 1 treatment group), and PD results. Categorical variables (eg, gender and events) will be summarised in frequency tables (frequency and proportion of subjects in the analysis set). Missing data will result in a reduced sample size for that parameter. Missing data will not be imputed. A subject who withdraws prior to the last planned observation in a study period will be included in the analyses up to the time of withdrawal.

12.2.2 Subject characteristics

Continuous variables (eg, age, height, etc) will be summarised using descriptive statistics (number of observations [n], mean, standard deviation [SD], minimum [min], median, and maximum [max]). Categorical variables (eg, gender, race, etc) will be summarised in frequency tables (frequency and proportion).

12.2.3 Safety and tolerability

All safety data (scheduled and unscheduled) will be presented in the data listings.

Continuous variables will be summarised using descriptive statistics (n, mean, SD, min, median, max). Categorical variables will be summarised in frequency tables (frequency and proportion). Graphical presentations may be used as appropriate. Examples may include line graphs showing individual or mean development over time, and shift plots showing pre-treatment values on horisontal axis and post-treatment values on vertical axis.

All AEs will be collected for each subject from the time when informed consent is obtained (Visit 1) until the follow-up visit. Adverse events that occur before dosing will be reported separately.

Adverse events will be summarised by Preferred Term (PT) and System Organ Class (SOC) using MedDRA vocabulary by dose/treatment group. Furthermore, listings of SAEs and AEs that led to withdrawal will be made and the number of subjects who had any AEs, SAEs, DAEs, and AEs with severe intensity will be summarised.

For ECG parameters, the QT correction factor will be based on the Fridericia's formula. Further categorical summaries of absolute QT and QTcF values (>450 ms, >480 ms, >500 ms) and change from baseline values in QT and QTcF values (>30 ms, >60 ms) may also be produced.

Tabulations and listings of data for vital signs (blood pressure and pulse), clinical laboratory tests, ECGs, and physical examination findings will be presented. Where applicable, data will be summarised for the absolute value at each scheduled assessment, and for the corresponding change from baseline. For clinical laboratory tests, listings of values for each subject will be presented with abnormal or out-of-range values flagged. Clinical laboratory data will be reported in Système International units in the CSR.

Extra measurements (such as unscheduled or repeat assessments) will not be included in the descriptive statistics, but will be included in subject listings. All AEs, ECG outliers, and clinical laboratory outliers that occur following the first dose of investigational product will be included in the tabulations of AEs and outlier events, including episodes that occur at unscheduled evaluations or during the washout period between treatments.

12.2.4 Pharmacokinetic and pharmacodynamic analysis

The PK and PD summaries, individual figures, data listings, as well as the statistical analysis of the PK and PD variables will be the responsibility of the study biostatistician at Quintiles (using SAS® version 9.2 or higher and, where appropriate, additional validated software).

Quantitative continuous variables will be summarised using descriptive statistics, including n, mean, SD, median, minimum and maximum values. Additionally, for PK parameters, (except for t_{max}), geometric means and geometric coefficient of variation (GCV%) will be reported. The geometric mean is calculated as the exponential of the arithmetic mean calculated from data on a log scale. The GCV% is calculated as $100 \cdot \sqrt{\left(\exp\left(s^2\right)-1\right)}$ where 's' is the SD of the data on a log scale. Mean, SD, geometric mean, and GCV% will not be calculated for t_{max}. In general, descriptive statistics will follow the rounding convention in Quintiles' Phase I SOPs.

Relationship between AZD5069 plasma concentration and effect on circulating neutrophils will be presented graphically.

12.2.4.1 Pharmacodynamics

All recorded circulating neutrophil count in blood will be listed by treatment group and visit. The absolute value, along with change and percentage change from baseline, will be summarised by treatment group and visit. Neutrophil functional assays data (normal,

abnormal) will be listed for each subject (for all randomised subjects), by treatment, time, and period. Separate listing for phagocytosis and oxidative burst will be produced. In addition, by treatment and period shift from baseline listings will be produced for the change in status from baseline to day 4 (steady state) and from baseline to 7 days after end of treatment. The change from baseline will be categorised as follows: normal to normal = 0, normal to abnormal = 1, abnormal to normal = 2, abnormal to abnormal = 3.

For each subject who had a reduction in circulating neutrophil count post-baseline, the maximum reduction and maximum % reduction from baseline will be summarised by treatment group together with the visit that the maximum reduction occurred at.

The following plots will also be produced:

- Plots of mean neutrophil values for AZD5069 and placebo against time for:
  - Exercise challenge (pre-dose, 10 min, 2, 4 hours post exercise)
  - G-CSF challenge (pre-dose, 2, 6, 12, 24, 36 hours post-subcutaneous G-CSF)
  - Daily morning pre-dose, ie, 8 am, (Days 1 to 7)
  - Daily evening pre-dose, ie, 8 pm, (Days 1 to 6)
  - 24 hours profile (Day 3 – pre-dose, 1, 2, 3, 5, 8, 12 hours post-dose)
- Plots of mean absolute change from baseline (Day 1) in neutrophil values for AZD5069 and placebo against time, and mean percentage change from baseline (Day 1) in neutrophil values for AZD5069 and placebo against time for:
  - Daily morning pre-dose, ie, 8 am, (Days 1 to 7)
- Plots of individual curves for absolute neutrophils values for AZD5069 and placebo against time (one plot per sequence in order to make the plots less busy) for:
  - Exercise challenge (pre-dose, 10 min, 2, 4 hours post-exercise)
  - G-CSF challenge (pre-dose, 2, 6, 12, 24, 36 hours post-subcutaneous G-CSF)
  - 24 hours profile (Day 3 – pre-dose, 1, 2, 3, 5, 8, 12 hours post-dose)

Analyses of circulating neutrophils *during exercise challenge*: Analysis will be performed by fitting a mixed effect linear model, using $AUC_{(0-4)}/4$ (and ANC_{max}) as the response variable, the last circulating neutrophils measurement observed just prior to the challenge in each treatment period (ie, the measurement on Day 1 obtained in each treatment period) as a continuous covariate, treatment (placebo as reference), sequence and period as explanatory fixed factors, and subject within sequence as a random factor. Transformed back from the

logarithmic scale, the estimates of the geometric means from the fitted model, together with corresponding 95% confidence interval (CI) (2-sided) will be presented. Also the ratio of the geometric means for AZD5069 versus placebo will be presented together with corresponding 95% CI (2-sided).

Analyses of circulating neutrophils *during G-CSF challenge*: Analysis will be performed by fitting a mixed effect linear model, using $AUC_{(0-36)}/36$ (and ANC_{max}) as the response variable, the last circulating neutrophils measurement observed just prior to the challenge in each treatment period (ie, the measurement on Day 1 obtained in each treatment period) as a continuous covariate, treatment (placebo as reference), sequence and period as explanatory fixed factors, and subject within sequence as a random factor. Transformed back from the logarithmic scale, the estimates of the geometric means from the fitted model, together with corresponding 95% CI (2-sided) will be presented. Also the ratio of the geometric means for AZD5069 versus placebo will be presented together with corresponding 95% CI (2-sided).

To assess the potential differences between evening and morning PD parameters on Day 3, $AUC_{(0-12)}$ and ANC_{min} will be analysed using a repeated measures analysis of variance model. The model will include time (evening, morning) as a repeated-fixed effect. The results will be back-transformed and presented as geometric least squares means, geometric mean ratios, and associated 95% CIs. Evening will be the test and morning will be the reference for the purposes of these analyses.

If appropriate, PD parameters may be transformed (ie, natural log) prior to performing statistical analyses modelling.

12.2.4.2 Pharmacokinetics

For descriptive statistics the handling of concentrations below LLOQ values will be handled as follows:

- At a time point where less than or equal to 50% of the values are below the LLOQ (BLQ), all BLQ values will be set to LLOQ, and all descriptive statistics will be calculated.

- At a time point where more than half of the values are BLQ, the mean, SD, geometric mean and CV% will be set to Not Determined (ND). The maximum value will be reported from the individual data, and the minimum and median will be set to BLQ.

- If all values are BLQ at a time point, no descriptive statistics will be calculated for that time point. Not applicable (NA) will be written in the field for SD, CV%, and BLQ will be written in fields for mean, geometric mean, minimum, median, and maximum.

- The number of BLQ values will be reported for each time point.

Data from volunteers excluded from an analysis set will be included in the data listings, but not in the summaries.

A listing of PK blood sample collection times as well as derived sampling time deviations will be provided. A listing of all concentration-time data will be presented. Figures of arithmetic mean (SD) concentration-time data will be presented. Individual subject concentration-time data will be graphically presented on linear and semi-logarithmic scales. Mean, median, and individual $AUC_{(0\text{-}12),ss}$, $C_{ss,min}$ and $C_{ss,max}$ values will be presented versus dose regimen. Additional graphical presentations of PK data may be added at the discretion of the PK scientist.

To assess the potential differences between evening and morning PK parameters, $AUC_{(0\text{-}12),ss}$, $C_{ss,min}$, and $C_{ss,max}$ will be analyzed using a repeated measures analysis of variance model on the log-transformed data. The model will include time (evening, morning) as a repeated-fixed effect. The results will be back-transformed and presented as geometric least squares means, geometric mean ratios, and associated 90% CIs. Evening will be the test and morning will be the reference for the purposes of these analyses.

12.2.4.3 Pharmacokinetic/pharmacodynamic correlations

For Day 3, absolute neutrophil counts (ANC) and derived PD parameters including $AUC_{(0\text{-}12)}$ and ANC_{min} after morning and evening doses will be summarised using descriptive statistics. Pharmacodynamic parameters will be assessed and compared for morning and evening doses. Mean neutrophil counts will be graphically presented.

The relationship between plasma concentrations and circulating neutrophils after the morning and evening doses on Day 3 will be investigated using appropriate graphical methodology. A population PK/PD model may be fitted to the data. The PD parameters $AUC_{(0\text{-}12)}$ and ANC_{min} values will be compared to the pharmacokinetic (PK) parameters $AUC_{(0\text{-}12),ss}$ and $C_{ss,max}$ values, respectively, for both morning and evening values. The relationships will be depicted graphically and a regression model may be fitted to the data if appropriate.

12.3 Determination of sample size

A formal sample size calculation has not been performed for this study. A sample size of 30 with 28 completers is considered adequate to investigate effect on neutrophils and the effect of the different challenges.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such, see Section 6.3.4.**

In the case of a medical emergency the Principal Investigator may contact the AstraZeneca CPA Physician. If the AstraZeneca CPA Physician is not available, contact the CPA Programme Director as detailed below.

Name	**Role in the study**	**Address & telephone number**
	CPA Physician	AstraZeneca Pharmaceuticals
	Programme Director	AstraZeneca Pharmaceuticals
HMR emergency contact telephone number :		

13.2 Overdose

There is no known antidote to AZD5069. Investigators will be advised that any subject who inadvertently received a dose in excess of that specified within the CSP should be treated with appropriate supportive care until recovery and be followed up expectantly.

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the CRF and on the Overdose CRF module.
- An overdose without associated symptoms is only reported on the Overdose CRF module.

If an overdose on an AstraZeneca investigational product occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives **within one day**, ie, immediately but no later than **the end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site.

For overdoses associated with SAE, standard reporting timelines apply, see Section 6.3.4. For other overdoses, reporting should be done within 30 days.

13.3 Pregnancy

All outcomes of pregnancy should be reported to AstraZeneca.

13.3.1 Maternal exposure

Women of childbearing potential are not allowed to be included in this study. Should a pregnancy still occur, the investigational product should be discontinued immediately and the pregnancy reported to AstraZeneca.

13.3.2 Paternal exposure

Male subjects should refrain from fathering a child or donating sperm during the study and until 3 months after the follow-up visit (Visit 6).

Pregnancy of a subject's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should if possible be monitored and documented from the first administration until 3 months after the last administration of the investigational product.

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