Clinical Study Report Synopsis

Drug Substance	AZD5069
Study Code	D3550C00017
Edition Number	1
Date	
EudraCT Number	2011-004813-18

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

Study dates: First subject enrolled: 4 May 2012
Last subject last visit: 15 July 2012

Phase of development: Clinical pharmacology (I)

International Co-ordinating Investigator:

Sponsor's Responsible Medical Officer:

This study was performed in compliance with Good Clinical Practice, including the archiving of essential documents.

Study centre(s)

One study centre in the United Kingdom.

Publications

None at the time of writing this report.

Objectives and criteria for evaluation

Table S1 Objectives and outcome variables

Objective			Outcome Variable
Priority	**Type**	**Description**	**Description**
Primary	Pharmacodynamics	To investigate the effect of AZD5069 on neutrophil number and function (phagocytosis and oxidative burst) in the circulation of subjects	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	To investigate the effect of AZD5069 and placebo on circulating neutrophil numbers following a burst of strenuous exercise	Circulating neutrophil counts, pre-challenge-adjusted counts, and percent change-from pre-challenge counts during exercise challenge. Pre-challenge-adjusted and percent change-from pre-challenge $AUEC_{(0-4)}$, ANC_{max}, ANC_{min}, $ANCt_{max}$, $ANCt_{min}$, and $AUEC_{(0-4)}/4$
	Pharmacodynamics	To investigate the effect of AZD5069 and placebo on circulating neutrophils following subcutaneous injection of G-CSF	Circulating neutrophil counts, pre-challenge-adjusted counts, and percent change-from pre-challenge counts following subcutaneous injection of G-CSF. Pre-challenge-adjusted and percent change-from pre-challenge $AUEC_{(0-36)}$, ANC_{max}, ANC_{min}, $ANCt_{max}$, $ANCt_{min}$, and $AUEC_{(0-36)}/36$
Secondary	Safety	To evaluate general safety and tolerability of AZD5069	Adverse events (AEs), 12-lead electrocardiogram (ECG), physical examination including signs of infection, haematology, clinical chemistry and urinalysis[a], daily neutrophil counts, vital signs and body temperature

Objective			Outcome Variable
Priority	**Type**	**Description**	**Description**
	Pharmacokinetics	To evaluate the steady state 24 hour pharmacokinetic (PK) profile of the AZD5069 capsule following bid dosing	For AZD5069 after both morning and evening doses: $AUC_{(0-12),ss}$, $C_{ss,max}$, $t_{ss,max}$, $C_{ss,min}$, $t_{ss,min}$, and C_{12}
	Pharmacodynamics	To evaluate the steady state 24 hour profile of circulating neutrophils following bid dosing of AZD5069 and its relationship to plasma concentration	After both morning and evening doses, circulating neutrophil counts, absolute change from baseline, baseline-adjusted counts, and percent change-from baseline counts. Baseline-adjusted and percent change-from baseline $AUEC_{(0-12)}$, ANC_{max}, ANC_{min}, $ANCt_{max}$, $ANCt_{min}$, and $AUEC_{(0-12)}/12$
Exploratory[a]	Pharmacodynamics	To assess which subpopulation of neutrophils is reduced on AZD5069	Not applicable
	Genetics	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	Not applicable
	Pharmacodynamics	To explore potential up-regulation of biomarkers, ie, interleukin (IL)-8, G-CSF, GRO-α and ENA-78	Not applicable

[a] Results from the exploratory analyses, if performed, will be reported separately from the Clinical Study Report.

ANC_{max} maximum neutrophil count; ANC_{min} minimum neutrophil count; $ANCt_{max}$ time to maximum neutrophil count; $ANCt_{min}$ time to minimum neutrophil count; $AUC_{(0-12),ss}$ area under the plasma concentration-time curve from 0 to 12 hours after multiple dose administration; $AUEC_{(0-4)}$, $AUEC_{(0-12)}$, $AUEC_{(0-36)}$ area under the neutrophil value-time curve from 0 to 4, 12, and 36 hours, respectively; C_{12} plasma concentration 12 hours postdose $C_{ss,max}$ maximum plasma concentration after multiple dose administration; CSP Clinical study protocol; $C_{ss,min}$ minimum plasma concentration after multiple dose administration; $t_{ss,max}$ time to reach maximum plasma concentration; $t_{ss,min}$ time to reach minimum plasma concentration.

Study design

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

All potentially suitable subjects attended the study centre for an information visit where the study was explained and informed consent collected (Visit 1) before starting study specific screening (Visit 2). Visits 1 and 2 could have occurred on the same day as per the study centre requirements. Screening was conducted within 28 days of randomisation (Visit 3). After screening, subjects who fulfilled all the inclusion criteria and none of the exclusion criteria were 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 was very important due to diurnal variation in neutrophils. Investigational product administration was to be at the time points stated above ±30 minutes. Each subject received the investigational product at the same time point on each occasion with a maximum difference of 15 minutes. The investigational product was administered orally, twice daily (bid), for 6 days during Treatment Period 1. After Treatment Period 1 there was a washout period for a minimum of 21 days after which the subjects were admitted for another 6 days of treatment (Treatment Period 2).

On Day 3 (after dose 4) the circulating neutrophil profile over 24 hours and a corresponding pharmacokinetic (PK) profile to define the steady state kinetics in each subject were determined. Subjects had to perform an exercise challenge on Day 4. On Day 5, after the morning dose, the subjects were administered 300 μg G-CSF subcutaneously.

At three time points during each treatment period (baseline, at steady state [Day 4], and 7 days after end of treatment) fresh neutrophils were collected to run functional assays of phagocytosis and oxidative burst. At these time points there was also an evaluation of flow cytometry to evaluate which subpopulation of neutrophils was affected. Blood samples for explorative biomarkers (G-CSF, GRO-α, ENA-78 and IL-8) were also taken at these times (results from these exploratory analysis are reported separately from this CSR). The subjects remained in the study centre during the whole treatment period and circulating neutrophils were 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 took place 7 days after the last day of investigational product administration, when neutrophil function and leukocyte differential counts were assessed at the same time of the day as the other samples were collected. Neutrophil function was 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 and sample size

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, who provided written informed consent.

Planned: 30 healthy volunteers

Randomised: 30 healthy volunteers

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

Table S2 Details of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer	Batch number
AZD5069	50 mg capsules	AstraZeneca	P Lot: 11-003104AZ F Lot: 10-003546AZ
Placebo	Capsules to match AZD5069	AstraZeneca	P Lot: 11-003103AZ F Lot: 08-001091AZ

Duration of treatment

The study duration for each subject was 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 received a total of 12 doses of AZD5069 and 12 doses of placebo:

- Treatment A: 100 mg AZD5069 (2 x 50 mg capsule) orally twice daily for 6 days
- Treatment B: Placebo matching AZD5069 capsule twice daily for 6 days

Statistical methods

All statistical analyses were performed by Quintiles, Overland Park, Kansas (USA) and Quintiles, Bangalore (India) using SAS® Version 9.2.

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

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

Data are 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) were summarised in frequency tables (frequency and proportion of subjects in the analysis set).

Analyses of circulating neutrophils *during exercise challenge*: Analysis was performed by fitting a mixed effect linear model, using the logarithm of $AUEC_{(0-4)}/4$ (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) are presented. Also the ratio of the geometric means for AZD5069 versus placebo is presented together with corresponding 95% CI (2-sided).

Analyses of circulating neutrophils *during G-CSF challenge* were similar to those performed for the circulating neutrophils *during exercise challenge*.

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

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

The relationship between plasma concentrations and circulating neutrophils after the morning and evening doses on Day 3 were investigated using appropriate graphical methodology.

Adverse events were 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 were 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 were summarised.

Tabulations and listings of data for vital signs (blood pressure and pulse), clinical laboratory tests, electrocardiograms, and physical examination findings are presented. Where applicable, data were summarised for the absolute value at each scheduled assessment, and for the corresponding change from baseline.

Subject population

In total, 39 healthy volunteers signed the informed consent form and 30 healthy volunteers were randomised into the study. All randomised healthy volunteers were received investigational product.

One subject (3.3%) was withdrawn on his own decision and 2 subjects (6.7%) were withdrawn due to AEs (tonsillitis and orchitis). All 30 subjects (100.0%) received the scheduled investigational product as per the randomisation scheme, but only 27 subjects (90.0%) completed the study.

Summary of pharmacodynamic/pharmacokinetic results

Neutrophil function

All individual phagocytosis and oxidative burst function results were normal, where normal is defined as more than 80% of neutrophils observed phagocytosing and oxidising when stimulated with E. Coli at 37°C for both AZD5069 and placebo at each scheduled time point

(Day 1, Day 4, and at follow-up, 7 days after end of treatment). This shows that AZD5069 did not affect the function of neutrophils with respect to phagocytosis and oxidative burst in healthy subjects in this study.

Table S3 Number of subjects with neutrophil function (phagocytosis and oxidative burst) change in status normal/abnormal

		Oxidative burst		Phagocytosis	
Treatment group		**Change from baseline to Day 4**	**Change from baseline to Follow-up**	**Change from baseline to Day 4**	**Change from baseline to Follow-up**
AZD5069	Normal to normal	27	28	27	27
	Normal to abnormal	0	0	0	0
	Abnormal to normal	0	0	0	0
	Abnormal to abnormal	0	0	0	0
Placebo	Normal to normal	28	29	29	29
	Normal to abnormal	0	0	0	0
	Abnormal to normal	0	0	0	0
	Abnormal to abnormal	0	0	0	0

Day 4, exercise challenge

The arithmetic mean (±SD) circulating neutrophil count versus time profiles and percent change from pre-challenge neutrophil count versus time profiles are presented below.

Figure S1 **Arithmetic mean (± SD) neutrophil count (x10^9/L) versus time after the exercise challenge for each treatment on Day 4.**

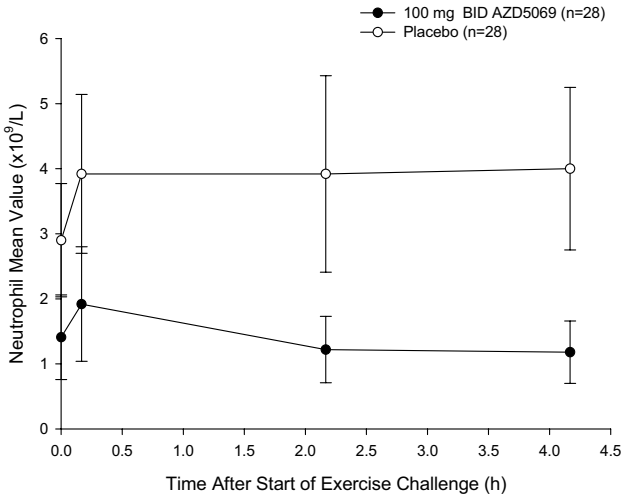
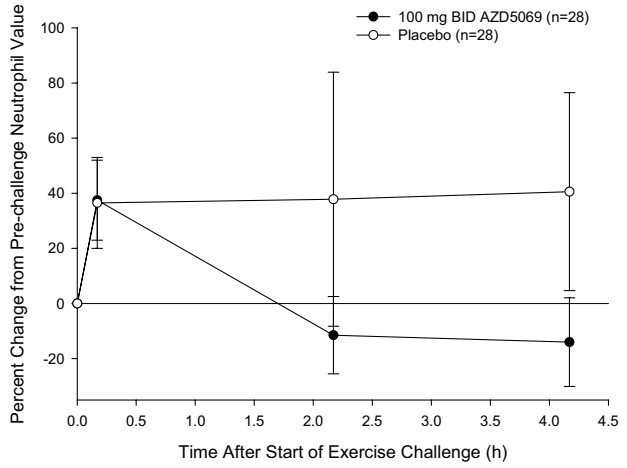


Figure S2 **Arithmetic mean (± SD) neutrophil percent change from pre-exercise challenge value versus time after the exercise challenge for each treatment on Day 4.**



Immediately after the 10 minute exercise challenge, mean neutrophil counts increased from pre-challenge observations during both AZD5069 and placebo treatments. During the 4-hour sampling period after the exercise challenge, mean neutrophil counts decreased in subjects receiving AZD5069 while in subjects receiving placebo, neutrophil counts remained elevated

Point estimates of the geometric least-squares mean ratios and associated 95% confidence intervals for the pre-challenge value adjusted primary pharmacodynamic parameters for Day 4, exercise challenge are presented in Table S3.

Table S4 Statistical comparison of primary pre-challenge value adjusted pharmacodynamic parameters during the Day 4, exercise challenge

					Comparison of AZD5069 to placebo		
Parameter	**Treatment**	**n**	**Geometric LS mean**	**95% CI**	**Ratio (%)**	**95% CI**	**Geo CV (%)**
ANC_{max}	AZD5069	28	1.317	(1.214, 1.428)	81.30	(71.19, 92.84)	15.9
	Placebo	28	1.620	(1.495, 1.756)			
$AUEC_{(0-4)}/4$	AZD5069	28	0.9570	(0.8827, 1.038)	68.02	(59.82, 77.35)	14.4
	Placebo	28	1.407	(1.298, 1.524)			

Following exercise challenge, there was an estimated 19% reduction in ANC_{max} (AZD5069 versus Placebo), and an estimated 32% reduction in $AUEC_{(0-4)}/4$ (AZD5069 vs placebo).

Day 5, granulocyte-colony stimulating factor challenge

The arithmetic mean (±SD) circulating neutrophil count versus time profiles and percent change from pre-challenge neutrophil count versus time profiles are presented below.

Figure S3 Arithmetic mean (± SD) neutrophil count ($x10^9$/L) versus time during the G-CSF challenge for each treatment on Day 5.

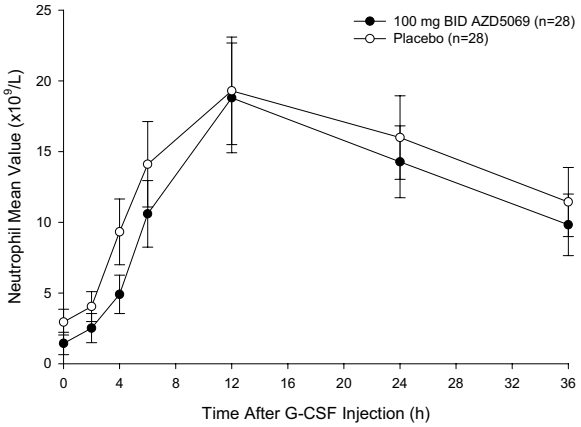
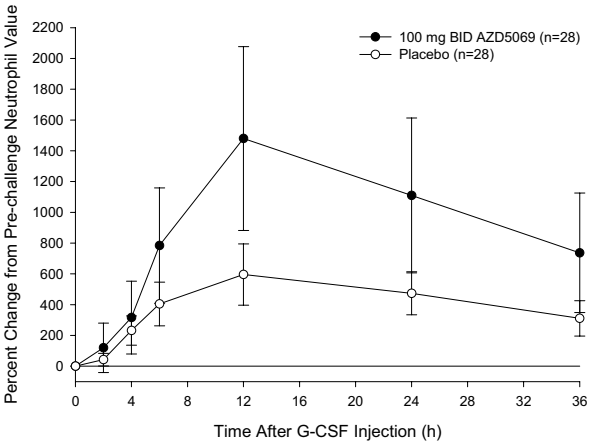


Figure S4 **Arithmetic mean (± SD) neutrophil percent change from pre-G-CSF injection challenge value versus time during the G-CSF challenge for each treatment on Day 5.**



Following AZD5069 administration, subjects had on average a larger percent increase from pre-challenge neutrophil values.

Point estimates of the geometric least-squares mean ratios and associated 95% confidence intervals for the pre-challenge value adjusted primary pharmacodynamic parameters for Day 5, G-CSF challenge are presented in Table S4.

Table S5 **Statistical comparison of primary pre-challenge value adjusted pharmacodynamic parameters during the Day 5, G-CSF challenge**

					Comparison of AZD5069 to placebo		
Parameter	**Treatment**	**n**	**Geometric LS mean**	**95% CI**	**Ratio (%)**	**95% CI**	**Geo CV (%)**
ANC_{max}	AZD5069	28	10.54	(9.669, 11.49)	111.98	(99.33, 126.25)	11.7
	Placebo	28	9.411	(8.643, 10.25)			
$AUEC_{(0-36)}/36$	AZD5069	28	7.127	(6.616, 7.678)	101.78	(91.92, 112.70)	9.6
	Placebo	28	7.003	(6.507, 7.536)			

Following G-CSF challenge there was an estimated 12% increase in ANC_{max} (AZD5069 versus Placebo) and an estimated 2% increase in $AUEC_{(0-36)}/36$ (AZD5069 vs placebo).

Day 3, 24-hour profile of circulating neutrophils

The arithmetic mean (±SD) circulating neutrophil count versus time profiles and percent change from baseline neutrophil value versus time profiles for Day 3 are presented below.

Figure S5 **Arithmetic mean (± SD) neutrophil count versus time by treatment, Day 3 on linear scale.**

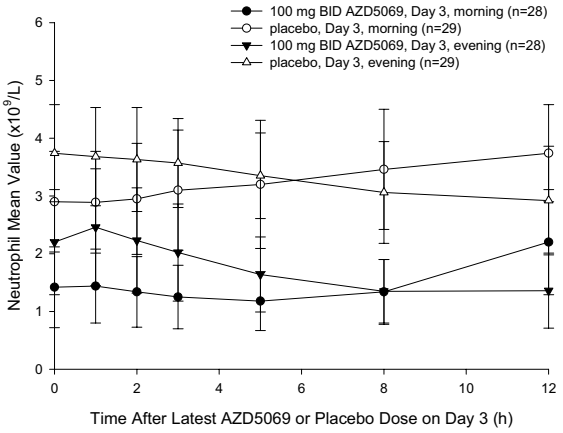
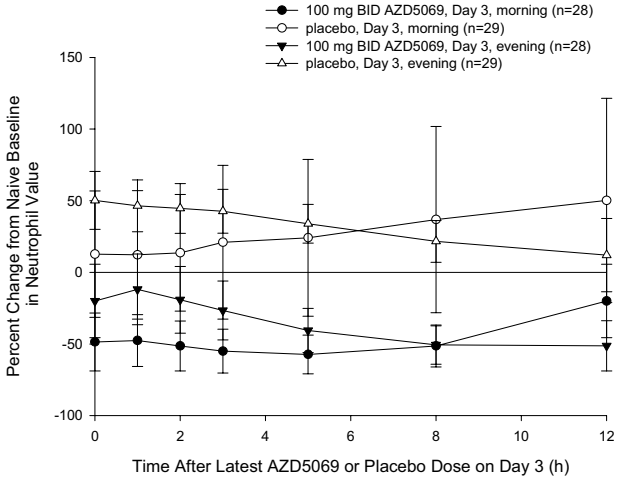


Figure S6 **Arithmetic mean (± SD) neutrophil percent change from naïve baseline versus time by treatment, Day 3 on linear scale.**



Baseline is the period related baseline at Day 1, morning dose (naïve baseline)

On Day 3 following both morning and evening administration of AZD5069, mean neutrophil counts were lower in subjects receiving AZD5069 as compared with placebo throughout the 12 hours after dosing. The median time to ANC_{min} occurred at 5 hours after the morning AZD5069 dose. Mean neutrophil counts remained below the naïve baseline in subjects receiving AZD5069 throughout the sampling period for both morning and evening. The pattern of response over time after treatment with AZD5069 indicates that a diurnal variation was maintained.

Point estimates of the geometric least-squares mean ratios and associated 95% confidence intervals for the naïve baseline-adjusted primary pharmacodynamic parameters for Day 3 are presented in Table S5.

Table S6 Statistical comparison of baseline-adjusted pharmacodynamic parameters for Day 3 by treatment

						Comparison of evening to morning		
Parameter	**Treatment**	**Time**	**n**	**Geometric LS mean**	**95% CI**	**Ratio (%)**	**95% CI**	**Geo CV (%)**
ANC_{min}	AZD5069	evening	28	0.4339	(0.3871, 0.4863)	110.03	(103.74, 116.69)	10.8
		morning	28	0.3943	(0.3518, 0.4420)			
	Placebo	evening	29	1.038	(0.9268, 1.162)	104.17	(99.31, 109.27)	8.9
		morning	29	0.9961	(0.8897, 1.115)			
$AUEC_{(0-12)}$	AZD5069	evening	28	7.055	(6.322, 7.874)	117.30	(112.07, 122.77)	8.3
		morning	28	6.015	(5.390, 6.713)			
	Placebo	evening	29	14.73	(13.08, 16.58)	100.98	(96.71, 105.44)	8.0
		morning	29	14.59	(12.96, 16.42)			

Baseline is the period related baseline at Day 1, morning dose (naïve baseline)

For Day 3, ANC_{min} baseline-adjusted geometric least-squares mean values were approximately 10% and 4% higher after the evening dose as compared with the morning dose in the AZD5069 and placebo treatments, respectively.

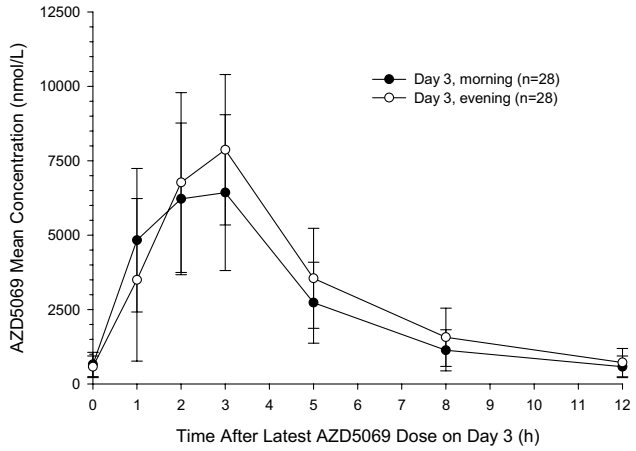
For Day 3, $AUEC_{(0-12)}$ baseline-adjusted geometric least-squares mean values were approximately 17% and 1% higher after the evening dose as compared with the morning in the AZD5069 treatment group and placebo treatment group, respectively.

Pharmacokinetic results

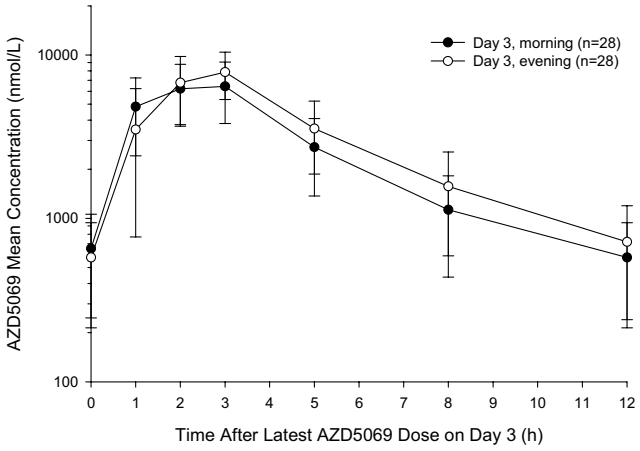
The arithmetic mean (±SD) AZD5069 plasma concentration-time profiles for subjects receiving AZD5069 on Day 3, morning and evening are presented below.

Figure S7 Arithmetic mean (±SD) plasma concentration (nmol/L) of AZD5069 versus time by treatment on linear and semi-logarithmic scales

Linear



Semi-logarithmic



For much of the 12-hour sampling period, AZD5069 plasma concentrations were lower on average following the morning dose in comparison with the concentrations following the evening dose.

Point estimates of the geometric least-squares mean ratios and associated 90% confidence intervals for the AZD5069 primary pharmacokinetic parameters for Day 3 are presented in Table S6.

Table S7 Statistical comparison of primary AZD5069 pharmacokinetic parameters

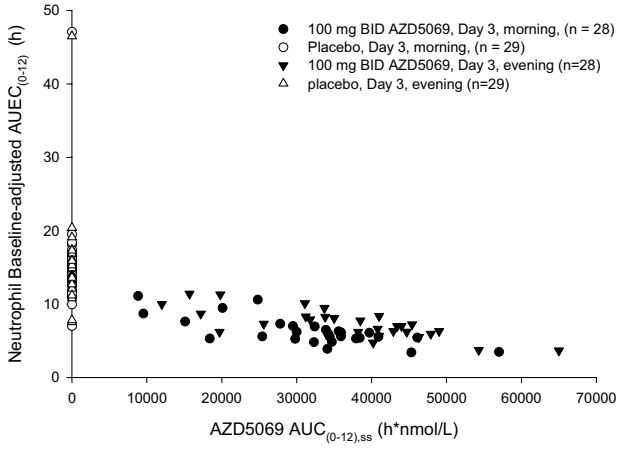
					Comparison of evening to morning		
Parameter	**AZD5069/ Time**	**n**	**Geometric LS mean**	**95% CI**	**Ratio (%)**	**90% CI**	**Geo CV (%)**
$AUC_{(0\text{-}12),ss}$	Day 3 evening	28	34370	(29260, 40360)	116.64	(112.55, 120.88)	7.9
(h·nmol/L)	Day 3 morning	28	29470	(25090, 34600)			
$C_{ss,max}$	Day 3 evening	28	8543	(7554, 9661)	115.02	(106.90, 123.76)	16.2
(nmol/L)	Day 3 morning	28	7427	(6568, 8399)			
$C_{ss,min}$	Day 3 evening	28	445.6	(332.0, 598.2)	100.93	(98.72, 103.19)	4.9
(nmol/L)	Day 3 morning	28	441.5	(328.9, 592.7)			

There were no major differences in systemic exposure of AZD5069 after the morning and evening doses. $AUC_{(0\text{-}12),ss}$ was on average 17% (90% CI: 13% to 21%) and $C_{ss,max}$ 15% (90% CI: 7% to 24%) higher after dosing in the evening.

Summary of pharmacokinetic/pharmacodynamic relationships

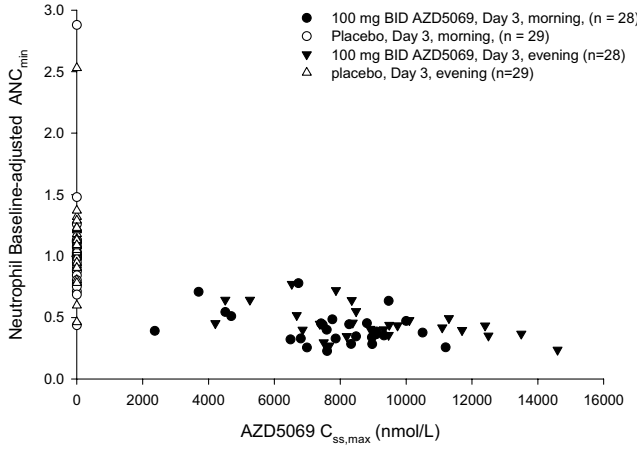
The relationships between Day 3 baseline-adjusted neutrophil $AUEC_{(0\text{-}12)}$ and AZD5069 $AUC_{(0\text{-}12),ss}$ and between Day 3 baseline-adjusted neutrophil ANC_{min} and AZD5069 $C_{ss,max}$ were explored graphically.

Figure S8 Individual subject $AUEC_{(0\text{-}12)}$ of baseline-adjusted circulating neutrophil on Day 3 (morning and evening) versus $AUC_{(0\text{-}12),ss}$.



Baseline is the period related baseline at Day 1, morning dose (naïve baseline).

Figure S9 **Individual subject ANC_{min} of baseline-adjusted circulating neutrophil on Day 3 (morning and evening) versus $C_{ss,max}$.**



Baseline is the period related baseline at Day 1, morning dose (naïve baseline).

There was a trend in the figures towards lower baseline adjusted neutrophil counts with increasing AZD5069 exposure.

Summary of safety results

The incidence of subjects reporting AEs was similar for on treatment with both AZD5069 and Placebo. The most commonly reported AEs were back pain and headache. Most of the subjects who reported events of back pain reported these events within 24 hours after receiving the G-CSF subcutaneous injection.

Two subjects prematurely withdrew from the study due to AEs, 1 subject due to tonsillitis on Day 2, Period 1 while on AZD5069 treatment and 1 subject due to orchitis after Placebo treatment during Period 1. The events were considered to be not related to the investigational product administration. The incidence of subjects with at least one AE considered to be related to the investigational product administration was higher on treatment with AZD5069 (27.6%) than Placebo (17.2%).

Most of the AEs were considered to be mild (23.2% overall; 17.2% for AZD5069 and 31.0% for Placebo) to moderate (40.0% overall; 37.9% for AZD5069 and 24.1% for Placebo) in intensity by the investigator. An event of orchitis, recorded for Subject E0002017 (3.3%; Placebo), was considered to be severe in intensity.

A reduction in neutrophils was observed, as expected, however the values returned to baseline after the investigational product administration was stopped.

Overall, no clinically relevant changes in clinical chemistry, vital signs or 12-lead ECG results were observed.