St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

Quality Statement

This non-GLP study was conducted using sound scientific principles and established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Preclinical Pharmacokinetic Shared Resource and St. Jude Children's Research Hospital, Memphis, TN, USA. This report accurately reflects the data obtained during the course of this study.

These results represent part of an early phase preclinical pharmacology program. This study has been conducted to provide preliminary insights into the pharmacokinetic (PK) properties of the compound(s) in the indicated preclinical model(s). This study and its results are not intended to provide a comprehensive PK evaluation of the compound(s). The applied bioanalytical method was validated/qualified to support this specific study and discovery-style sample analyses.

Substantial study-to-study and inter-animal variability in preclinical PK exists. Such variability depends upon the in vivo scientists' experience, variations in compound purity and formulation, animal strains, sex and age, and other situational fixed effects (i.e. husbandry conditions, presence or absence of disease, concomitant drugs). As such, the actual PK, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program (DBSTP)

P-PKSR Study 103489-1063453

STUDY TITLE:

INITIAL PLASMA PHARMACOKINETICS OF AZD6738 IN FEMALE CD1 NU/NU MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**AZD6738 Initial PK**	
TEST ARTICLE:	AZD6738	
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)	
PRINCIPAL INVESTIGATOR(S)	Stewart, Elizabeth	
SJCRH SRM2 O/R:	103489-1063453	Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA	NA
IN VIVO SCIENTIST(S)	Stewart, Elizabeth Ocarz, Monica Gordon, Brittney	
BIOANALYTICAL SCIENTIST:	Wang, Lindsey	
REPORT AUTHOR(S):	Freeman, Burgess Wang, Lindsey Stewart, Elizabeth	
REPORT FORMAT:	Nonregulated Report	
REPORT STATUS:	FINAL	
DATE:	2018-06-04	

FOR P-PKSR APPROVED USE AND DISTRIBUTION

Quality Statement

This non-GLP study was conducted using sound scientific principles and established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Preclinical Pharmacokinetic Shared Resource and St. Jude Children's Research Hospital, Memphis, TN, USA. This report accurately reflects the data obtained during the course of this study.

These results represent part of an early phase preclinical pharmacology program. This study has been conducted to provide preliminary insights into the pharmacokinetic (PK) properties of the compound(s) in the indicated preclinical model(s). This study and its results are not intended to provide a comprehensive PK evaluation of the compound(s). The applied bioanalytical method was validated/qualified to support this specific study and discovery-style sample analyses.

Substantial study-to-study and inter-animal variability in preclinical PK exists. Such variability depends upon the in vivo scientists' experience, variations in compound purity and formulation, animal strains, sex and age, and other situational fixed effects (i.e. husbandry conditions, chow constituents, presence or absence of disease, concomitant drugs). As such, the actual PK, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

Signatures (Nonregulated Report)

Approved By:

Burgess B. Freeman III, PharmD
Director
Preclinical Pharmacokinetic Shared Resource
St. Jude Children's Research Hospital

Date

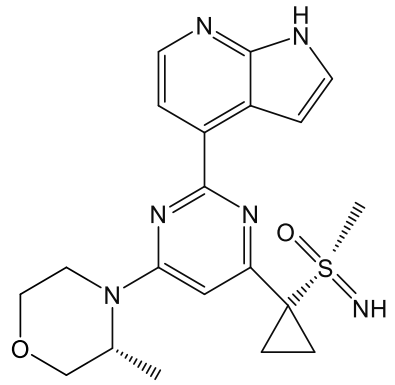
Table of Contents

1.0 INTRODUCTION

The ATR inhibitor AZD6738 (*AstraZeneca*) is being investigated by the DBSTP (PI Dr. Elizabeth Stewart) for single agent and combination therapies in several pediatric solid tumors, including neuroblastoma (NBL). The goal of this study is to determine the plasma PK characteristics of AZD6738 in 10% DMSO / 40% propylene glycol / 50% UP water in nontumor bearing mice.

2.0 MATERIALS AND METHODS

2.1 Test Articles



Compound	AZD6738
Molecular Weight	412.51
SJ REG #	SJ000861428-2
CAS #	1352226-88-0
Vendor	LC Labs
Lot #	L15794B001
Exp. Date	NA
Purity	91.3% (CBT-HTAC QC)* NA (Vendor)

* QC documentation is included in Attached File 6.1.

2.2 Formulations

Formulation: AZD6738 in 10% DMSO / 40% Propylene Glycol / 50% UP water, 5 mg/mL free base equivalent final nominal concentration, 10 mL/kg gavage volume, 50 mg/kg dosage.

Item	Vendor	Lot #	Exp. Date
DMSO	Fisher	150785	2020-04
Propylene Glycol	Dickson Company	NA	2016-01
DDI / UP H_2O	Millipore	NA	NA

The AZD6738 stock solution at 20 mg/mL in DMSO was prepared by Lindsey Wang on 2017-03-20 using a standardized procedure (see **Appendix 7.1**). Briefly, 0.2 mL of AZD6738 DMSO stock was added into a 2 mL volumetric flask, followed by 0.8 mL of propylene glycol, then the total volume was brought up to 2 mL with DDI/UP water. The volumetric flask was indirectly sonicated in a water bath for 30 minutes, then the formulation was transferred into glass dosing vial. The dosing solution appeared visually homogenous, and was stored at ambient temperature until use (see **§ 2.4 Dosing**). NOTE: Some formulating materials were beyond the re-test / expiration dates; however, the probability of this deviation affecting the study findings is minimal, in the opinion of the P-PKSR scientists.

2.3 Animals

Six (6) female CD1 nu/nu mice (Jax Laboratories), aged 12-16 weeks and weighing from 21.0-26.5 g, were used. Mice were permitted standard chow and purified water *ad libitium* during the study, and were housed under SJCRH IACUC-approved animal husbandry conditions.

2.4 Dosing

Animals were dosed once with 50 mg/kg AZD6738 free base equivalents (5 mg/mL in final formulation) via a 10 mL/kg oral gavage using a 20 gauge flexible plastic feeding tube (Instech FTP-20-38) attached to a 1 mL syringe. Individual dosages were determined based upon the total body weight of each animal recorded on the day of dosing. The calculated volume gavaged in mL was rounded to the nearest hundredth decimal place. Animals were dosed starting on Day 1 at 2017-03-20T15:30:00-40:00 and ending at Day 2 on 2017-03-21T08:00:00-10:00.

2.5 Plasma Sample Collection

A batch sampling study design was used, whereby each animal provided four (4) non-terminal blood samples by retro-orbital bleed and one terminal blood sample via cardiac puncture upon sacrifice with an IACUC-approved technique. **Table 2.1** below lists the scheduled sampling schema. For more information, see **Appendix 7.1. and 7.2.**

Table 2.1 Sample Collection Schedule

Time after dose (hr)	0.167	0.25	0.5	1	2	4	8	16	24
Animal IDs: Group 1		M1 M2 M3	M1* M2* M3*	M1 M2 M3				M1 M2 M3	M1 M2 M3
Animal IDs: Group 2	M4 M5 M6				M4 M5 M6	M4 M5 M6	M4 M5 M6		M4 M5 M6

* Sample time deviation: M1-3 0.5 hr obtained at 0.67 hr. All other sampled at nominal scheduled times.

At each survival sampling time point, the mouse was anesthetized with isoflurane as per IACUC approved protocol. Then ~50 µL of whole blood was collected from the retro-orbital plexus using a Minivette capillary device (Sarstedt, cat no. 17.2113.150, lot 5281601) pre-coated with K3EDTA and transferred into a plastic microcentrifuge tube (1.5 mL, Fisher cat # 05-408-129 or equivalent). For terminal samples, mice were anesthetized with 0.6 mL of Avertin (tribromoethanol, 12.5 mg/mL) by intraperitoneal injection, and 0.5 – 1 mL of whole blood was collected from closed cardiac puncture using a 25 gauge needle attached to a 1 mL syringe. Cardiac blood was then transferred into a Microvette K3EDTA microcentrifuge tube (Sarstedt, cat no. 20.1341.102, lot 5750411M), and gently vortexed. All blood samples were immediately centrifuged at ambient temperature for 2 min at 10000 rpm to generate plasma. Each plasma supernatant was transferred into an appropriately labeled microcentrifuge tube, placed on dry ice for remainder study, and transferred to -80 ºC until analysis.

2.6 Bioanalytical Summary

Matrix calibrators and quality controls were spiked with analyte from stock solutions prepared in methanol using AZD6738 (BioVision, 3A27B11670, Purity 98%). Matrix samples, 25 µL each, were protein precipitated with 100 µL of acetonitrile and 25 µL of 50 ng/mL GDC-0941 (courtesy of CBT) in methanol as an internal standard. A 5 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a Shimadzu SIL-20AC XR autosampler. The LC separation was performed using a Phenomenex Kinetex C18 (2.6 µm, 50 mm x 2.0 mm) column maintained at 40 °C with gradient elution at a flow rate of 0.25 mL/min. The binary mobile phase consisted of 0.1% formic acid in water in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase was maintained at 20% B for half minute, with a linear increase to 50% B in half minute, then to 100% B in half minute. The column was then rinsed for half minute at 100% B and then

equilibrated at the initial conditions for two minutes. The total run time was four minutes. Under these conditions, the analyte and IS eluted at 2.32 and 2.41 minutes, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 4400 in the positive ESI mode and the following mass transitions were monitored: AZD6738 413.2 -> 264.2, GDC-0941 514.2 -> 338.1.

The method qualification and bioanalytical runs all passed P-PKSR's acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the calibrators across the 1 to 100 ng/mL range, with a correlation coefficient (R) of ≥ 0.9959. The lower limit of quantitation (LLOQ), defined as a peak area signal-to-noise ratio of 5 or greater verses a matrix blank with IS, was 1 ng/mL. The intra-run precision and accuracy was < 9.61% CV and 93.93% to 111.8%, respectively.

For more information, please refer the bioanalytical method qualification and run report archived by the P-PKSR. NOTE: Bioanalytical validation, qualification, and/or run reports are marked "COMPANY CONFIDENTIAL," and are not for distribution outside SJCRH as per P-PKSR policy.

2.7 Data and Statistical Analyses

The bioanalytical concentration results were processed by run and matrix using Analyst 1.6.2 software (SCIEX, Framingham, MA) and outputted as standardized tab delimited text (.txt) files. These .txt files were subsequently processed using R software [1]. The concentrations for analytes were grouped by compound, matrix, day, and nominal sample time, and arithmetic means (Mean) and standard deviations (SD) were generated. If at any time point, ≥ 2/3rds of the results were below the assay LLOQ (BLOQ), then the entire time point was treated as missing. Otherwise, any data BLOQ were replaced with a value of ½ LLOQ, and the concentration Mean and SD values calculated.

2.8 Pharmacokinetic (PK) Analyses

The AZD6738 concentration-time (Ct) data was subjected to noncompartmental pharmacokinetic analysis (NCA) using Phoenix WinNonlin 6.4 (Certara USA, Inc., Princeton, NJ). The extravascular model (Model 202) was applied, and area under the Ct curve (AUC) values were estimated using the "linear up log down" method. The terminal phase was defined as at least three time points at the end of the Ct profile, and the elimination rate constant (Ke) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Ke, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + Clast (predicted)/Ke. Other parameters estimated included observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of Clast (Tlast), apparent clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F).

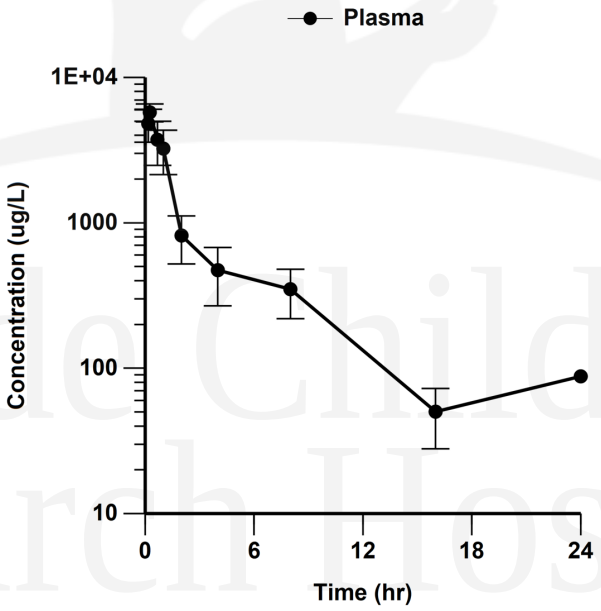
3.0 RESULTS AND DISCUSSION

AZD6738 showed rapid absorption, with the Cmax observed at 0.25 hr. Overall, the inter-animal variability in Ct observations was modest, %CV between 13.9% and 44.4%; however, high variability was noted at 24 hr time point: %CV 115.0%. The mean plasma concentration at 24 hr (N=6) also appeared to be higher than that at 16 hr (N=3), possibly due the difference in sampling approach, as the final sample for each mouse at 24 hr was terminal by cardiac puncture, while all other time pointes were survival sampling by retro-orbital sinus bleeding. The most commonly reported preclinical formulation of 10% DMSO / 40% propylene glycol / 50% UP water was found to be adequate and stable for 10 days at ambient temperature, with a mean assessed concentration of 4.75 mg/mL and %CV of 4.98% across 6 aliquots. **Figure 3.1** presents the mean (SD) Ct profiles, while the corresponding NCA PK parameter estimates are reported in **Table 3.1**.

Table 3.1 Noncompartmental PK Parameter Estimates

		Matrix
		Plasma
Parameter	**Units**	**Estimate**
Cmax	ug/L	5760
Tmax	hr	0.250
AUClast	hr*ug/L	10400
AUCinf	hr*ug/L	10600
Kel	1/hr	0.162
T1/2	hr	4.29
CL/F	L/hr/kg	4.71
Vz/F	L/kg	29.1
Clast	ug/L	87.9
Tlast	hr	24.0

Figure 3.1 Mean (SD) Ct Profiles



Compared with the limited mouse PK data published for AZD6738 to date, our plasma exposures appeared to be slightly lower in the current described study. When contrasted with a mouse oral plasma PK profile simulated from parameters reported in Checkley et al. [2], we demonstrated lower Cmax (5760 vs 17900 ug/L) and AUCinf (10600 vs 82600 hr-ug/L) values, respectively. The terminal half-life in our current study was also much shorter, 4.29 vs ~18 hr. However, compared with limited mouse plasma data reported by Clack [3], our PK parameter estimates were more aligned. It is difficult to determine the source of variance between theses preclinical PK studies, but we speculate it could be due to different forms or formulations, or poor assumptions (i.e. linear, proportional PK with dose in mice).

To date, detailed human plasma PK information is unavailable in the literature, and is limited to one abstract [4], in which only the Tmax (1.5 hr) and terminal half-life (11 hr) were reported. Multiple combinations and regimens of AZD6738 were studied in this reported Phase 1 study, with AZD6738 160 mg PO QD D1-7 as the apparent recommended Phase 2 dose (RP2D), in combination with olaparib 300 mg PO QD D1-28. No clinically relevant dosage (CRD) recommendation for AZD6738 in mice can be made, as insufficient clinical PK data are available. However, preclinical studies in mice have used doses of to 25 to 50 mg/kg PO QD for 20 to 28 days. Such regimens were generally well-tolerated, depending on whether it was single agent or combination therapy (e.g. cisplatin, radiation).

4.0 CONCLUSIONS

- Free base AZD6738, 50 mg/kg, in 10% DMSO / 40% PG / 50% UP water solution, displayed rapid absorption, a distinct distribution and elimination phase (T1/2 = 4.29 hr) in plasma, and low to modest inter-mouse variability for all but the 24 hour point.
- Plasma exposure of AZD6838 in this study was slightly lower than the limited previously published data in mice.
- A PK-based clinically relevant dose (CRD) for AZD6738 could not be derived, as there is insufficient clinical PK available in literature.
- Given literature, candidate mouse regimens include AZD6738 25 mg/kg PO QD or BID or 50 mg/kg PO QD.

5.0 REFERENCES

1. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2016. Available from: https://www.R-project.org/
2. Checkley S, MacCallum L, Yates J, Jasper P, Luo H, Tolsma J, Bendtsen C. Bridging the gap between in vitro and in vivo: Dose and schedule predictions for the ATR inhibitor AZD6738. Sci Rep. 2015 Aug 27;5:13545.
3. Clack G, Lau A, Pierce A, Smith S, Stephens C. ATR inhibitor AZD6738. Ann Oncol [Internet]. 2015 Mar 1 [cited 2017 Nov 27];26(suppl_2):ii8-ii8. Available from: https://academic.oup.com/annonc/article/26/suppl_2/ii8/151854
4. Yap TA, Krebs MG, Postel-Vinay S, Bang YJ, El-Khoueiry A, Abida W, Harrington K, Sundar R, Carter L, Castanon-Alvarez E, Im SA, Berges A, Khan M, Stephens C, Ross G, Soria JC. Phase I modular study of AZD6738, a novel oral, potent and selective ataxia telangiectasia Rad3-related (ATR) inhibitor in combination (combo) with carboplatin, olaparib or durvalumab in patients (pts) with advanced cancers. Eur J Cancer [Internet]. 2016 Dec 1 [cited 2017 Nov 27];69:S2. Available from: http://www.ejcancer.com/article/S0959-8049(16)32607-7/abstract

6.0 ATTACHED FILES

Attached File 6.1. A15794_AZD6738_20170323_HTAC_completed.zip

7.0 APPENDICES

Appendix 7.1 AZD6738 Initial Plasma PK.docx

Murine Pharmacokinetics (PK) of AZD6738
Client Investigators: Dr. E. Stewart

Date: TBD

Title: Initial plasma PK of oral AZD6738

Animals: Female CD1 nu mice. Aged approx. 12 weeks at study execution.

Dosages: 50 mg/kg AZD6738 by oral gavage, single dose

Formulation: AZD6738 free base equivalents in 10% DMSO / 40% propylene glycol / 50% UP Water

Design: A total of 6 mice will be dosed, with 3 mice providing blood at each time point. Five (5) blood samples will be collected from each mouse after dosing. Survival sampling (S) will be performed by retro-orbital, facial vein, saphenous vein, or tail vein bleed at the indicated time points. The final sample for each mouse will be terminal by cardiac puncture (T).

Group #s	Dose Level	Mouse #s	Mouse Ear Tag IDs	Sample Times (hr)
1	50 mg/kg	1-3		S: 0.25, 0.5, 1, 16 T: 24
2	50 mg/kg	4-6		S: 0.167, 2, 4, 8 T: 24

Summary:

Materials:

- At least 24 Minivette POCT K3EDTA capillary devices (50 uL, Sarstedt 17.2113.150) for survival blood collections.
- 6 appropriately labeled Microvette 500 K3EDTA microcentrifuge screw top tubes (500 uL, Sarstedt 20.1341.100) for terminal cardiac blood collection and spin down.
- One set of 24 standard 0.5 mL screw-top microcentrifuge tubes (Fisher Cat# 02-681-333 or equivalent), pre-labeled with AZD6738 IniPK, mouse #, and nominal time point in hrs for survival plasma collection.
- One set of 6 standard 2.0 mL screw-top microcentrifuge tubes (Fisher Cat# 02-681-343 or equivalent), pre-labeled with AZD6738 IniPK, mouse #, and nominal time point in hrs for terminal plasma collection.
- DMSO, propylene glycol, ultra pure water
- ~50 mg of AZD6738 free base equivalents
- Mouse gavage needle and 1 mL syringes for PO administration
- 25 gauge needles and TB/insulin syringes for cardiac punctures
- Centrifuge (10000g) w/ microcentrifuge rotor (4°C preferred, but room temp. will suffice)
- Container of wet ice
- Styrofoam cooler with labeled cardboard vial box and **dry** ice

Procedure:

1. The day before the study, sort mice into groups, 3 mice per cage with 2 cages and perform weighing. Tattoo tails for identification, or refer to mouse ear tag numbers. Label cages with group number, mouse numbers, and nominal time points. Each tattooed stripe represents the number of mouse in the cage's sequence. For example, the mouse with 1 stripe in Cage/Group 2 would be mouse #4 and the mouse with 2 stripes would be mouse #5, and so forth. Weigh each mouse, record weight in grams on the Study Worksheet, and calculate planned doses in mL.
2. Prior to the study, compound the 50 mg/mL AZD6738 DMSO stock solution

Murine Pharmacokinetics (PK) of AZD6738

Client Investigators: Dr. E. Stewart

a. In a 1 mL glass volumetric flask, to 50 mg of AZD6738 free base equivalents, add QS DMSO to 1 mL mark.
b. Vortex for several minutes, and sonicate directly in flask in water bath sonicator for up to 30 min until solution is clear.
c. SelleckChem claims the DMSO stock solution of AZD6738 to be stable for 2 years stored at -80 °C
d. Always allow the stock to come to ambient temperature and vortex prior to any use in formulations.

3. The day before or the morning of the study, formulate AZD6738 in vehicle as a solution for oral gavage (5 mg/mL, 0.25 mL for a 25 g mouse = 50 mg/kg)
    a. In a 2 mL volumetric flask, to 0.2 mL of the AZD6738 DMSO stock solution, add 0.8 mL of propylene glycol at ambient temperature. Pipette and gently shake/stir the interim solution of AZD6738.
    b. Add ~1 mL or QS ultra pure water to bring up to 2 mL total
    c. Vortex and/or sonicate for up to 30 min to ensure a homogenous solution. Immediately prior to administration, vortex and check for visual homogeneity.

4. Execute in vivo study according to the Study Worksheet
    a. NOTE: All actual times for dosing and samples should be referenced to the same study clock.
    b. Dose mice by PO gavage; record the actual dose volume administered in mL and the actual times of administration.
    c. At each time point, collect the blood sample by the indicated means and record the actual sample time (from the start of the collection), and make notes of any issues.
        i. S: Survival bleed – Anesthetize the mouse per IACUC protocol. Using a Microvette POCT, proceed to collect 50 uL of whole blood from the designated sampling site with the device. Gently agitate to mix the anticoagulant. Place blood into appropriate pre-labeled tube. All samples should be processed to plasma ASAP, but if necessary, put on wet ice until centrifugation.
        ii. T: Terminal cardiac puncture – Anesthetize the mouse per IACUC protocol. Proceed to collect 500 uL of whole blood from aorta. Place blood into appropriate pre-labeled Microvette K3EDTA tube and gently agitate. All samples should be processed to plasma ASAP, but if necessary, put on wet ice until centrifugation.
    d. Centrifuge the whole blood samples at 10000g for 2 min. to generate plasma.
    e. Remove plasma supernatant, place in appropriate pre-labeled tube from Set #2; place in vial box in cooler on dry ice and transfer to -80°C as soon as possible.
    f. Please submit remaining formulation in the original dosing vial stored at ambient temperature for stability assessment.

Appendix 7.2 AZD6738 PK_non tumor.docx

AZD6738 PK Study: 50 mg/kg oral gavage, [5mg/ml]

Mouse #	Mouse weight	Mouse Dose	Dose Time (3/20/17)	Bleed Time 15 min (3/20/17)	Bleed Time 30 min* (3/20/17)	Bleed Time 1 hour (3/20/17)	Bleed Time 16 hr (3/21/17)	Harvest Time 24 hr (3/21/17)
1	22.4	**0.22**	3:30p	3:45p	4:10p (40 min)	4:30p	7:30a	3:30p
2	26.5	**0.27**	3:35p	3:50p	4:15p (40 min)	4:35p	7:35a	3:35p
3	23.9	**0.24**	3:40p	3:55p	4:20p (40 min)	4:40p	7:40a	3:40p

*Tube states 30 min timepoint, actual time point is 40 min

Mouse #	Mouse weight	Mouse Dose	Dose Time (3/21/17)	Bleed Time 10 min (3/21/17)	Bleed Time 2 hr (3/21/17)	Bleed Time 4 hour (3/21/17)	Bleed Time 8 hr (3/21/17)	Harvest Time 24 hr (3/22/17)
4	21	**0.21**	8:00a	8:10a	10:00a	12:00p	4:00p	8:00a
5	21.8	**0.22**	8:05a	8:15a	10:05a	12:05p	4:05p	8:05a
6	22.5	**0.23**	8:10a	8:20a	10:10a	12:10p	4:10p	8:10a

3/20/17 - 3/22/17

Study Done by: Beth Stewart, Brittney Gordon, Monica Ocarz, Kaley Blankenship, Victoria Honnell

Mice: CD-1 nude Females, non tumor bearing, ~ 12 wks

Drug: Formulated by Lindsey Wang on 3/20/17, stored at room temp

Bleeds: all done in retro-orbital location using standard 50 microliter EDTA minivette tubes except for the terminal bleed which was done with a cardiac puncture after avertin into a 500 microliter EDTA tube. Tubes immediately spun and plasma portion placed onto dry ice in labeled tubes.

Samples transferred to Freeman freezer on 3/22/17

Appendix 7.3 Listing of Ct Data

Component_Name	Matrix	Subject	Time (hr)	Concentration (ug/L)
AZD6738	Plasma	1.00	0.25	6022.00
AZD6738	Plasma	1.00	0.67	3056.50
AZD6738	Plasma	1.00	1.00	3008.40
AZD6738	Plasma	1.00	16.00	48.23
AZD6738	Plasma	1.00	24.00	78.58
AZD6738	Plasma	2.00	0.25	6393.00
AZD6738	Plasma	2.00	0.67	5176.90
AZD6738	Plasma	2.00	1.00	4436.10
AZD6738	Plasma	2.00	16.00	73.47
AZD6738	Plasma	2.00	24.00	284.06
AZD6738	Plasma	3.00	0.25	4857.50
AZD6738	Plasma	3.00	0.67	2959.60
AZD6738	Plasma	3.00	1.00	2280.30
AZD6738	Plasma	3.00	16.00	29.03
AZD6738	Plasma	3.00	24.00	51.66
AZD6738	Plasma	4.00	0.17	3458.00
AZD6738	Plasma	4.00	2.00	607.35
AZD6738	Plasma	4.00	4.00	394.40
AZD6738	Plasma	4.00	8.00	225.16
AZD6738	Plasma	4.00	24.00	85.69
AZD6738	Plasma	5.00	0.17	5117.30
AZD6738	Plasma	5.00	2.00	689.04
AZD6738	Plasma	5.00	4.00	318.76
AZD6738	Plasma	5.00	8.00	485.31
AZD6738	Plasma	5.00	24.00	4.03
AZD6738	Plasma	6.00	0.17	5856.30
AZD6738	Plasma	6.00	2.00	1157.50
AZD6738	Plasma	6.00	4.00	704.10
AZD6738	Plasma	6.00	8.00	338.16
AZD6738	Plasma	6.00	24.00	23.39

Appendix 7.4 Extended Summary Statistics of Ct Data

		Matrix
		Plasma
Time (hr)		Concentration (ug/L)
0.167	N	3
	Mean	4810
	SD	1230
	Min	3460
	Median	5120
	Max	5860
	CV%	25.5
	Geometric Mean	4700
	CV% Geometric Mean	27.9
0.250	N	3
	Mean	5760
	SD	801
	Min	4860
	Median	6020
	Max	6390
	CV%	13.9
	Geometric Mean	5720
	CV% Geometric Mean	14.5
0.670	N	3
	Mean	3730
	SD	1250
	Min	2960
	Median	3060
	Max	5180
	CV%	33.6
	Geometric Mean	3600
	CV% Geometric Mean	32.2
1.00	N	3
	Mean	3240
	SD	1100
	Min	2280
	Median	3010
	Max	4440
	CV%	33.8
	Geometric Mean	3120
	CV% Geometric Mean	34.4

AZD6738 Initial PK

		Matrix
		Plasma
Time (hr)		Concentration (ug/L)
2.00	N	3
	Mean	818
	SD	297
	Min	607
	Median	689
	Max	1160
	CV%	36.3
	Geometric Mean	785
	CV% Geometric Mean	35.2
4.00	N	3
	Mean	472
	SD	204
	Min	319
	Median	394
	Max	704
	CV%	43.2
	Geometric Mean	446
	CV% Geometric Mean	42.8
8.00	N	3
	Mean	350
	SD	130
	Min	225
	Median	338
	Max	485
	CV%	37.3
	Geometric Mean	333
	CV% Geometric Mean	39.9
16.0	N	3
	Mean	50.2
	SD	22.3
	Min	29.0
	Median	48.2
	Max	73.5
	CV%	44.4
	Geometric Mean	46.9
	CV% Geometric Mean	49.1
24.0	N	6
	Mean	87.9

AZD6738 Initial PK

		Matrix
		Plasma
Time (hr)		Concentration (ug/L)
	SD	101
	Min	4.03
	Median	65.1
	Max	284
	CV%	115.0
	Geometric Mean	45.9
	CV% Geometric Mean	264