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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.

PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 155124-1630748

STUDY TITLE:

SCREENING PLASMA PHARMACOKINETICS OF AZD1390 IN ATHYMIC NUDE MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**AZD1390 Screening Plasma PK (SPPK)**	
TEST ARTICLE:	AZD1390	
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)	
PRINCIPAL INVESTIGATOR(S)	Dyer, Michael	
SJCRH SRM2 O/R:	155124-1630748	Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA	CIVIT
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REPORT FORMAT:	Study Summary	
REPORT STATUS:	FINAL	
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FOR P-PKSR APPROVED USE AND DISTRIBUTION

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1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The plasma pharmacokinetic (PK) profile of ATM inhibitor AZD1390 was evaluated in normal female Athymic nude mice (Charles River), approximately 12 weeks in age. AZD1390 (SJ000982893-2, Chemgood, purity 99.92%) was suspended 0.5% hydroxypropyl methylcellulose (HPMC) / 0.1% Tween 80 in ultrapure water, 2 mg/mL for a 10 mL/kg gavage. Two survival blood samples were obtained from each mouse via retro-orbital plexus using 50 µL Minivette POCT K3EDTA capillary devices (Sarstedt), and a third final sample by cardiac puncture. Samples were obtained at various times up to 24 hours post-dose, immediately processed to plasma, and stored at -80 °C until analysis. The remaining dosing solution was submitted for verification of potency, and chemical and physical stability during the study period.

1.2 Bioanalysis

Plasma samples were analyzed for AZD1390 (SJ000982893-2, Chemgood, purity 99.92%) with a qualified LC MS/MS assay. Plasma calibrators and quality controls were spiked with solutions prepared in methanol and corrected for salt content. Plasma samples, 25 µL each, were protein precipitated with 100 µL of 50 ng/mL AZD0156 (SJ000879910-3, Chemietek, CT-A0156) in methanol as an internal standard. A 1 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a LEAP CTC PAL autosampler. The LC separation was performed using a Phenomenex Kinetex EVO C18 (2.6 µm, 50 mm x 2.1 mm) column maintained at 50 °C with gradient elution at a flow rate of 0.50 mL/min. The binary mobile phase consisted of 20 mM ammonium acetate in water-acetonitrile (90:10 v/v) in reservoir A and 20 mM ammonium acetate in water-acetonitrile (10: 90 v/v) in reservoir B. The initial mobile phase consisted of 10% B with a linear increase to 77% B in 3 min. The column was then rinsed for 2 min at 100% B and then equilibrated at the initial conditions for 2 min for a total run time of 7 min. Under these conditions, the analyte and IS eluted at 1.7 and 1.2 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX 5500 QTRAP in the positive ESI mode and the following mass transitions were monitored: AZD1390 478.30 -> 126.00, AZD0156 462.25 -> 85.80. The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the calibrators across the 2.5 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9991. 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 2.5 ng/mL. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 4.18% CV and 93.7% to 100%, respectively.

1.3 Pharmacokinetic (PK) Analysis

AZD1390 plasma Ct data were grouped by nominal time point, and the mean Ct values were subjected to noncompartmental analysis (NCA) using Phoenix WinNonlin 8.1 (Certara USA, Inc., Princeton, NJ). The extravascular model was applied, and area under the Ct curve (AUC) was 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 (Kel) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Kel, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + Clast (predicted)/Kel. 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 oral clearance (CL/F = Dose/AUCinf), and the apparent oral terminal volume of distribution (Vz/F).

2.0 RESULTS

The AZD1390 plasma concentrations exhibited moderate-to-high variability, particularly at later time points. AZD1390 was rapidly absorbed (Tmax = 0.5 hr), and exhibited an approximate bi-exponential profile after Cmax, with a shallow distribution phase ending at 4 to 6 hr. The estimated apparent terminal half-life using the last 4 time points was 4.06 hr. The total plasma apparent oral clearance (CL/F) was high at 5.95 L/hr/kg or 99 mL/min/kg. The apparent oral terminal volume of distribution (Vz/F) was also high at 34.8 L/kg. The oral bioavailability (F) of AZD1390 in mice is unknown, but has been reported to be 74% in rats [1]. The plasma exposure of AZD1390 was approximately half of that observed in the published mouse PK by Durant (Supplemental Figure S4a) [1]. The formulation met specification (19.9 ± 0.719 mg/mL) and was stable over a 14-day period.

In vitro plasma protein binding of AZD1390 was assessed using rapid equilibrium dialysis by CBT ATC, yielding fractions unbound in plasma (Fu,p) of 0.0324 and 0.0543 for mice and humans, respectively. The human binding value was substantially different than that reported in literature by Durant (0.261).

At the time of this writing, human plasma PK of AZD1390 has not been publicly reported. Therefore, a clinically relevant dose (CRD) based off of pharmacokinetics cannot be made. Typical doses used in mice have ranged from 5 to 20 mg/kg PO QD [1].

3.0 REFERENCES

1. Durant ST, Zheng L, Wang Y, Chen K, Zhang L, Zhang T, Yang Z, Riches L, Trinidad AG, Fok JHL, Hunt T, Pike KG, Wilson J, Smith A, Colclough N, Reddy VP, Sykes A, Janefeldt A, Johnström P, Varnäs K, Takano A, Ling S, Orme J, Stott J, Roberts C, Barrett I, Jones G, Roudier M, Pierce A, Allen J, Kahn J, Sule A, Karlin J, Cronin A, Chapman M, Valerie K, Illingworth R, Pass M. The brain-penetrant clinical ATM inhibitor AZD1390 radiosensitizes and improves survival of preclinical brain tumor models. Sci Adv. 2018 Jun 1;4(6):eaat1719.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profile of AZD1390 by Group

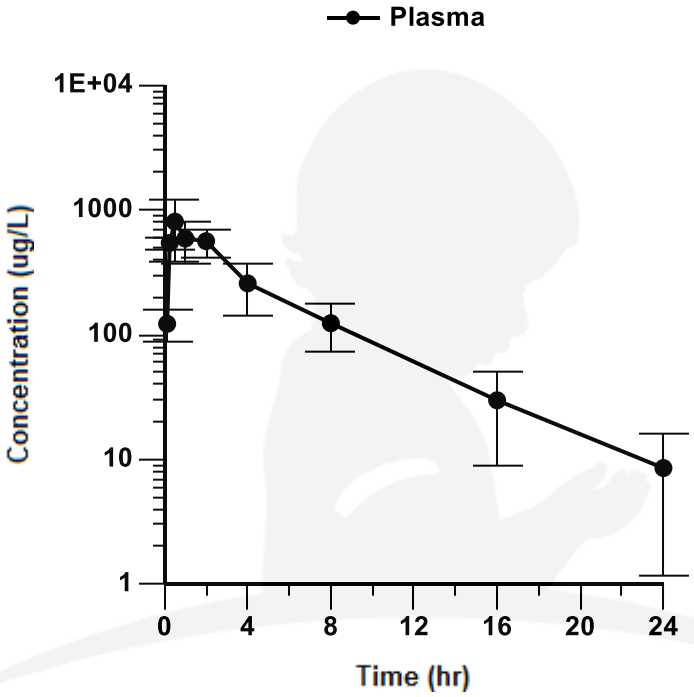

Table 4.1: NCA PK Parameter Estimates of AZD1390 by Group

		Analyte
		AZD1390
		Group
		Plasma
Parameter	**Units**	**Estimate**
Cmax	ug/L	805
Tmax	hr	0.500
AUClast	hr*ug/L	3310
AUCinf	hr*ug/L	3360
Kel	1/hr	0.171
T1/2	hr	4.06

		Analyte
		AZD1390
		Group
		Plasma
Parameter	**Units**	**Estimate**
CL/F	L/hr/kg	5.95
Vz/F	L/kg	34.8
Clast	ug/L	8.52
Tlast	hr	24.0

Table 4.2: Full Summary Statistics of AZD1390 Ct Data by Group

		Analyte
		AZD1390
		Group
		Plasma
Time (hr)		**Concentration (ug/L)**
0.125	N	3
	Mean	122
	SD	35.7
	Min	81.1
	Median	139
	Max	146
	CV%	29.2
	Geometric Mean	118
	CV% Geometric Mean	33.6
0.250	N	3
	Mean	544
	SD	55.7
	Min	505
	Median	519
	Max	608
	CV%	10.2
	Geometric Mean	542
	CV% Geometric Mean	10.0

AZD1390 Screening Plasma PK (SPPK)

		Analyte
		AZD1390
		Group
		Plasma
Time (hr)		Concentration (ug/L)
0.500	N	3
	Mean	805
	SD	415
	Min	356
	Median	887
	Max	1170
	CV%	51.5
	Geometric Mean	718
	CV% Geometric Mean	69.0
1.000	N	3
	Mean	589
	SD	216
	Min	456
	Median	474
	Max	838
	CV%	36.6
	Geometric Mean	566
	CV% Geometric Mean	35.1
2.000	N	3
	Mean	562
	SD	145
	Min	428
	Median	542
	Max	716
	CV%	25.9
	Geometric Mean	550
	CV% Geometric Mean	26.3
4.000	N	3
	Mean	258
	SD	117

AZD1390 Screening Plasma PK (SPPK)

		Analyte
		AZD1390
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	Min	167
	Median	216
	Max	390
	CV%	45.4
	Geometric Mean	242
	CV% Geometric Mean	45.5
8.000	N	3
	Mean	124
	SD	50.6
	Min	67.0
	Median	139
	Max	165
	CV%	41.0
	Geometric Mean	115
	CV% Geometric Mean	50.7
16.000	N	3
	Mean	29.8
	SD	21.0
	Min	10.4
	Median	26.9
	Max	52.0
	CV%	70.4
	Geometric Mean	24.4
	CV% Geometric Mean	96.3
24.000	N	3
	Mean	8.52
	SD	7.33
	Min	2.78
	Median	5.99
	Max	16.8

		Analyte
		AZD1390
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	CV%	86.1
	Geometric Mean	6.54
	CV% Geometric Mean	112

Table 4.3: AZD1390 Ct Data Listings by Subject, Analyte, Group, and Time

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	AZD1390	Plasma	0.13	145.99
M1	AZD1390	Plasma	0.50	886.75
M1	AZD1390	Plasma	16.00	52.02
M2	AZD1390	Plasma	0.13	139.36
M2	AZD1390	Plasma	0.50	1173.00
M2	AZD1390	Plasma	16.00	10.38
M3	AZD1390	Plasma	0.13	81.10
M3	AZD1390	Plasma	0.50	355.84
M3	AZD1390	Plasma	16.00	26.89
M4	AZD1390	Plasma	0.25	607.55
M4	AZD1390	Plasma	1.00	837.96
M4	AZD1390	Plasma	24.00	16.78
M5	AZD1390	Plasma	0.25	519.36
M5	AZD1390	Plasma	1.00	455.59
M5	AZD1390	Plasma	24.00	5.99
M6	AZD1390	Plasma	0.25	504.62
M6	AZD1390	Plasma	1.00	474.16
M6	AZD1390	Plasma	24.00	2.78
M7	AZD1390	Plasma	2.00	716.14
M7	AZD1390	Plasma	4.00	389.90
M7	AZD1390	Plasma	8.00	139.03
M8	AZD1390	Plasma	2.00	427.50
M8	AZD1390	Plasma	4.00	167.36

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M8	AZD1390	Plasma	8.00	66.97
M9	AZD1390	Plasma	2.00	542.44
M9	AZD1390	Plasma	4.00	216.14
M9	AZD1390	Plasma	8.00	164.57

Table 4.4: AZD1390 Ct Summary (Mean, SD, N) by Group

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	AZD1390	Plasma	0.13	122.15	35.70	3.00
Concentration	ug/L	AZD1390	Plasma	0.25	543.84	55.66	3.00
Concentration	ug/L	AZD1390	Plasma	0.50	805.20	414.64	3.00
Concentration	ug/L	AZD1390	Plasma	1.00	589.24	215.60	3.00
Concentration	ug/L	AZD1390	Plasma	2.00	562.03	145.31	3.00
Concentration	ug/L	AZD1390	Plasma	4.00	257.80	116.97	3.00
Concentration	ug/L	AZD1390	Plasma	8.00	123.52	50.62	3.00
Concentration	ug/L	AZD1390	Plasma	16.00	29.76	20.97	3.00
Concentration	ug/L	AZD1390	Plasma	24.00	8.52	7.33	3.00

5.0 ATTACHED FILES

Attached File 5.1 AZD1390 Screening Plasma PK V1.0.docx – *Final in vivo study plan as executed*
Attached File 5.2 155124-1630748_AZD_SPPK_2019-03-20.xlsx – *Submitted in vivo study digital data collection form (DCF)*
Attached File 5.3 AZD-1390 Study Sheet one.jpeg – *Submitted in vivo study worksheet #1*
Attached File 5.4 AZD-1390 Study Sheet two.jpeg – *Submitted in vivo study worksheet #2*
Attached File 5.5 AZD1390 SPPK TLFs 20190403.docx– *Report TLFs as a Word document for manipulation, plotting, and further presentation*