PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 45904-385084

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS OF AZD1775 IN FEMALE CD1 NU/NU MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**AZD1775 Screening Plasma and Tumor PK (SPTPK)**
TEST ARTICLE:	AZD1775 (adavosertib)
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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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 (P-PKSR) and St. Jude Children's Research Hospital (SJCRH), 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.

1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The total plasma and tumor homogenate PK of AZD1775 in female CD1 nu/nu mice (Jax Laboratories, aged 8-16 weeks) was assessed after a single oral gavage dose of 120 mg/kg. AZD1775 (AbMole, M2143, purity >98%, MolWt 500.60) was suspended in 0.5% methylcellulose (type 400 cPs, Sigma) at a nominal concentration of 12 mg/mL for a 10 mL/kg gavage volume. Mice were sacrificed using an IACUC-approved method at 30 min, 1, 2, 4, and 8 hr post-dose, with 3 mice per time point. Whole blood was collected with sodium heparin via cardiac puncture, immediately centrifuged to plasma, and stored on dry ice for remainder of study. Mice were then perfused with PBS via the aorta, the EX-8 orthotopic xenografts excised from the femur, rinsed with PBS, and placed on dry ice. At the end of the in vivo procedures, all samples were transferred from dry ice and placed at -80 °C until analysis.

1.2 Bioanalysis

Total plasma and tumor homogenate AZD1775 concentrations were assessed using a sensitive and specific liquid chromatography, tandem mass spectrometry assay. First, tumor samples were macerated, diluted with a 5:1 volume of Ringer Solution (Frey Scientific), and homogenized with a bead-based technique [1] on a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). Steel lysing matrix beads (MP Biomedicals, Metal Bead Lysing Matrix, 3 mg per mg of tumor) were added to the microcentrifuge tubes containing samples. The samples were then subjected to three 60 M/S vibratory cycles of 1 min each on the FastPrep-24 system. To prevent over-heating due to friction, samples were placed on wet ice for 5 min between each cycle. The homogenates were then stored at -80 °C until analysis.

AZD1775 (AbMole, M2143, purity >98%) stock solutions were prepared in methanol and used to spike matrix calibrators and quality controls. Plasma and tumor homogenate samples, 25 µL each, were protein precipitated with 100 µL of 5 ng/mL crizotinib (ApexBio, A8802, Batch 1, purity >99%) in methanol as an internal standard. A 2 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 Synergi Hydro-RP 80Å LC column (4.0 µm, 30 mm x 2.0 mm) maintained at 60 °C with gradient elution at a flow rate of 0.35 mL/min. The binary mobile phase consisted of 0.1% formic acid in ultra-pure water in reservoir A and 0.1% formic acid in methanol in reservoir B. The initial mobile phase consisted of 5% B with a linear increase to 70% B in 1.5 minutes. The column was then rinsed for 2.5 minutes at 100% B and then equilibrated at the initial conditions for 2 minutes for a total run time of 6 minutes. Under these conditions, the analyte and IS eluted at 0.98 and 0.93 minutes, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 5500 Q-TRAP in the positive ESI mode with monitoring of the following mass transitions: AZD1775 501.20 -> 442.20, crizotinib 450.10 -> 260.20.

The method qualification and bioanalytical runs all passed our acceptance criteria for non-GLP assay performance. A quadratic model ($1/X^2$ weighting) fit the calibrators across the 0.5 to 100 ng/mL range, with a correlation coefficient (R) of ≥0.9982. 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 0.5 ng/mL. The intra-run precision and accuracy was < 6.28% CV and 88.2% to 107%, respectively.

1.3 Pharmacokinetic (PK) Analysis

The resultant AZD1775 concentration-time (Ct) data were grouped by matrix and time point, and summary statistics generated using Phoenix WinNonlin 6.4 (Certara USA, Inc., Princeton, NJ). The AZD1775 arithmetic mean Ct data for each matrix was then subjected to noncompartmental pharmacokinetic analysis (NCA) using WinNonlin. 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 the 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,pred/Ke.

Other NCA 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). The average concentration over a dosing interval (Cavg) was estimated as AUCinf / dosing interval in hours. The apparent partition coefficient of AZD1775 from the plasma to tumor (Kp,tumor) was estimated as the ratio of the AUCinf, tumor to AUCinf plasma when available. To estimate a clinically relevant mouse dosage, the resultant mouse plasma AUCinf and Cavg was compared with the reported human plasma PK values at the putative single agent AZD1775 maximum tolerated dose or Phase 2 recommended dose [2,3]. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

2.0 RESULTS

AZD1775 concentrations showed appreciable variability, demonstrating average coefficients of variation of 74.3% and 63.2% respectively for plasma and tumors across the sampling time points. The earliest time points, during the putative absorption and distribution phases, exhibited the most variability. Tumor penetration appeared to be rapid and generally mirrored the plasma Ct profile, with a Kp,tumor value of 1.42. The T1/2 of AZD1775 in the plasma was 1.97 hr.

Analysis of the reported human AZD1775 plasma PK in a Phase 1 study from Do et al. revealed a Day 3 $AUC_{0\text{-}8hr}$ of 4690 hr-ng/mL after twice daily oral dosing of 225 mg. Graphical analysis of the human Ct profile figure published suggested an approximate apparent oral clearance of 31.4 L/hr, an AUC over the 12-hour dosing interval of 7170 hr-ng/mL and a Cavg of 569 ng/mL. The half-life of AZD1775 has been estimated at between 10 and 24 hr in clinical reports, and is supported by the accumulation of drug over a 3-day dosing period in the Phase 1 study. Assuming a minimal difference in plasma protein binding between mice and humans, a murine dosage of 120 mg/kg daily would provide a similar AUC and Cavg as that achieved at the single agent MTD of 225 mg PO BID for 3 days after the fifth dose. Given the short half-life of AZD1775 in mice compared with humans, a 60 mg/kg BID dosage would be preferred. This will minimize peak-to-trough fluctuations, maintaining a more consistent Cavg in tumor, and would better emulate human plasma and putative tumor concentrations with respect to wee1 pharmacodynamic coverage. Of note, other preclinical studies of AZD1775 in mice have used this 60 mg/kg BID regimen, where it was found to be efficacious and well-tolerated in addition to being clinically relevant by our inter-species PK evaluation [4,5].

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: AZD1775 Mean (SD) Ct Profile by Group

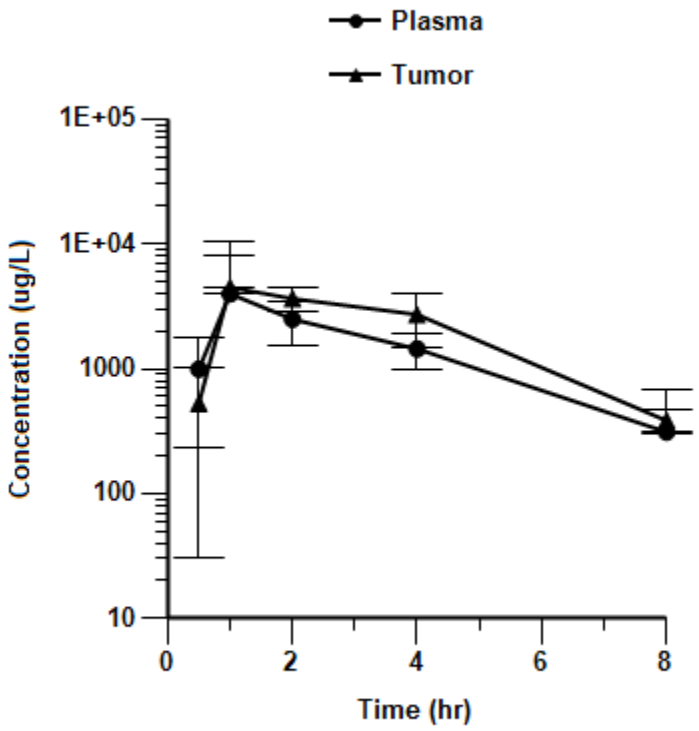

Table 4.1: NCA PK Parameter Estimates of AZD1775 by Group

		Analyte	
		AZD1775	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	3980	4480
Tmax	hr	1.00	1.00

		Analyte	
		AZD1775	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
AUClast	hr*ug/L	11400	16500
AUCinf	hr*ug/L	12400	17600
Kel	1/hr	0.352	0.391
T1/2	hr	1.97	1.77
CL/F	L/hr/kg	9.71	6.82
Vz/F	L/kg	27.6	17.4
Clast	ug/L	311	384
Tlast	hr	8.00	8.00
Cavg,12hr	ug/L	1030	1470
Kp,tumor	-	-	1.42

Table 4.2: Full Summary Statistics of AZD1775 Ct Data by Group

		Analyte	
		AZD1775	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.500	N	3	3
	Mean	992	529
	SD	756	498
	Min	434	122
	Median	690	380
	Max	1850	1080
	CV%	76.2	94.2
	Geometric Mean	821	369
	CV% Geometric Mean	85.6	152
1.000	N	3	3
	Mean	3980	4480
	SD	4120	5880
	Min	1120	122
	Median	2100	2140
	Max	8700	11200
	CV%	104	131
	Geometric Mean	2740	1430
	CV% Geometric Mean	142	1360

		Analyte	
		AZD1775	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
2.000	N	3	3
	Mean	2490	3640
	SD	942	812
	Min	1450	2900
	Median	2730	3520
	Max	3290	4510
	CV%	37.8	22.3
	Geometric Mean	2350	3580
	CV% Geometric Mean	44.9	22.4
4.000	N	3	3
	Mean	1440	2720
	SD	470	1250
	Min	1080	1960
	Median	1270	2030
	Max	1970	4160
	CV%	32.6	46.0
	Geometric Mean	1390	2550
	CV% Geometric Mean	31.9	44.4
8.000	N	3	3
	Mean	311	384
	SD	377	85.2
	Min	12.7	320
	Median	185	351
	Max	735	481
	CV%	121	22.2
	Geometric Mean	120	378
	CV% Geometric Mean	833	21.6

Table 4.3: AZD1775 Ct Data Listings by Subject, Analyte, Group, and Time

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
1	AZD1775	Plasma	0.50	1851.75
1	AZD1775	Tumor	0.50	1084.18
2	AZD1775	Plasma	0.50	689.93

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
2	AZD1775	Tumor	0.50	379.58
3	AZD1775	Plasma	0.50	433.91
3	AZD1775	Tumor	0.50	122.09
4	AZD1775	Plasma	1.00	8702.66
4	AZD1775	Tumor	1.00	11171.37
5	AZD1775	Plasma	1.00	2103.55
5	AZD1775	Tumor	1.00	122.30
6	AZD1775	Plasma	1.00	1122.44
6	AZD1775	Tumor	1.00	2144.66
7	AZD1775	Plasma	2.00	1451.84
7	AZD1775	Tumor	2.00	2897.35
8	AZD1775	Plasma	2.00	3288.94
8	AZD1775	Tumor	2.00	4507.49
9	AZD1775	Plasma	2.00	2731.15
9	AZD1775	Tumor	2.00	3519.22
10	AZD1775	Plasma	4.00	1972.49
10	AZD1775	Tumor	4.00	4156.00
11	AZD1775	Plasma	4.00	1080.34
11	AZD1775	Tumor	4.00	2026.21
12	AZD1775	Plasma	4.00	1270.44
12	AZD1775	Tumor	4.00	1964.26
13	AZD1775	Plasma	8.00	735.09
13	AZD1775	Tumor	8.00	350.68
14	AZD1775	Plasma	8.00	12.71
14	AZD1775	Tumor	8.00	320.06
15	AZD1775	Plasma	8.00	184.60
15	AZD1775	Tumor	8.00	480.55

Table 4.4: AZD1775 Ct Summary (Mean, SD, N) by Group

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	AZD1775	Plasma	0.50	991.86	755.61	3.00
Concentration	ug/L	AZD1775	Plasma	1.00	3976.22	4122.51	3.00
Concentration	ug/L	AZD1775	Plasma	2.00	2490.64	941.87	3.00
Concentration	ug/L	AZD1775	Plasma	4.00	1441.09	469.92	3.00
Concentration	ug/L	AZD1775	Plasma	8.00	310.80	377.36	3.00
Concentration	ug/L	AZD1775	Tumor	0.50	528.62	498.06	3.00
Concentration	ug/L	AZD1775	Tumor	1.00	4479.44	5882.93	3.00
Concentration	ug/L	AZD1775	Tumor	2.00	3641.35	811.99	3.00
Concentration	ug/L	AZD1775	Tumor	4.00	2715.49	1247.90	3.00
Concentration	ug/L	AZD1775	Tumor	8.00	383.76	85.20	3.00

5.0 ATTACHED FILES

Attached File 5.1 AZD1775 Prelim PK.docx – *Final in vivo study plan as executed*

Attached File 5.2 AZD_1775 PK study sheet 11_21_14 – *Submitted in vivo study digital data collection form (DCF)*

Attached File 5.3 AZD1775 Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*