PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 291926 - 2550541

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS OF AZD-5305 IN ATHYMIC NUDE MICE BEARING EWING SARCOMA (ES8) OTXS AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**AZD-5305 Screening Plasma and Tumor PK (SPTPK)**
TEST ARTICLE:	AZD-5305 (Saruparib, AZD5305), free base
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH SRM2 O/R:	291926 - 2550541 Preclinical Pharmacokinetics Shared Resource
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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 (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 plasma and tumor pharmacokinetic (PK) profiles of AZD-5305 were evaluated in normal female Athymic nude mice (Charles River) bearing Ewing Sarcoma (ES8) orthotopic xenograft tumors, approximately 8 to 12 weeks in age. The test article AZD-5305 (SJ001041299-5, MedChemExpress, CAT# HY-132167, LOT# 157156) was suspended in 0.5% w/v hydroxypropyl methylcellulose (HPMC) / 0.1% Tween80 in water, adjusted to final pH 3–3.2 with HCl at 0.1 mg/mL for a 10 mL/kg oral gavage, and a 1 mg/kg dosage. One terminal blood sample was obtained from each mouse via cardiac puncture with KEDTA as the anticoagulant. Samples were obtained at various times up to 24 hours post-dose, immediately processed to plasma, and stored at -80 °C until analysis. Tumors were extracted after vascular perfusion with PBS. Remaining dosing suspension was submitted for verification of potency, chemical and physical stability during the study period.

1.2 Bioanalysis

Tumor samples were weighed in 2.0, 4.5 mL or 15 mL tubes (depending on tumor size) containing Lysing matrix D (MP Biomedicals, Santa Ana, CA), diluted with a 1:5 volume of 0.1 M PBS pH 7.4, and homogenized using a FastPrep-24 system (MP Biomedicals, Santa Ana, CA) for three cycles of 1 min vibration at 6.0 M/S speed, with 5 min in wet ice bath between each cycle to prevent over-heating. The homogenates were then stored at -80 °C for 24 hours until analysis.

Plasma and tumor homogenate samples were analyzed for AZD-5305 (SJ001041299-5, MedChemExpress, CAT# HY-132167, LOT# 157156) using a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Plasma calibrators and quality controls were spiked with solutions, corrected for salt content and purity as necessary, prepared in DMSO. Plasma samples, 25 µL each, were protein precipitated with 100 µL of 30 ng/mL AZD-9574 (GLPBIO, CAT# GC64099, LOT# 1) in methanol as an internal standard (IS). A 2 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a Shimadzu LEAP CTC PAL autosampler. The LC separation was performed using a Phenomenex Kinetex C18 (2.6 µm, 50 mm x 2.1 mm) column maintained at 50 °C with gradient elution at a flow rate of 0.6 mL/min. The binary mobile phase consisted of H2O-MeOH-0.2 M ammonium acetate pH 5 (80:10:10 v/v/v) in reservoir A and MeOH-0.2 M ammonium acetate pH 5.0 (90:10 v/v) in reservoir B. The initial mobile phase consisted of 20% B followed by a linear increase to 60% B in 2 min. The column was then rinsed for 2 min at 100% B and then equilibrated at the initial conditions for 2.0 min for a total run time of 6 min. Under these conditions, the analyte and IS eluted at 0.85 and 1.03 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX QTRAP 5500 in the positive ESI mode and the following mass transitions were monitored: AZD-5305 407.2 → 376.2, AZD-9574 429.2 → 398.2. 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 0.5 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9961. 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 for plasma and 3 ng/mL for tumor homogenate. Tumor homogenate was quantitated using the plasma calibration curve. Sample dilution integrity was confirmed. No significant matrix effects, ion enhancement or suppression, were detected in blank EDTA plasma or tumor homogenates harvested from untreated female Athymic nude mice. The intra-run precision and accuracy was ≤ 10.3% CV and 93.0% to 107%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Plasma and tumor concentration-time (Ct) data for AZD-5305 were grouped by matrix and nominal time point. Manual imputation of data below the lower limit of quantitation (BLOQ) was as follows: IF at any time point ≥ 2/3rds of the Ct results were above the LLOQ, the BLOQ data were replaced with a value of

½ LLOQ, ELSE the entire time point's data were treated as missing. Summary statistics were calculated and the arithmetic 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) 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 (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 clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F). The apparent plasma-to-tumor partition coefficient (Kp_inf) was estimated as the ratio of AUCinf in tumor to AUCinf in plasma, with Kp_last similarly estimated using AUClast values.

Additionally, the AZD-5305 fraction unbound in mouse and human plasma (Fu,p,m and Fu,p,h) was determined using rapid equilibrium dialysis (RED, 8000 MWCO, Pierce Biotechnology, ThermoFisher Scientific, Waltham, MA). Briefly, blank plasma was spiked with compound and diluted with 100 mM PBS (6 replicates per group), achieving a back-calculated final concentration of 5 µM in blank undiluted plasma. The diluted samples were placed in donor wells of the RED apparatus and permitted to equilibrate for 6 hours at 37 °C with 5% CO_2, ~85% humidity, and orbital shaking at 250 rpm. Compounds were assayed in donor and receiver well samples using the qualified LC-MS/MS assay, with the fraction unbound calculated as the ratio of concentration in receiver to donor adjusted for the dilution [1]

A clinically relevant dose (CRD) for mice was estimated from unbound plasma PK and exposure. The CRD was defined as the mouse dose achieving a predicted mean steady state unbound plasma AUC (AUCu) similar to humans at the single agent maximum tolerated dose (MTD), recommended Phase II dose (RP2D), or FDA-approved dose. Dose proportional, linear, and time-invariant PK across species was assumed. Human and mouse plasma protein binding were assumed similar when data were not available. This is similar to the clinical relevance approach proposed by Spilker [2] which uses unbound plasma average steady state concentrations. Some latitude in dose rounding was permitted in the CRD recommendation, and an unbound exposure within 2-fold of the clinical target was considered acceptable. Additional considerations influenced the final recommended mouse dose, including mouse dosing regimens prevalent in the literature and the tolerability of the compound in mice.

2.0 RESULTS

The AZD-5305 plasma Ct data demonstrated moderate variability between and within mice, with coefficients of variation ranging from 12.5% to 93.2%. Most of the variability was seen during the later time points. The absorption rate of AZD-5305 was rapid, with the Tmax occurring at 0.500 hours post-dose. After Cmax, plasma concentrations diminished in a mono-exponential manner and there were no BLOQ observations. The apparent plasma terminal half-life of AZD-5305 was 4.72 hours. The apparent plasma clearance (CL/F) of AZD-5305 was low at 0.49 mL/min/kg, or approximately 0.54% of murine hepatic blood flow. The apparent plasma terminal volume of distribution (Vz/F) for AZD-5305 was low at 0.200 L/kg, below total body water for a mouse. The oral bioavailability of AZD-5305 was unknown in the current study but has been previously reported to be 77% in mice [3]. The formulation met specification (0.0984 ± 0.00255 mg/mL) and was stable over the 2-day usage period.

The results of RED plasma protein binding for AZD-5305 were: Fu,p,m = 0.0170 ± 0.00212; Fu,p,h = 0.0505 ± 0.00549. The plasma protein binding appeared substantially higher in mice by approximately 3-fold, as presented in the previous plasma only PK report (RPT.291925-2550540).

While plasma exposure was low, AZD-5305 appeared to have a slow rate (Tmax of 4 hours) and moderate extent (Kp_last of 0.338 and Kp_inf of 0.425) of tumor penetration, with a prolonged retention (T1/2 = 11.2 hours).

Compared with our previous screening plasma PK study at the same dose (RPT.291925-2550540), the plasma AUCinf in the current study is about 18% higher, and the plasma apparent terminal half-life is shorter by 11%.

The PK profile of AZD-5305 in the current study differs from previously published findings in mice, when considering our estimated Fu,p,m value. Illuzzi et al. reported an unbound plasma AUC of 1057 hr-ng/mL (2.6 uM-hr) for AZD-5305 after daily oral dosing of 1 mg/kg AZD-5305 for 14 days in mice [4]. The estimated unbound plasma AUC in the current study was 364 hr-ng/mL, and is approximately 3-fold lower in comparison. Notably, digital extrapolation of the unbound AZD-5305 plasma Ct data from Illuzzi revealed a slightly longer apparent T1/2 of 5.85 hours vs. our currently observed 4.72-hour value.

At the time of this report, no human PK data for AZD-5305 are publicly available; therefore, a PK-guided CRD cannot be recommended.

3.0 REFERENCES

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2. Spilker ME, Chen X, Visswanathan R, Vage C, Yamazaki S, Li G, Lucas J, Bradshaw-Pierce EL, Vicini P. Found in Translation: Maximizing the Clinical Relevance of Nonclinical Oncology Studies. Clin Cancer Res. 2017 Feb 15;23(4):1080–90.

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profiles by Analyte and Group

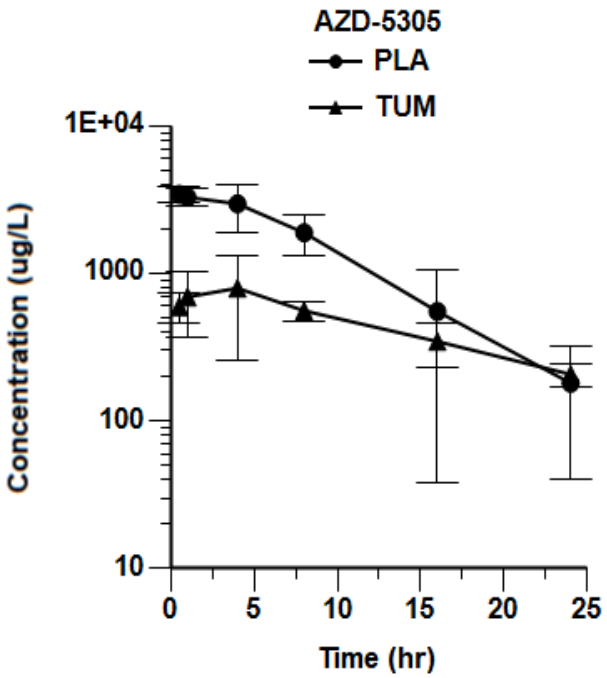

Table 4.1: NCA Parameter Estimates by Analyte and Group

		Analyte	
		AZD-5305	
		Group	
		PLA	TUM
Parameter	Unit	Value	
Cmax	ug/L	3440	794
Tmax	hr	0.500	4.00
AUClast	hr*ug/L	32800	11100
AUCinf	hr*ug/L	34000	14400
Kel	1/hr	0.147	0.0621
T1/2	hr	4.72	11.2
CL/F	L/hr/kg	0.0294	0.0692
Vz/F	L/kg	0.200	1.12
Clast	ug/L	180	207
Tlast	hr	24.0	24.0
Kp_last			0.338
Kp_inf			0.425

Table 4.2: Full Summary Statistics of Ct Data by Analyte and Group

		Analyte	
		AZD-5305	
		Group	
		PLA	TUM
Time (hr)		Concentration (ug/L)	
0.500	N	3	3
	Mean	3440	597
	SD	431	139
	Min	2940	505
	Median	3640	529
	Max	3730	757
	CV%	12.5	23.3
	Geometric Mean	3420	587
	CV% Geometric Mean	13.1	22.4
1.00	N	3	3
	Mean	3290	693
	SD	436	326
	Min	2990	378
	Median	3080	672
	Max	3790	1030
	CV%	13.3	47.1
	Geometric Mean	3270	639
	CV% Geometric Mean	12.9	53.7
4.00	N	3	3
	Mean	2970	794
	SD	1070	535
	Min	1810	203
	Median	3180	937
	Max	3910	1240
	CV%	36.0	67.3
	Geometric Mean	2820	618
	CV% Geometric Mean	41.7	126
8.00	N	3	3
	Mean	1890	558
	SD	570	89.2
	Min	1430	455
	Median	1700	605
	Max	2530	614
	CV%	30.2	16.0
	Geometric Mean	1830	553
	CV% Geometric Mean	29.7	17.0
16.0	N	3	3
	Mean	553	346

		Analyte	
		AZD-5305	
		Group	
		PLA	TUM
Time (hr)		Concentration (ug/L)	
	SD	515	118
	Min	206	229
	Median	308	344
	Max	1140	465
	CV%	93.2	34.1
	Geometric Mean	417	332
	CV% Geometric Mean	111	36.6
24.0	N	3	3
	Mean	180	207
	SD	140	35.2
	Min	96.2	178
	Median	103	197
	Max	342	246
	CV%	77.5	17.0
	Geometric Mean	150	205
	CV% Geometric Mean	81.3	16.8

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	AZD-5305	PLA	0.500	3638.30
M1	AZD-5305	TUM	0.500	504.79
M2	AZD-5305	PLA	0.500	3727.70
M2	AZD-5305	TUM	0.500	756.99
M3	AZD-5305	PLA	0.500	2941.20
M3	AZD-5305	TUM	0.500	529.44
M4	AZD-5305	PLA	1.00	3785.60
M4	AZD-5305	TUM	1.00	672.03
M5	AZD-5305	PLA	1.00	2989.80
M5	AZD-5305	TUM	1.00	377.76
M6	AZD-5305	PLA	1.00	3080.10
M6	AZD-5305	TUM	1.00	1029.60
M7	AZD-5305	PLA	4.00	1806.40
M7	AZD-5305	TUM	4.00	202.89
M8	AZD-5305	PLA	4.00	3911.80
M8	AZD-5305	TUM	4.00	1243.60
M9	AZD-5305	PLA	4.00	3180.90
M9	AZD-5305	TUM	4.00	936.80
M10	AZD-5305	PLA	8.00	1433.90

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M10	AZD-5305	TUM	8.00	455.29
M11	AZD-5305	PLA	8.00	2526.50
M11	AZD-5305	TUM	8.00	613.91
M12	AZD-5305	PLA	8.00	1701.00
M12	AZD-5305	TUM	8.00	605.39
M13	AZD-5305	PLA	16.0	1144.50
M13	AZD-5305	TUM	16.0	465.17
M14	AZD-5305	PLA	16.0	205.77
M14	AZD-5305	TUM	16.0	343.50
M15	AZD-5305	PLA	16.0	307.72
M15	AZD-5305	TUM	16.0	229.47
M16	AZD-5305	PLA	24.0	103.03
M16	AZD-5305	TUM	24.0	196.99
M17	AZD-5305	PLA	24.0	341.57
M17	AZD-5305	TUM	24.0	245.90
M18	AZD-5305	PLA	24.0	96.20
M18	AZD-5305	TUM	24.0	177.52

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

Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
AZD-5305	PLA	0.500	3440	431	3
AZD-5305	PLA	1.00	3290	436	3
AZD-5305	PLA	4.00	2970	1070	3
AZD-5305	PLA	8.00	1890	570	3
AZD-5305	PLA	16.0	553	515	3
AZD-5305	PLA	24.0	180	140	3
AZD-5305	TUM	0.500	597	139	3
AZD-5305	TUM	1.00	693	326	3
AZD-5305	TUM	4.00	794	535	3
AZD-5305	TUM	8.00	558	89.2	3
AZD-5305	TUM	16.0	346	118	3
AZD-5305	TUM	24.0	207	35.2	3

5.0 ATTACHED FILES

Attached File 5.1 AZD-5305 Screening Plasma PK V1.0.docx – *Final in vivo study plan as executed*

Attached File 5.2 291925_AZD-5305_SPPK_TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*

Attached File 5.3 291926- 2550541_AZD-5305_SPTPK_2023-04-06a.xlsx – *Completed electronic data collection form for the PK study from in vivo scientist.*

Attached File 5.4 Saruparib-COA-157156-MedChemExpress.pdf – *Certificate of analysis for the AZD-5305 lot used in vivo and for bioanalysis.*