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PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 155057-1630075

STUDY TITLE:

SCREENING PLASMA AND TISSUE PHARMACOKINETICS (SPTPK) OF IDASANUTLIN IN FEMALE ATHYMIC NUDE MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Idasanutlin Screening Plasma and Tissue PK (SPTPK)**
TEST ARTICLE:	Idasanutlin (free base)
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Brennan, Rachel <Rachel.Brennan@STJUDE.ORG>
SJCRH SRM2 O/R:	155057-1630075 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA
IN VIVO SCIENTIST(S)	Gordon, Brittney <Brittney.Gordon@STJUDE.ORG> Blankenship, Kaley B <Kaley.Blankenship@STJUDE.ORG> Hoffmann, Lauren <Lauren.Hoffmann@STJUDE.ORG> Zhang, Jiakun <Jiakun.Zhang@STJUDE.ORG>
BIOANALYTICAL SCIENTIST:	Caufield, William <William.Caufield@STJUDE.ORG>
REPORT AUTHOR(S):	Freeman, Burgess <Burgess.Freeman@STJUDE.ORG> Caufield, William <William.Caufield@STJUDE.ORG> Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>
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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 pharmacokinetic (PK) profile of Idasanutlin free base was evaluated in normal female Athymic nude mice (Charles River), approximately 12 weeks in age. Idasanutlin (SJ000833632-3, AdooQ, A14211) was suspended in a cosolvent vehicle consisting of 10% ethanol / 30% polyethylene glycol 400 (PEG400) / 60% Phosal PG50. The final concentration was 30 mg/mL free base equivalents in an organic phospholipid solution for a 5 mL/kg oral gavage. Terminal blood samples, under IP Avertin (tribromoethanol) anesthesia, were obtained at various times up to 24 hours post-dose, immediately processed to plasma, and stored at -80 °C until analysis. Following terminal bleeds, animals were perfused with PBS to flush blood from the vasculature. Tissues were then extracted, rinsed with PBS as necessary, and then placed in appropriately labeled microcentrifuge tubes in a cooler on dry ice. Tissue samples were then transferred to a -80°C freezer as soon as possible. Remaining dosing solution was submitted for verification of potency, and chemical and physical stability during the study period.

1.2 Bioanalysis

Retina samples were weighed in tared microcentrifuge tubes, diluted with a 5:1 volume of ultrapure water, and homogenized with a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). The use of ceramic beads was not necessary to facilitate homogenization. The samples were then subjected to three 6.5 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.

Plasma, vitreous humor and retina homogenate samples were analyzed for idasanutlin (SJ000833632-3, AdooQ) with a qualified LC MS/MS assay. Plasma calibrators and quality controls were spiked with solutions prepared in methanol. Plasma samples, 25 µL each, were protein precipitated with 100 µL of 25 ng/mL Nutlin3a (SJ000359792-18, APEx Bio) in methanol as an internal standard. A 3 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 C18 (2.6 µm, 50 mm x 2.1 mm) column maintained at 50 °C with gradient elution at a flow rate of 0.5 mL/min. The binary mobile phase consisted of 0.1% formic acid in water-acetonitrile (90:10 v/v) in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase consisted of 30% B with a linear increase to 100% B in 2.5 min. The column was then rinsed for 1.5 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 2.09 and 0.89 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: Idasanutlin 616.16 -> 421.00, Nutlin3a 581.17 -> 439.10. The method qualification passed acceptance criteria for non-GLP assay performance. Of the two subsequent bioanalytical runs, one run (plasma and vitreous humor) passed acceptance criteria for non-GLP assay performance. The calibration curves for the second bioanalytical run (retinas and vitreous humor BLOQ re-runs) had 11 out of 16 calibrators (68.75%) that met the 15% (20% for LLOQ) accuracy requirement and, therefore, did not achieve the ≥ 75% acceptance criteria. However, the issue did not have a significant effect on the overall data integrity. A linear model ($1/X^2$ weighting) fit the calibrators across the 1 to 500 ng/mL range, with a correlation coefficient ® of ≥ 0.9948. 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. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 5.92% CV and 98.4% to 113%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Idasanutlin 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) 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-tissue partition coefficient of (Kp) was estimated as the ratio of the AUCinf in tissue to AUCinf plasma, whereas Kp* was similarly estimated using AUClast values.

2.0 RESULTS

Idasanutlin plasma, retina, and vitreous concentrations showed low variability at early time points, with the later time points showing higher variability. The mean plasma Ct profile appeared nearly monophasic, with indication of a shallow distribution phase, with a terminal phase beginning at 16 to 24 hours post-dose. Vitreous concentrations beyond 8 hours were below the lower limit of quantitation (BLOQ) of a dilution adjusted value ca. 6 ng/mL. As such, terminal phase dependent parameters could not be estimated for vitreous.

Absorption of idasanutlin was fast, with the Tmax occurring at the first sampling time of 1 hr. The apparent terminal plasma half-life, using the last four observed time points, was 3.7 hours, with the retina exhibiting a similar half-life. The total apparent oral plasma clearance (CL/F) was high at 9.73 L/hr/kg or 162 mL/min/kg. The apparent volume of distribution at steady state (Vz/F) was also high at 52.0 L/kg. While the oral bioavailability (F) of idasanutlin in this study is unknown, it has been previously reported to be as high as 80% in mice [1]. The applied formulation was found to be adequate and stable over a 15-day period at ambient temperature, with a idasanutlin concentration of 29.8 ± 1.71 mg/mL

Plasma PK of idasanutlin in our mice with our cosolvent formulation was distinct from that observed with an amorphous micro-bulk precipitate suspension [2,3]. We demonstrated a rapid Tmax and a 6- to 10-fold lower dose normalized Cmax (0.023 vs 0.15-0.23 kg*ug/L/ug). Our dose normalized AUCinf was 10-fold lower at 0.103 vs 0.947 hr*kg*ug/L/ug, and our mice exhibited a shorter apparent half-life (3.70 vs 5.59 hr). As we tested only one dose level, it's unclear whether our current EtOH / PEG400 / Phosal PG50 cosolvent formulation yields dose proportional exposures, and both solubility limited absorption and saturable metabolism may occur. It's apparent that additional oral formulations will need to be trialed to optimize exposure of oral, commercially sourced, idasanutlin in mice. Either a 4% DMA, 30% PEG400, 66% Gelucire 44-14 [1] or an attempt at an amorphous dispersion should be undertaken.

The idasanutlin total plasma AUCinf was 15400 hr*ug/L – approximately 4-fold lower than the estimated human AUCinf at the recommended Phase 2 dose (RP2D) of 300 mg with the optimized spray dried powder tablet (64900 hr*ug/L) [4]. As plasma protein binding is very high (99.99%) and similar between mice and humans [5], total plasma concentrations can be used to define a pharmacokinetically clinically relevant dose (CRD), using the Cavg or AUCtau at steady state [6]. To achieve the same AUC0-24hr as humans at steady state with 300 mg PO QD dosing, and assuming linear and dose proportional PK, mice would require 300 mg/kg PO BID idasanutlin in the current cosolvent formulation.

If a precise CRD for oral idasanutlin is necessary with commercially sourced compound, it is highly recommended that repeat PK studies are performed with an improved and qualified formulation.

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

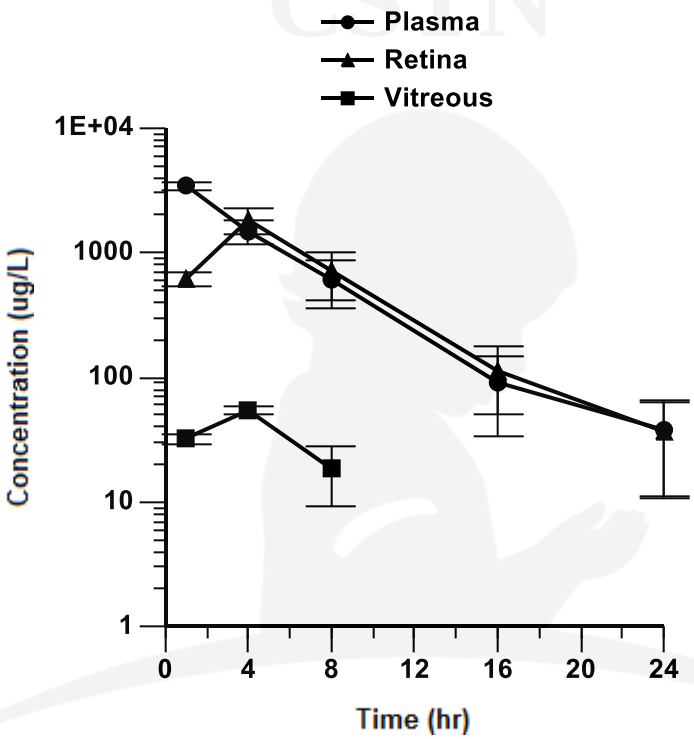

Table 4.1: NCA PK Parameter Estimates of Idasanutlin by Group

		Analyte		
		Idasanutlin		
		Group		
		Plasma	Retina	Vitreous
Parameter	Units	Estimate		
Cmax	ug/L	3450	1830	54.3
Tmax	hr	1.00	4.00	4.00
AUClast	hr*ug/L	15300	11900	279
AUCinf	hr*ug/L	15400	12000	-
Kel	1/hr	0.187	0.186	-
T1/2	hr	3.70	3.73	-

		Analyte		
		Idasanutlin		
		Group		
		Plasma	Retina	Vitreous
Parameter	Units	Estimate		
CL/F	L/hr/kg	9.73	12.5	-
Vz/F	L/kg	52.0	67.2	-
Clast	ug/L	37.9	36.7	18.5
Tlast	hr	24.0	24.0	8.00
Kp*	-	-	0.778	0.0182
Kp	-	-	0.779	-

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

		Analyte		
		Idasanutlin		
		Group		
		Plasma	Retina	Vitreous
Time (hr)		Concentration (ug/L)		
1.000	N	3	3	3
	Mean	3450	616	32.5
	SD	263	88.5	3.00
	Min	3150	562	29.6
	Median	3580	567	32.2
	Max	3630	718	35.6
	CV%	7.62	14.4	9.24
	Geometric Mean	3450	612	32.4
	CV% Geometric Mean	7.81	13.9	9.23
4.000	N	3	3	3
	Mean	1470	1830	54.3
	SD	322	430	3.87
	Min	1110	1340	51.6
	Median	1560	1980	52.4
	Max	1740	2160	58.7
	CV%	21.9	23.6	7.14

Idasanutlin Screening Plasma and Tissue PK (SPTPK)

		Analyte		
		Idasanutlin		
		Group		
		Plasma	Retina	Vitreous
Time (hr)		Concentration (ug/L)		
	Geometric Mean	1450	1790	54.2
	CV% Geometric Mean	23.5	25.8	7.02
8.000	N	3	3	3
	Mean	607	715	18.5
	SD	254	297	9.32
	Min	412	462	7.76
	Median	515	640	23.2
	Max	894	1040	24.5
	CV%	41.8	41.6	50.4
	Geometric Mean	574	676	16.4
	CV% Geometric Mean	41.5	42.7	72.3
16.000	N	3	3	
	Mean	90.8	112	
	SD	57.3	61.8	
	Min	46.9	68.5	
	Median	69.9	85.7	
	Max	156	183	
	CV%	63.1	55.0	
	Geometric Mean	79.9	102	
	CV% Geometric Mean	67.2	55.2	
24.000	N	3	3	
	Mean	37.9	36.7	
	SD	27.0	26.1	
	Min	22.1	19.7	
	Median	22.6	23.6	
	Max	69.0	66.8	
	CV%	71.1	71.3	
	Geometric Mean	32.5	31.4	
	CV% Geometric Mean	72.7	73.8	

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Idasanutlin	Plasma	1.00	3582.30
M1	Idasanutlin	Retina	1.00	566.67
M1	Idasanutlin	Vitreous	1.00	29.62
M2	Idasanutlin	Plasma	1.00	3625.70
M2	Idasanutlin	Retina	1.00	717.78
M2	Idasanutlin	Vitreous	1.00	35.60
M3	Idasanutlin	Plasma	1.00	3150.10
M3	Idasanutlin	Retina	1.00	562.40
M3	Idasanutlin	Vitreous	1.00	32.18
M4	Idasanutlin	Plasma	4.00	1562.00
M4	Idasanutlin	Retina	4.00	2161.50
M4	Idasanutlin	Vitreous	4.00	58.72
M5	Idasanutlin	Plasma	4.00	1737.90
M5	Idasanutlin	Retina	4.00	1976.30
M5	Idasanutlin	Vitreous	4.00	52.44
M6	Idasanutlin	Plasma	4.00	1113.80
M6	Idasanutlin	Retina	4.00	1341.20
M6	Idasanutlin	Vitreous	4.00	51.64
M7	Idasanutlin	Plasma	8.00	412.04
M7	Idasanutlin	Retina	8.00	462.36
M7	Idasanutlin	Vitreous	8.00	7.76
M8	Idasanutlin	Plasma	8.00	514.68
M8	Idasanutlin	Retina	8.00	1042.80
M8	Idasanutlin	Vitreous	8.00	24.52
M9	Idasanutlin	Plasma	8.00	893.54
M9	Idasanutlin	Retina	8.00	639.62
M9	Idasanutlin	Vitreous	8.00	23.20
M10	Idasanutlin	Plasma	16.00	46.90
M10	Idasanutlin	Retina	16.00	68.53
M11	Idasanutlin	Plasma	16.00	69.87
M11	Idasanutlin	Retina	16.00	85.68
M12	Idasanutlin	Plasma	16.00	155.59
M12	Idasanutlin	Retina	16.00	183.16

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M13	Idasanutlin	Plasma	24.00	22.09
M13	Idasanutlin	Retina	24.00	23.56
M14	Idasanutlin	Plasma	24.00	69.03
M14	Idasanutlin	Retina	24.00	66.79
M15	Idasanutlin	Plasma	24.00	22.61
M15	Idasanutlin	Retina	24.00	19.71

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Idasanutlin	Plasma	1.00	3452.70	262.96	3.00
Concentration	ug/L	Idasanutlin	Plasma	4.00	1471.23	321.80	3.00
Concentration	ug/L	Idasanutlin	Plasma	8.00	606.75	253.61	3.00
Concentration	ug/L	Idasanutlin	Plasma	16.00	90.79	57.28	3.00
Concentration	ug/L	Idasanutlin	Plasma	24.00	37.91	26.95	3.00
Concentration	ug/L	Idasanutlin	Retina	1.00	615.62	88.50	3.00
Concentration	ug/L	Idasanutlin	Retina	4.00	1826.33	430.22	3.00
Concentration	ug/L	Idasanutlin	Retina	8.00	714.93	297.46	3.00
Concentration	ug/L	Idasanutlin	Retina	16.00	112.46	61.83	3.00
Concentration	ug/L	Idasanutlin	Retina	24.00	36.69	26.14	3.00
Concentration	ug/L	Idasanutlin	Vitreous	1.00	32.46	3.00	3.00
Concentration	ug/L	Idasanutlin	Vitreous	4.00	54.26	3.87	3.00
Concentration	ug/L	Idasanutlin	Vitreous	8.00	18.49	9.32	3.00

5.0 ATTACHED FILES

Attached File 5.1 Idasanutlin Screening Plasma and Tissue PK V2.0.docx – *Final in vivo study plan as executed*

Attached File 5.2 155057-1594754_IDA_SPTPK_2019-03-06 (002).xlsx – *Submitted in vivo study digital data collection form (DCF)*

Attached File 5.3 IdasanutlinPKDosing.jpeg – *Submitted in vivo study worksheet #1*

Attached File 5.4 IdasunutlinPKTimepointScheduleandDetails.jpeg – *Submitted in vivo study worksheet #2*

Attached File 5.5 Idasanutlin Screening Plasma PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*