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

PRECLINICAL PHARMACOKINETIC REPORT

Hematological Malignancies Program

P-PKSR Study 148062-1554511

STUDY TITLE:

SCREENING PLASMA PHARMACOKINETICS (SPPK) OF VENETOCLAX IN FEMALE NRGSGM3 MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Venetoclax Screening Plasma PK (SPPK) NRG-SGM3**
TEST ARTICLE:	Venetoclax
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Klco, Jeffery M <Jeffery.Klco@STJUDE.ORG>
SJCRH SRM2 O/R:	148062-1554511 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA CIVIT
IN VIVO SCIENTIST(S)	Thorne, Rebecca L <Rebecca.Thorne@STJUDE.ORG>
BIOANALYTICAL SCIENTIST:	Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>
REPORT AUTHOR(S):	Freeman, Burgess <Burgess.Freeman@STJUDE.ORG>
REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2019-06-18

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 venetoclax was evaluate in normal female NRG-SGM3 mice (Jackson Laboratories), approximately 12 weeks in age. Venetoclax (SJ000821632-9, MedKoo) was dissolved in 6:3:1 Phosal PG50 / PEG400 / EtOH, for a 100 mg/kg free base equivalents dose as a 10 mL/kg oral 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. 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 venetoclax with a qualified LC-MS/MS method using navitoclax as the internal standard. 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 5 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9996. 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 5 ng/mL. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 6.48% CV and 97.9% to 104%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Venetoclax 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).

2.0 RESULTS

The plasma PK venetoclax showed moderate variability, particularly between mice, with the 24 hour time point being the most variable. The absorption of venetoclax was very slow, with the first few time points being below the limit of quantitation in plasma, and a Tmax of 8 hours. The NSG-SGM3 mice showed a low apparent clearance of venetoclax at 46.7 mL/min/kg, roughly half of hepatic blood flow. The apparent terminal volume of distribution was high at 12.3 L/kg. Venetoclax displayed a moderate apparent terminal half-life of around 3 hours. The oral bioavailability of venetoclax in this study is unknown, but it has been reported to be 27% in mice [1]. The remaining formulation met specification (9.20 ± 0.310 mg/mL) and was stable for 7 days.

The population plasma PK of venetoclax has been reported in adult patients [2]. There appears to be a saturable absorption effect when escalating from 400 mg to 800 mg PO, as well as a significant food effect. The human plasma venetoclax PK was simulated from the population parameters for a 800 mg dose assuming a “fed” state, under the assumption that the PK in adults and children will be similar (or at least they will, after weight /BSA or age based dosing – venetoclax is highly metabolized by ontogenic CYPs). At steady state, this yielded an AUC of 50900 hr-ug/L, Cmax 3200 ug/L, Ctrough 897 ug/L, and a Cavg of 2120 ug/L.The plasma protein binding between mice and humans has found to be similarly high (>99.99%), and therefore adjustments for interspecies protein binding are not necessary. A strict clinically

relevant dose (CRD) was estimated using the plasma AUC or Cavg at steady state estimated from this PK data and humans [4], under the assumption of linear, dose-proportional PK, and was found to be 140 mg/kg. However, the most commonly applied and tolerated regimen of venetoclax in mice is 100 mg/kg PO QD, and therefore this common dosage is recommended.

3.0 REFERENCES

1. Drug Approval Package: Venclexta tablets (venetoclax) NDA # 208573 Pharmacology Review [Internet]. Drugs@FDA. [cited 2019 Feb 19]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/208573Orig1s000TOC.cfm
2. Jones AK, Freise KJ, Agarwal SK, Humerickhouse RA, Wong SL, Salem AH. Clinical Predictors of Venetoclax Pharmacokinetics in Chronic Lymphocytic Leukemia and Non-Hodgkin's Lymphoma Patients: a Pooled Population Pharmacokinetic Analysis. AAPS J. 2016 Sep 1;18(5):1192–202.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS) Figure 4.1:

Mean (SD) Ct Profile of Venetoclax by Group

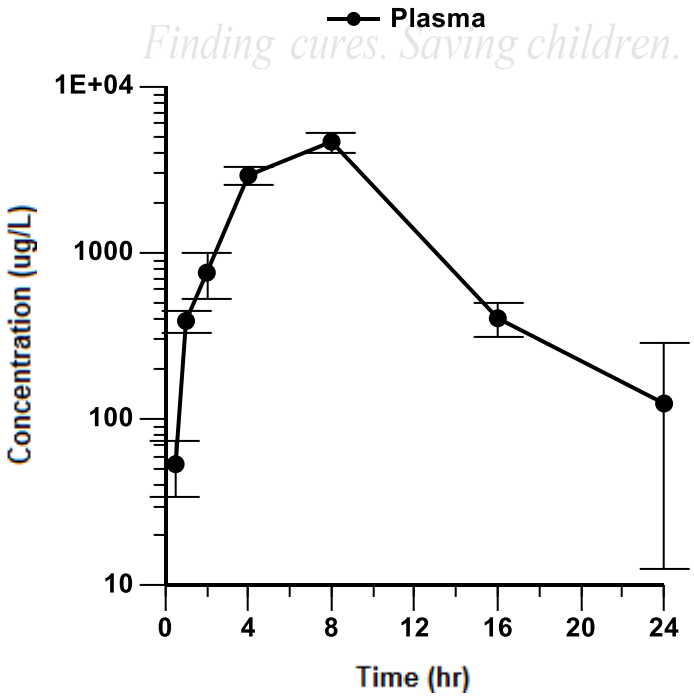

Table 4.1: NCA PK Parameter Estimates of Venetoclax by Group

		Analyte
		Venetoclax
		Group
		Plasma
Parameter	**Units**	**Estimate**
Cmax	ug/L	4650
Tmax	hr	8.00
AUClast	hr*ug/L	35300
AUCinf	hr*ug/L	35800
Kel	1/hr	0.227
T1/2	hr	3.06
CL/F	L/hr/kg	2.80
Vz/F	L/kg	12.3
Clast	ug/L	124
Tlast	hr	24.0

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

		Analyte
		Venetoclax
		Group
		Plasma
Time (hr)		**Concentration (ug/L)**
0.125	N	0
	Mean	
	SD	
	Min	
	Median	
	Max	
	CV%	
	Geometric Mean CV%	
	Geometric Mean	
0.250	N	0
	Mean	

Venetoclax Screening Plasma PK (SPPK) NRG-SGM3

		Analyte
		Venetoclax
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	SD	
	Min	
	Median	
	Max	
	CV%	
	Geometric Mean	
	CV% Geometric Mean	
0.500	N	2
	Mean	53.6
	SD	19.8
	Min	39.6
	Median	53.6
	Max	67.5
	CV%	37.0
	Geometric Mean	51.7
	CV% Geometric Mean	39.2
1.000	N	3
	Mean	389
	SD	63.3
	Min	330
	Median	382
	Max	456
	CV%	16.3
	Geometric Mean	386
	CV% Geometric Mean	16.3
2.000	N	3
	Mean	759
	SD	230
	Min	595
	Median	660

Venetoclax Screening Plasma PK (SPPK) NRG-SGM3

		Analyte
		Venetoclax
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	Max	1020
	CV%	30.3
	Geometric Mean	738
	CV% Geometric Mean	29.3
4.000	N	3
	Mean	2920
	SD	360
	Min	2620
	Median	2820
	Max	3320
	CV%	12.3
	Geometric Mean	2910
	CV% Geometric Mean	12.1
8.000	N	3
	Mean	4650
	SD	663
	Min	3950
	Median	4750
	Max	5260
	CV%	14.3
	Geometric Mean	4620
	CV% Geometric Mean	14.7
16.000	N	3
	Mean	404
	SD	91.7
	Min	299
	Median	442
	Max	470
	CV%	22.7
	Geometric Mean	396

		Analyte
		Venetoclax
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	CV% Geometric Mean	24.9
24.000	N	3
	Mean	124
	SD	164
	Min	25.5
	Median	32.9
	Max	314
	CV%	132
	Geometric Mean	64.1
	CV% Geometric Mean	239

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Venetoclax	Plasma	0.13	
M1	Venetoclax	Plasma	1.00	329.63
M1	Venetoclax	Plasma	16.00	470.15
M2	Venetoclax	Plasma	0.13	
M2	Venetoclax	Plasma	1.00	455.54
M2	Venetoclax	Plasma	16.00	299.03
M3	Venetoclax	Plasma	0.13	
M3	Venetoclax	Plasma	1.00	381.64
M3	Venetoclax	Plasma	16.00	441.84
M4	Venetoclax	Plasma	0.25	
M4	Venetoclax	Plasma	2.00	659.77
M4	Venetoclax	Plasma	24.00	25.54
M5	Venetoclax	Plasma	0.25	
M5	Venetoclax	Plasma	2.00	595.44
M5	Venetoclax	Plasma	24.00	32.91
M6	Venetoclax	Plasma	0.25	

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M6	Venetoclax	Plasma	2.00	1022.10
M6	Venetoclax	Plasma	24.00	313.72
M7	Venetoclax	Plasma	0.50	39.56
M7	Venetoclax	Plasma	4.00	2824.20
M7	Venetoclax	Plasma	8.00	3945.60
M8	Venetoclax	Plasma	0.50	
M8	Venetoclax	Plasma	4.00	2618.40
M8	Venetoclax	Plasma	8.00	4754.60
M9	Venetoclax	Plasma	0.50	67.55
M9	Venetoclax	Plasma	4.00	3318.60
M9	Venetoclax	Plasma	8.00	5260.40

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Venetoclax	Plasma	0.13			0.00
Concentration	ug/L	Venetoclax	Plasma	0.25			0.00
Concentration	ug/L	Venetoclax	Plasma	0.50	53.55	19.79	2.00
Concentration	ug/L	Venetoclax	Plasma	1.00	388.94	63.27	3.00
Concentration	ug/L	Venetoclax	Plasma	2.00	759.10	230.02	3.00
Concentration	ug/L	Venetoclax	Plasma	4.00	2920.40	359.88	3.00
Concentration	ug/L	Venetoclax	Plasma	8.00	4653.53	663.20	3.00
Concentration	ug/L	Venetoclax	Plasma	16.00	403.67	91.72	3.00
Concentration	ug/L	Venetoclax	Plasma	24.00	124.06	164.29	3.00

5.0 ATTACHED FILES

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

Attached File 5.2 148062-1554511_VEN_SPPK_2019-04-10.xlsx – *Submitted in vivo study digital data collection form (DCF)*

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