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1. Poster #: T2070; Abstract #: AM-17-1064
Ren, C.; Fox, D.; Maniar, M. Enhancement of Oral Absorption of Rigosertib.
Session Date: November 14, 2017
Session Time: 10:00 am – 11:00 am

ABSTRACT #: AM-17-1604
Pharmacokinetics/Pharmacodynamics/Drug Metabolism

Enhancement of Oral Absorption of Rigosertib

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Purpose. To enhance the oral bioavailability of Rigosertib by evaluating the pH effect on the formulation. Rigosertib is a small molecule kinase inhibitor that inhibits cellular signaling in cancer cells by acting as a Ras mimetic and binding to the Ras-binding domain (RBD). Oral Rigosertib is in Phase II clinical trial as a single agent for the treatment of lower risk MDS and in combination with azacitidine for the treatment of MDS and AML.

Methods. Different formulations of Rigosertib (ON 01910.Na) were dosed to fasted non-naïve male beagle dogs. High pH formulation was prepared by pre-treating PEG 400 vehicle with base and then dissolving the Rigosertib API powder slowly and mixing for ~2 hrs till a homogenous solution was obtained. The pH of the final formulation was adjusted to pH 9.5-10.5. Two different lots of API were evaluated in pH adjusted formulation. The control formulation was a solution of API in PEG 400 without any pH adjustment. In the first animal study, the formulations were dosed at 25 mg/kg via oral gavage. The second study comprised of three groups: Group 1 was dosed with enteric polymer based capsules filled with 280 mg API powder; Group 2 was dosed with enteric polymer based capsules filled with 140 mg pH adjusted formulation and Group 3 was dosed with soft gelatin capsules encapsulated with 280 mg non-pH adjusted formulation . Each dog received either one or two capsules for a total dose of 280 mg. The blood samples were collected as a function of time and plasma were isolated. The drug concentrations in plasma were determined through multiple reaction monitoring (MRM) by a validated LC-MS/MS assay. The formulation effect was characterized by determining the absorption maxima and overall drug exposure.

Results. The absorption maxima and overall drug exposure of Rigosertib in dogs after dosing with different formulations are summarized in **Table 1**. Study 1 showed higher absorption in dogs dosed with pH adjusted oral solutions compared to regular pH formulation. In study 2, the dogs dosed with enteric polymer based capsules filled with API powder showed very low absorption. The absorption was improved about 10 fold with soft gelatin capsules filled with non-pH adjusted formulation. The absorption was further improved almost 2 fold using enteric polymer based capsules filled with pH adjusted formulation.

Conclusions. The oral absorption of Rigosertib was significantly enhanced using pH adjusted formulation in conjunction with a capsule made of enteric polymer.

Table 1: Oral Absorption of Rigosertib in Dogs: Mean ± STD			
	Formulation	**Cmax (ng/mL)**	**AUC (ng-hr/mL)**
Study 1 (Dose: 25 mg/kg)	75 mg/mL Oral Solution (Non- pH adjusted ; pH = 6.98)	1738.12 ± 1553.33	3159.86 ± 1902.47
	75 mg/mL Oral Solution (pH adjusted-1; pH = 9.74)	3022.39 ± 2299.27	4075.08 ± 3276.70
	75 mg/mL Oral Solution (pH adjusted-2; pH = 9.94)	3493.68 ± 1594.09	5738.26 ± 1520.87
Study 2 (Dose: 280mg)	Enteric polymer based capsule filled with 280 mg API powder	139.53 ± 37.54	673.20 ± 457.45
	Enteric polymer based capsule filled with 140 mg pH adjusted, non-aqueous formulation	5366.67 ± 3276.31	11833.30 ± 2398.17
	Soft gelatin capsule filled with 280 mg of non-pH adjusted, non-aqueous formulation	3146.67 ± 1005.00	6700.30 ± 354.53

2. Poster #: W4103; Abstract #: AM-17-3113;
Session Date: November 15, 2017
Session Time: 12:00 pm – 01:00 pm
Ren, C.; Maniar, M. Liver Microsomal Stability of ON 123300 and Identification of Metabolites.

ABSTRACT #: AM-17-3113
Pharmacokinetics/Pharmacodynamics/Drug Metabolism

Liver Microsomal Stability of ON 123300 and Identification of Metabolites

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Purpose. ON 123300 is a novel small molecule, dual inhibitor of the c-MYC activated kinases ARK5 and CDK4/6. Inhibition of ARK5 by ON 123300 results in the collapse of oncogene-altered energy metabolism, leading to cell death. ON 123300 targets CDK4/6 leading to G1 arrest, inhibiting MYC-driven cell cycle activation and DNA synthesis. Liver microsomal stability studies were undertaken to understand the metabolism of the compound in different species and to identify the potential metabolites.

Methods. ON 123300 (final concentration 10 μM) was incubated with mouse, rat, dog, rabbit, minipig and human liver microsomes at 37 ºC water bath. The reaction mixture contained 0.5 mg/mL of microsomal protein in 100 mM potassium phosphate pH 7.4 buffer and 1.3 mM

cofactors NADPH. The control reaction mixture did not contain NADPH. At specified incubation time 0, 5, 10, 20, 40 and 60 minutes, 100 µL of sample was withdrawn, and the reaction was immediately quenched with same volume of ice cold acetonitrile. The mixture was then vortexed and centrifuged at 12000 rpm for 5 min. The supernatant was injected into HPLC-UV system for analysis. Data were converted to %remaining by dividing the area under the curve (AUC) at different time points by AUC at time zero. The half-life $T_{1/2}$ and intrinsic clearance (CLint) was calculated by fitting the data to a first-order decay model. The isolated supernatant was also injected into LC-MS/MS system in full scan mode in order to identify any potential metabolites. The mixture was separated by Agilent XDB C18 column (150 x 4.6 mm, 5 µm) under isocratic elution for 16 min. The wavelength was set at 223 nm with a flow rate of 1.0 mL/min. The same separation condition was applied for the full scan LC-MS analysis in order to keep the same order of elution.

Results. The half-life and intrinsic clearance of the compound in various species is summarized in **Table 1.** The control without NADPH cofactors showed no degradation of the parent peak. The compound is most stable in human and least in minipig liver microsomes, respectively.

Table 1. Summary of NADPH dependent intrinsic clearance in livermicrosomes

	Human	Minipig Male	Rabbit Male	Dog Male	Dog Female	Mouse Male	Mouse Female	Rat Male
Protein Content (mg/mL)	20	20	20	20	20	20	20	20
Total P450 (pmol/mg)	400	880	1400	590	530	730	940	650
Strains	UltraPool 150	Gottingen	New Zealand White	Beagle	Beagle	CD-1	CD-1	Sprague Dawley
$T_{1/2}$ (min)	47.5	1.7	9.8	9.2	8.8	23.7	15.3	9.9
CLint (µl/min/mg)	29.2	813.6	142.2	150.2	157.4	58.6	90.4	140.2

The full scan of the supernatant by LC-MS/MS identified two major metabolites. They are dealkylated and the oxidative metabolite of the parent compound.

Conclusions. The *in vitro* metabolism of ON 123300 was studied in liver microsomes of different species. The rate and the proportion of the formation of the two metabolites differed significantly across various species. The data provides significant insight into selection of potential species for toxicological studies.