A Phase Ib Study of the dual PI3K/mTOR inhibitor Dactolisib (BEZ235) Combined with Everolimus in Patients with Advanced Solid Malignancies.

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Supplementary Methods

<u>BEZ235 – Bioanalytical Method</u>

Standards

NVP-BEZ235 was provided by Novartis Pharma, Basel. NVP-BBD130 was used as internal standard and was purchased from Axon Medchem. All other reagents were of HPLC grade and obtained from Fisher scientific.

Chromatographic separation

Chromatographic separations were performed on a Waters Sunfire™ C18 column (2.1 mm × 150 mm). The chromatographic system consisted of Waters pump connected to autosampler and a model FP-1520 fluorescence detector operating at excitation and emission wavelengths 270 and 425 nm, respectively. The mobile phase consisted of methanol : 10 mM ammonium acetate buffer adjusted to pH 6.8 (75:25, v/v). The mobile phase was delivered at a flow rate of 0.6 ml/min.

Sample Preparation

To 200 µL of plasma (standard / sample) 50 µl of IS working solution and 1 ml Ethyl acetate was added. After vigorously mixing for 1 min, the samples were centrifuged at 2000 RPM for 10 min to separate the aqueous and organic layers. The upper organic layer was separated into another clean tube and evaporated to dryness in a Speed-Vac set at 50 °C and the residue was reconstituted in 100 µl methanol–water (70:30, v/v). After vortexing the sample was transferred to an HPLC autosampler vial and a volume of 50 µl was injected into a HPLC system.

Assay limit of quantification
The limit of quantification was 5 ng/mL and the assay was linear from 5 – 1000 ng/mL.

Everolimus – Bioanalytical Method

Standards
RAD001 was provided by Novartis Pharma, Basel. D4-RAD001 was used as internal standard and was purchased from TRC chemicals. All other reagents were of HPLC grade and obtained from Fisher scientific.

Chromatographic separation
Chromatographic separations were performed on a Waters Symmetry™ C18 column (2.1 mm × 100 mm) connected to a liquid chromatography Finnigan Surveyor pump equipped with a Finnigan Micro AS autosampler connected to a linear ion trap-Fourier Transform LTQ-FT™ mass spectrometer.

Sample Preparation
Sample preparation solution consisted of methanol: 0.1 M zinc sulfate (70:30, v/v) and internal standard at 20 ng/mL concentration. To 500 µL of whole blood (standard / sample) 2 ml of sample preparation solution was added and vigorously mixed for 1 min. The samples were centrifuged at 2000 RPM for 10 min and the upper clean layer was separated and loaded on to Oasis HLB SPE cartridges preconditioned with 3 ml methanol and 3 ml water. Cartridges were washed with 3 mL water, 3 mL methanol: water (50:50, v/v) and 1 mL heptane. Samples were eluted using 1 mL of heptane:2-propranol (50:50, v/v) and evaporated to dryness in a Speed-Vac set at 50 °C. The residue was reconstituted in 100 µl methanol–water (70:30, v/v). After vortexing the sample was transferred to an HPLC autosampler vial.

Assay limit of quantification
The limit of quantification was 1 ng/mL and the assay was linear from 1 – 500 ng/mL.

Population Pharmacokinetic Modeling:

The population PK parameters of everolimus were obtained from clinical pharmacology review section of the everolimus NDA. Population PK model was developed using data from various phase I, Ib and pivotal phase III trials of everolimus at doses ranging from 5, 10 mg once daily and 15, 30 mg weekly regimens. There were a total of 398 subjects with 1667 concentrations in the population PK model building. A two compartment model of disposition fit the everolimus data well and the final parameters including the inter-individual variability is reported in the Table 1a. A visual predictive check (VPC) with 2000 replicates were simulated for 2.5 mg once daily everolimus for 28 days. The predicted median concentration of everolimus and 90% prediction intervals were plotted and overlaid with the observations from the current study to evaluate for potential change in the everolimus exposure.

Supplementary Table 1: Dose Cohorts

Dose Level	BEZ235 (mg/d)	Everolimus (mg/d)
1	200	2.5
2	400	2.5
3	800	2.5

Supplementary Table 2: Common Adverse Events

Adverse Event (AE)	BEZ235 200 mg RAD001 2.5 mg N (%)	BEZ235 400 mg RAD001 2.5 mg N (%)	BEZ235 800 mg RAD001 2.5 mg N (%)	Total AE N
Any AE	4 (100)	7 (100)	8 (100)	19
Fatigue	3 (75)	5 (71)	6 (75)	13
Fever	0 (0)	2 (29)	2 (25)	4
Dehydration	0 (0)	2 (29)	3 (38)	5
Anorexia	1 (25)	6 (86)	2 (25)	9
Arthralgias/back pain	0 (0)	1 (14)	5 (63)	6
Thrombocytopenia	1 (25)	2 (29)	2 (25)	5
Anemia	1 (25)	5 (71)	3 (38)	9
AST elevation	2 (50)	5 (71)	5 (63)	12
ALT elevation	1 (25)	1 (14)	4 (50)	6
Alkaline phosphatase elev.	0 (0)	3 (43)	2 (25)	5
Hypokalemia	0 (0)	4 (57)	2 (25)	6
Hyperglycemia	0 (0)	1 (14)	3 (38)	4

Rash	1 (25)	0 (0)	3 (38)	4
Abdominal pain	2 (50)	1 (14)	2 (25)	5
Nausea	0 (0)	5 (71)	7 (88)	12
Vomiting	0(0)	2 (29)	4 (50)	6
Diarrhea	2 (50)	5 (71)	7 (88)	14
Sore throat/mucositis	0 (0)	2 (29)	6 (75)	8
Acute Renal Failure	0(0)	3 (43)	2 (25)	5

Supplementary Table 3: Response Evaluation

Cohort	**SD**	**PD**
1	0	4
2	0	4
3	1	2

Supplementary Table 4: Reported Population PK Parameters of Everolimus

Pharmacokinetic Parameters	**Reported population mean (inter-individual variability, %CV)**
Clearance, CL/F (L/h)	18.8 (48.8 %)
Central Volume, V1/F (L)	191 (47.2 %)
Inter compartmental clearance, Q (L/h)	46.2 (36.3 %)
Peripheral volume, V2/F (L)	517 (51.5 %)
First order absorption rate, ka (h^{-1})	6.07
Error Proportional	0.283
Error Additional	0.075

Supplementary Figure 1: Dose proportionality in BEZ235 C_{max} and AUC