LB-353: Pediatric Preclinical Testing Program (PPTP) Stage 1 Evaluation of Cabozantinib

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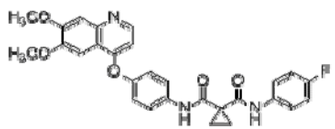
CABOZANTINIB

- Cabozantinib is a potent orally bioavailable small molecule inhibitor of MET and VEGFR2. It additionally inhibits AXL, KIT, FLT3,and TIE-2.
- Cabozantinib molecular targets play important roles in angiogenesis as well as in tumor cell proliferation and survival for selected cancers.
- Cabozantinib is FDA-approved for the treatment of patients with progressive metastatic medullary thyroid cancer.

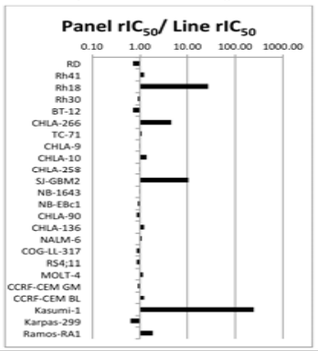


Cabozantinib
N-(4-(6,7-dimethoxyquinolin-4-yloxy)phenyl)-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide

CABOZANTINIB IN VITRO ACTIVITY

- The median relative IC_{50} (rIC_{50}) for cabozantinib against the PPTP cell lines was 8.1 µM (range 34 nM to > 10 µM).
- The most sensitive cell line, Kasumi-1, is an AML cell line that with an activating KIT mutation. Its rIC_{50} (34 nM) was 200-fold lower than the median for the PPTP Panel.
- The next most sensitive cell lines all have rIC_{50} values of 300 nM or greater, well above the range at which cabozantinib inhibits its target kinases.

COMPARE-Like plot for cabozantinib against PPTP cell lines



PPTP IN VITRO & IN VIVO TESTING METHODS

In vitro: In vitro testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates (Kang MH, et al. Pediatr Blood Cancer 56:239-249, 2011). Testing was for 96 hours at concentrations from 1.0 nM to 10.0 µM with replicates of 6-12 per data point. Data were analyzed by fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values vs. the concentration.

In vivo: Standard PPTP methods for in vivo testing were employed (http://pptp.nchresearch.org/documents/detailedAnalysisMethods.pdf).

Cabozantinib was tested *in vivo* using at a dose of 30 mg/kg administered orally daily for 21 to 28 days.

For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm^3. Two perpendicular tumor diameters were measured at either once or twice weekly intervals with digital vernier calipers Assuming tumors to be spherical, volumes were calculated from the formula $(\pi/6) \times d3$, where d represents the mean diameter.

The primary activity measures were the objective response measure (see legend to figure at right) and the EFS T/C measure. The EFS T/C value is defined by the ratio of the median time to event of the treatment group and the median time to event of the respective control group.

In vitro Activity of Cabozantinib against PPTP Cell Lines

Cell Line	Histotype	IC_{50} (µM)	Panel IC_{50}/ Line IC_{50}	Y_{min} (%) (Observed)
RD	Rhabdomyosarcoma	>10.0	0.7	56
Rh41	Rhabdomyosarcoma	6.4	1.3	34
Rh18	Rhabdomyosarcoma	0.3	27.0	17
Rh30	Rhabdomyosarcoma	9.1	0.9	41
BT-12	Rhabdoid	>10.0	0.7	54
CHLA-266	Rhabdoid	1.8	4.5	20
TC-71	Ewing sarcoma	8.0	1.0	36
CHLA-9	Ewing sarcoma	8.1	1.0	32
CHLA-10	Ewing sarcoma	5.7	1.4	16
CHLA-258	Ewing sarcoma	8.4	1.0	37
SJ-GBM2	Glioblastoma	0.8	10.1	15
NB-1643	Neuroblastoma	8.6	0.9	33
NB-EBc1	Neuroblastoma	8.9	0.9	42
CHLA-90	Neuroblastoma	9.5	0.8	43
CHLA-136	Neuroblastoma	6.6	1.2	28
NALM-6	ALL	7.8	1.0	23
COG-LL-317	ALL	9.6	0.8	47
RS4;11	ALL	9.2	0.9	41
MOLT-4	ALL	7.2	1.1	16
CCRF-CEM (1)	ALL	9.1	0.9	42
CCRF-CEM (2)	ALL	6.4	1.3	12
Kasumi-1	AML	0.034	236.5	14
Karpas-299	ALCL	>10.0	0.6	52
Ramos-RA1	NHL	4.4	1.8	17
Median		**8.1**	**1.0**	**33**
Minimum		**0.034**	**0.6**	**12**
Maximum		**>10.0**	**236.5**	**56**

CABOZANTINIB IN VIVO ACTIVITY

In vivo activity of Cabozantinib against PPTP Solid Tumor Xenograft Models

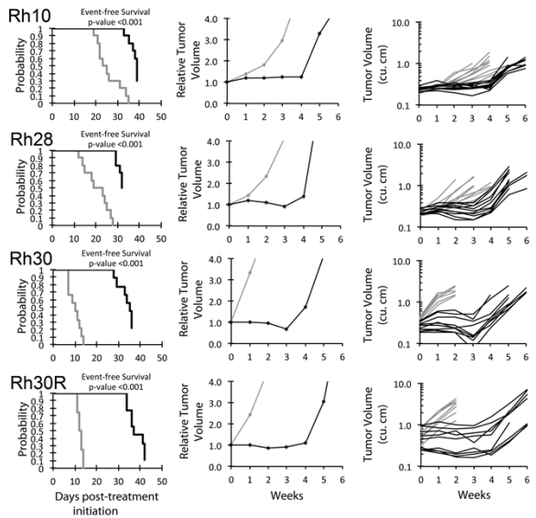
Line	Tumor Type	EFS T/C	P-value	Tumor Volume T/C	P-value	Day 21 RTV	Median Group Response
BT-29	Rhabdoid	> 1.8	<0.001	0.14	<0.001	0.5	PR
KT-16	Rhabdoid	3.8	<0.001	0.51	0.043	1.4	PD2
KT-14	Rhabdoid	> 1.3	<0.001	0.48	<0.001	2.0	PD2
KT-10	Wilms	2.9	<0.001	0.21	<0.001	1.9	PD2
KT-11	Wilms	2.3	<0.001	0.20	<0.001	1.0	SD
KT-13	Wilms	3.8	<0.001	0.12	<0.001	1.0	PD2
SK-NEP-1	Ewing	3.0	<0.001	0.20	<0.001	1.6	PD2
EW5	Ewing	3.1	<0.001	0.25	<0.001	2.3	PD2
EW8	Ewing	3.1	<0.001	0.18	<0.001	2.1	PD2
TC-71	Ewing	3.5	<0.001	0.40	<0.001	2.2	PD2
CHLA258	Ewing	1.9	<0.001	0.51	0.002	>4.0	PD2
Rh10	Alveolar RMS	1.6	<0.001	0.38	<0.001	1.2	PD2
Rh28	Alveolar RMS	1.6	<0.001	0.40	<0.001	1.0	PD2
Rh30	Alveolar RMS	3.6	<0.001	0.37	<0.001	0.6	PD2
Rh30R	Alveolar RMS	3.0	<0.001	0.19	<0.001	0.8	PD2
Rh41	Alveolar RMS	2.5	<0.001	0.30	<0.001	1.4	PD2
Rh18	Embryonal RMS	2.6	<0.001	0.46	<0.001	>4.0	PD2
BT-28	Medulloblastoma	3.4	<0.001	0.34	<0.001	1.0	PD2
BT-41	Ependymoma	.	1.000	0.36	<0.001	0.5	PR
GBM2	Glioblastoma	> 2.2	<0.001	0.40	<0.001	1.9	PD2
BT-39	Glioblastoma	1.6	0.023	0.69	0.083	>4.0	PD1
D645	Glioblastoma	2.8	<0.001	0.74	0.133	2.7	PD2
D456	Glioblastoma	2.5	0.004	0.45	0.009	2.2	PD2
NB-SD	Neuroblastoma	2.6	<0.001	0.31	<0.001	1.4	PD2
NB-1771	Neuroblastoma	2.2	<0.001	0.59	0.029	3.1	PD2
NB-1691	Neuroblastoma	1.4	0.004	0.63	0.011	>4.0	PD1
NB-EBc1	Neuroblastoma	2.7	<0.001	0.20	<0.001	>4.0	PD2
CHLA-79	Neuroblastoma	2.9	<0.001	0.45	0.014	2.2	PD2
OS-1	Osteosarcoma	1.7	<0.001	0.47	<0.001	1.7	PD2
OS-2	Osteosarcoma	1.1	0.123	0.94	0.247	>4.0	PD1
OS-17	Osteosarcoma	2.1	<0.001	0.43	<0.001	1.8	PD2
OS-9	Osteosarcoma	1.7	<0.001	0.50	<0.001	1.8	PD2
OS-33	Osteosarcoma	2.1	<0.001	0.55	<0.001	2.8	PD2
OS-31	Osteosarcoma	1.1	0.063	0.82	0.019	>4.0	PD1

- **Red shading in the p-value columns indicates a significant difference in EFS distribution or Tumor Volume T/C between treated and control groups.**
- **Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), low (gray), or indeterminant (white) activity.**
- **PD1 (Progressive Disease 1):** >25% ↑ in tumor volume, TGD value ≤1.5;
- **PD2 (Progressive Disease 2):** >25% ↑ in tumor volume, TGD value >1.5;
- **SD (Stable Disease):** <25% ↑ in tumor volume, <50% regression
- **PR (Partial response):** a tumor volume regression ≥50%
- **CR (Complete response):** disappearance of measurable tumor mass (< 0.10 cm^3)

**Cabozantinib was provided for testing by Exelixis.
Testing was supported by NCI NO1CM42216.**

In vivo activity of Cabozantinib against Rhabdomyosarcoma Models (Treatment for 21 days)

Rh10 (ARMS)
Rh28(ARMS)
Rh30 (ARMS) (diagnosis)
Rh30R (ARMS (relapse)



Controls (gray lines)
Treated (black lines)

IN VIVO RESULTS AND CONCLUSIONS

- Cabozantinib was well tolerated (1.8% mortality) at the dose (30 mg/kg PO) and schedule (daily x 21 or daily x 28) evaluated, and 34 of 34 xenograft models were considered evaluable for efficacy.
- Cabozantinib induced significant differences in EFS distribution compared to control in 31 of 34 (91%) evaluable solid tumor xenografts.
- Cabozantinib induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity (EFS T/C > 2) in 22 of 31 (71%) of evaluable solid tumor xenografts.
- Cabozantinib induced objective responses in 2 of 34 (6%) of solid tumor models, including two brain tumor models, BT-29, (ATRT) and BT-41 (ependymoma).
- Tumor growth control was most pronounced for the alveolar rhabdomyosarcoma xenografts (ARMS), with 4 of 5 ARMS lines showing < 20% increase in tumor volume during the 21 days of treatment. Two of 3 Wilms tumor lines (KT-11 and KT-13) also showed complete tumor growth control at Day 21,
- The activity of cabozantinib against the PPTP solid tumor xenografts is consistent with its known anti-angiogenic activity.

This poster will be available at: http://pptp.nchresearch.org/presentations.html