Abstract C106

Pediatric Preclinical Testing Program (PPTP) Stage 1 evaluation of the PI3K inhibitor XL147 (SAR245408)

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XL147 (SAR245408)

- Orally bioavailable, potent, and specific inhibitor of class I PI3K lipid kinases with minimal TORC inhibitory activity.
- Inhibits PI3K signaling and downstream effects in vitro and in vivo under conditions similar to those studied by PPTP.
- Shows strong tumor growth inhibition as a single agent in multiple adult cancer xenograft tumor models associated with increased apoptosis and inhibition of tumor angiogenesis.
- Currently in adult phase 2 trials (single agent) and in trials in combination with chemotherapeutic and targeted agents.

XL147 (SAR245408) *IN VITRO* ACTIVITY

- The median relative IC_{50} (rIC_{50}) for XL147 against the PPTP cell lines was 10.9 µM, (range 2.7 µM - 24.5 µM).
- There was a trend for lower values for the rhabdomyosarcoma panel (median rIC_{50} 5.6 µM) and higher values for the neuroblastoma panel (median rIC_{50} 19.5 µM).
- XL147 induced Ymin values approaching 0% for PPTP cell lines at the highest concentration tested (100 µM).

Cell Line	Histotype	rIC_{50} (µM)	Panel RIC_{50}/ Line RIC_{50}	Ymin
RD	RMS	5.3	2.1	0.00
Rh41	RMS	5.3	2.1	0.00
Rh18	RMS	5.8	1.9	0.00
Rh30	RMS	11.3	1.0	0.00
BT-12	Rhabdoid	16.1	0.7	0.00
SJ-GBM2	GBM	18.9	0.6	0.00
CHLA-266	Rhabdoid	7.7	1.4	0.00
CHLA-9	Ewing	6.8	1.6	0.00
CHLA-10	Ewing	2.7	4.0	0.00
CHLA-258	Ewing	10.2	1.1	0.00
TC-71	Ewing	24.5	0.4	0.00
NB-1643	NB	20.3	0.5	0.00
NB-EBc1	NB	23.9	0.5	0.00
CHLA-90	NB	8.5	1.3	0.00
CHLA-136	NB	18.7	0.6	0.00
NALM-6	ALL	7.6	1.4	0.00
COG-LL-317	ALL	13.2	0.8	0.00
RS4;11	ALL	3.8	2.9	0.00
MOLT-4	ALL	10.6	1.0	0.00
CCRF-CEM1	ALL	14.5	0.8	0.00
CCRF-CEM2	ALL	14.3	0.8	0.00
Kasumi-1	AML	3.8	2.9	0.00
Karpas-299	ALCL	13.1	0.8	0.00
Ramos	NHL	21.5	0.5	0.00
Median		10.9	1.0	0.00
Minimum		2.7	0.4	0.00
Maximum		24.5	4.0	0.00

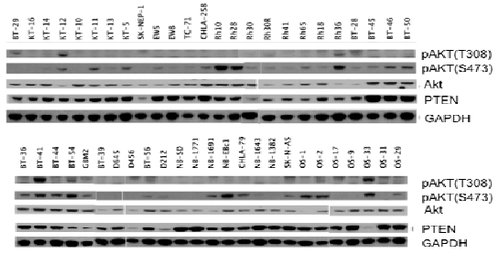
PPTP *IN VITRO* & *IN VIVO* TESTING METHODS

In vitro: *In vitro* testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system (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 mM with replicates of 6-12 per data point.

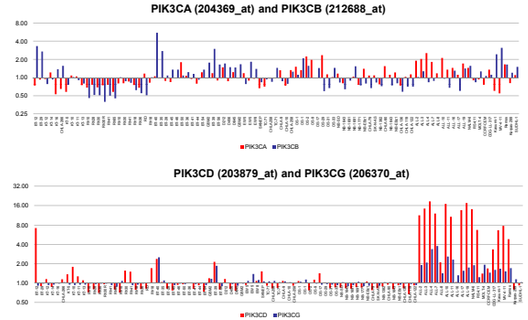
In vivo: Standard PPTP methods for in vivo testing were employed (see http://pptp.ncbresearch.org/documents/detailedAnalysisMethods.pdf). XL147 was tested *in vivo* using a 100 mg/kg dose administered orally daily for 14 days .

Solid tumor testing: 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.

Acute lymphoblastic leukemia (ALL) testing: For each xenograft line, 8 mice were inoculated with 3-5 x 10^6 mononuclear cells purified from the spleens of secondary recipient mice. Engraftment was monitored weekly by flow cytometry, and treatment was initiated when the proportion of human CD45+ cells in the peripheral blood reached 1%. The proportion of human CD45+ cells in the peripheral blood was monitored weekly throughout the course of treatment.



PI3K pathway activation is relatively uncommon among PPTP xenografts as assessed by phospho-AKT and by PTEN expression.



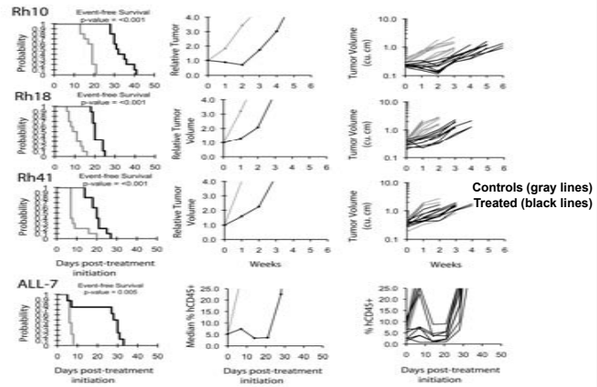
Expression of PI3K isoforms at the RNA level was notable for the pattern of expression of PIK3CD, which was virtually restricted to the ALL and lymphoma cell lines and xenografts.

XL147 (SAR245408) *IN VIVO* ACTIVITY

Line	Tumor Type	EFS T/C	P-value	T/C	P-Value	Response
KT-10	Wilms	1.6	<0.001	0.41	<0.001	PD2
KT-13	Wilms	1.6	0.014	0.62	0.011	PD1
SK-NEP-1	Ewing	1.6	0.004	0.37	<0.001	PD2
EW5	Ewing	1.3	0.045	0.68	0.029	PD1
EW8	Ewing	1.3	0.737	0.85	0.315	PD1
TC-71	Ewing	1.3	0.058	0.79	0.393	PD1
Rh10	ALV RMS	1.7	<0.001	0.25	<0.001	PD2
Rh28	ALV RMS	1.2	0.043	0.73	0.146	PD1
Rh30	ALV RMS	1.4	<0.001	0.49	<0.001	PD1
Rh30R	ALV RMS	1.7	<0.001	0.42	<0.001	PD2
Rh41	ALV RMS	2.9	<0.001	0.46	<0.001	PD2
Rh18	EMB RMS	2.1	<0.001	0.39	<0.001	PD2
BT-45	Medulloblastoma	1.4	0.010	0.54	0.019	PD1
GBM2	Glioblastoma	1.4	0.037	0.57	0.005	PD1
BT-39	Glioblastoma	1.3	0.006	0.73	0.019	PD1
D645	Glioblastoma	1.7	0.003	0.66	<0.001	PD2
D456	Glioblastoma	1.8	0.014	0.79	0.123	PD2
D212	Glioblastoma	1.9	0.070	0.57	0.029	PD2
NB-SD	Neuroblastoma	1.8	0.023	0.73	0.040	PD2
NB-1771	Neuroblastoma	1.6	0.004	0.77	0.014	PD2
NB-1691	Neuroblastoma	1.2	0.013	0.81	0.043	PD1
NB-EBc1	Neuroblastoma	2.4	<0.001	0.34	<0.001	PD2
CHLA-79	Neuroblastoma	1.9	<0.001	0.46	<0.001	PD2
NB-1643	Neuroblastoma	1.5	0.001	0.55	<0.001	PD1
OS-1	Osteosarcoma	.	0.791	0.79	0.001	PD2
OS-2	Osteosarcoma	1.2	<0.001	0.81	<0.001	PD1
OS-17	Osteosarcoma	1.1	0.008	0.79	0.015	PD1
OS-9	Osteosarcoma	1.3	<0.001	0.68	0.002	PD1
OS-33	Osteosarcoma	1.1	0.006	0.84	0.011	PD1
OS-31	Osteosarcoma	1.1	<0.001	0.84	0.006	PD1
ALL-4	ALL B-precursor	1.0	0.446	.		PD1
ALL-7	ALL B-precursor	4.5	0.005	.		PD2
ALL-8	ALL T-cell	0.8	0.830	.		PD1
ALL-17	ALL B-precursor	2.9	<0.001	.		PD2
ALL-19	ALL B-precursor	1.0	0.929	.		PD1
ALL-31	ALL T-cell	1.9	0.136	.		PD2
MLL-7	ALL B-precursor	1.2	0.415	.		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

XL147 (SAR245408) was provided for testing by sanofi-aventis. Testing was supported by NCI NO1CM42216.



IN VIVO RESULTS AND CONCLUSIONS

- XL147 was well tolerated at 100 mg/kg administered orally for 14 consecutive days.
- XL147 inhibited tumor growth in most PPTP solid tumor xenografts as shown by significant differences in EFS distribution compared to control in 26 of 30 (87%) evaluable solid tumor xenografts. Two of 7 (29%) evaluable ALL xenografts showed significant differences in EFS distribution.
- Three of 29 (10%) evaluable solid tumor xenografts metcriteria for intermediate EFS T/C activity (EFS T/C> 2): 2 of 6 rhabdomyosarcoma and 1 of 5 neuroblastoma. Objective responses (PR/CR) were not observed.
- Baseline levels of PI3K activation, as assessed by phospho-AKT levels, did not appear related to in vivo response to XL147.
- Under the conditions evaluated in this study, XL147 achieved modest single-agent activity against the PPTP preclinical models.
- Genomic activation of PI3K signaling (e.g., through PIK3CA mutation or PTEN deletion) appears relatively uncommon in pediatric solid tumors compared to adult cancers, which may contribute to the limited single agent XL147 activity observed.
- Preclinical evaluations of XL147 in combination with particular standard agents and with other pathway signaling inhibitors are planned.