Abstract 527

Pediatric Preclinical Testing Program (PPTP) Evaluation of the Multi-Targeted Kinase Inhibitor Sunitinib

Peter J. Houghton[1], John M. Maris[2], Henry S. Friedman[3], Stephen T. Keir[3], Richard B. Lock[4], Hernan Carol[4], Mayamin Tajbakhsh[4], Richard Gorlick[5], E. Anders Kolb[5], Nino Keshelava[6], C. Patrick Reynolds[6], Christopher Morton[1], Malcolm A. Smith[7].
[1]St. Jude Children's Research Hospital, [2]Children's Hospital of Philadelphia, [3]Duke University, [4]Children's Cancer Inst., Australia, [5]Albert Einstein College, [6]Children's Hospital of Los Angeles, [7]CTEP/NCI.

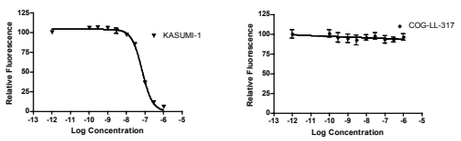
Abstract

Background: Sunitinib is an orally available, multi-targeted tyrosine kinase inhibitor with selectivity for PDGF receptors, VEGF receptors, FLT3, and KIT. Sunitinib inhibits the growth of a wide range of established adult tumor xenografts in a dose-dependent manner and is licensed for the treatment of renal cell carcinoma and GIST.
Methods: The PPTP includes an in vitro panel (n=27) as well as panels of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. Sunitinib in vitro testing used media containing 20% FCS and evaluated concentrations from 0.1 nM to 1 micromolar. Testing against the in vivo tumor panels used a dose of 53.5 mg/kg PO X 28 days. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting [e.g., partial response (PR), complete response (CR), etc.]; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4X increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: Kasumi-1 (gain-of-function KITAsn822Lys mutation) was the only cell line to show an in vitro response to sunitinib and had an EC_{50} of 71nM, a concentration well within the range achievable in humans. Sunitinib induced significant differences in EFS distribution in 19 of 35 (54%) of the solid tumor xenografts analyzed, and in 1 of 8 (13%) of the ALL xenografts analyzed. Using the PPTP time to event measure of efficacy, sunitinib had intermediate (13) and high (1) levels of activity against 14 of 34 evaluable solid tumor xenografts. One of 3 rhabdoid tumor xenografts exhibited high activity for the time to event measure, while another exhibited intermediate activity. Intermediate activity was also observed in 4 of 6 rhabdomyosarcoma, 4 of 5 Ewing tumor, 1 of 3 Wilms tumor, and 1 of 3 osteosarcoma. Following cessation of treatment for the 14 solid tumor xenografts that had not had tumor events by Day 28, tumor growth rate increased to that observed for the comparable control groups. The only objective response to sunitinib in the solid tumor panels was a CR in a rhabdoid tumor xenograft. For the ALL panel, sunitinib produced a partial response in 1 of 8 lines.
Conclusions: Sunitinib demonstrated broad activity against the PPTP's solid tumor panel, but only limited activity against the ALL panel. Antitumor activity was manifested primarily as tumor growth delay, consistent with an anti-angiogenic effect for sunitinib against many of the pediatric preclinical models evaluated. A pediatric phase 1 trial of sunitinib has been initiated. (Supported by NCI NO1CM42216)

In Vitro Test Results for Sunitinib

Methods: *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 (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005). Testing was for 96 hours at concentrations from 0.1 nM to 1.0 μM with replicates of 6-12 per data point. Data were analyzed using GraphPad Prism, fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values *vs.* the concentration. The PPTP in vitro panel contains cell lines for neuroblastoma (4), Ewing sarcoma (4), rhabdomyosarcoma (4), ALL (5), NHL (2), and others.
Results: Sunitinib showed little evidence for drug effect against the 23 cell lines of the PPTP *in vitro* panel at concentrations up to 1 μM. The exception was Kasumi-1 (AML), which is known to have a gain-of-function c-KITAsn822Lys mutation and which had an IC_{50} of 75.7 nM.

Examples of Sunitinib Dose Response Curves



Printed by Biomedical Communications

Methods for PPTP *In Vivo* Testing

Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD or at a dose selected based on PK/PD studies using adult preclinical models.

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. Assuming tumors to be spherical, volumes were calculated from the formula (π/6)×d3, where d represents the mean diameter.
Acute lymphoblastic leukemia 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.
Drug: Sunitinib was provided to the Pediatric Preclinical Testing Program by Pfizer Pharmaceuticals through the Cancer Therapy Evaluation Program (NCI). Sunitinib was dissolved in carboxymethylcellulose sodium 0.5% (w/v), sodium chloride 1.8% (w/v), benzyl alcohol 0.9% (w/v), pH 6, and administered by oral gavage daily for 28 days at a dose of 53.5 mg/kg. Sunitinib was provided to each testing site in coded vials for blinded testing according the PPTP's standard operating procedures.

Solid Tumor Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	>25% ↑ in tumor volume, TGD value ≤1.5	0
PD2 (Progressive Disease 2)	>25% ↑ in tumor volume, TGD value >1.5	2
SD (Stable Disease)	<25% ↑ in tumor volume, <50% regression	4
PR (Partial Response)	≥50% regression, but no CR	6
CR (Complete Response)	<0.1 cm3 tumor volume	8
MCR (Maintained CR)	<0.1 cm3 tumor volume at the end of study	10

Leukemia Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	No PR & TGD value of ≤1.5 & events at EOS	0
PD2 (Progressive Disease 2)	No PR & TGD value >1.5 & events at EOS	2
SD (Stable Disease)	No PR and no events at EOS	4
PR (Partial Response)	CD45% <1% for only 1 week	6
CR (Complete Response)	CD45% <1% for 2 consecutive weeks	8
MCR (Maintained CR)	CD45% <1% for last 3 weeks of study	10

Median Group Response: Each individual mouse in the treatment group was assigned a response score (see Tables above) and a median score for the treatment group was calculated and then each treatment group was assigned an overall response according to the table below.

If Median Score (MS) from (1):	Overall Group Response
0 ≤ MS ≤1	PD1
1 < MS ≤3	PD2
3 < MS ≤5	SD
5 < MS ≤7	PR
7 < MS ≤9	CR
9 < MS	MCR

Statistical Methods: Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant.

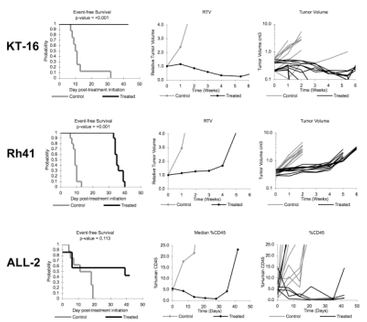
Sunitinib Activity: PPTP *In Vivo* Lines

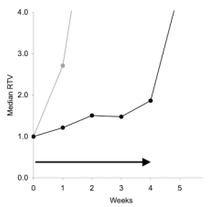
- Sunitinib significantly prolonged EFS in 19 of 35 (54%) of the solid tumor xenografts, and in 3 of 8 (38%) of the ALL, xenografts analyzed.
- Using the PPTP time to event measure of efficacy (EFS T/C), sunitinib had intermediate (coded light blue below with EFS T/C > 2) and high (coded dark blue below with EFS T/C > 2 and final RTV < 1) activity against 14 of 34 evaluable solid tumor xenografts, including 4 of 6 rhabdomyosarcoma, 4 of 5 Ewing tumor, and 2 of 3 rhabdoid tumor xenografts.
- KT-16 (rhabdoid tumor) achieved a complete response, and the ALL xenograft ALL-2 achieved a partial response.

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	Objective Response
BT-29	Rhabdoid	<0.001	3.7	>4	0.19	PD2
KT-16	Rhabdoid	<0.001	> 4.0	0.4	0.47	CR
KT-14	Rhabdoid	0.233	1.1	>4	0.92	PD1
KT-10	Wilms	<0.001	1.8	>4	0.54	PD2
KT-11	Wilms	0.002	3.2	>4	0.79	PD2
KT-13	Wilms	0.022	1.5	>4	0.76	PD1
SKNEP	Ewings	<0.001	5.9	>4	0.3	PD2
EW5	Ewings	<0.001	3.5	>4	0.22	PD2
EW8	Ewings	<0.001	5.3	>4	0.21	PD2
TC-71	Ewings	<0.001	6.1	>4	0.29	PD2
CHLA258	Ewings	0.069*	0.6	>4	1.15	PD1
Rh10	Alveolar RMS	0.255	2.1	>4	0.24	PD2
Rh28	Alveolar RMS	0.756	1	>4	1.42	PD1
Rh30	Alveolar RMS	<0.001	3.6	>4	0.25	PD2
Rh30R	Alveolar RMS	<0.001	4.8	>4	0.37	PD2
Rh41	Alveolar RMS	<0.001	3.8	>4	0.36	PD2
Rh18	Embryonal RMS	<0.001	3.8	>4	0.29	PD2
BT-28	Medulloblastoma	<0.001	3.2	>4	0.13	PD2
BT-45	Medulloblastoma	0.284	0.9	>4	0.93	PD1
BT-46	Medulloblastoma	0.181	1.4	>4	0.51	PD1
BT-44	Ependymoma	0.342	1.2	>4	1.2	PD1
GBM2	Glioblastoma	0.153	1.3	>4	1.05	PD1
BT-39	Glioblastoma	0.712	1	>4	0.98	PD1
D645	Glioblastoma	0.002	1.7	>4	0.59	PD2
D456	Glioblastoma	0.001	2.1	>4	0.67	PD2
NB-SD	Neuroblastoma	0.27	1.7	>4	0.58	PD2
NB-1771	Neuroblastoma	<0.001	1.8	>4	0.55	PD2
NB-1691	Neuroblastoma	0.689	1.1	>4	0.84	PD1
NB-EBc1	Neuroblastoma	0.123	1.1	>4	0.86	PD1
CHLA-79	Neuroblastoma	0.276	0.9	>4	1.19	PD1
NB-1643	Neuroblastoma	0.14	1.3	>4	0.69	PD1
OS-1	Osteosarcoma	<0.001	> 1.4	3.1	0.6	PD2
OS-2	Osteosarcoma	0.822	1.1	>4	1.08	PD1
OS-33	Osteosarcoma	<0.001	4.6	>4	0.18	PD2
OS-31	Osteosarcoma	0.171	1.7	>4	0.6	PD2
ALL-2	ALL B-precursor	0.04	> 2.9	>25	.	PR
ALL-3	ALL B-precursor	0.131	1.6	>25	.	PD2
ALL-4	ALL B-precursor	0.012*	0.5	>25	.	PD1
ALL-7	ALL B-precursor	0.483	1	>25	.	PD1
ALL-8	ALL T-cell	0.993	1	>25	.	PD1
ALL-16	ALL T-cell	0.645	1.1	>25	.	PD1
ALL-17	ALL B-precursor	<0.001	2.6	>25	.	PD2
ALL-19	ALL B-precursor	0.037	6.6	>25	.	PD2

Red shading in the p-value column indicates a significant difference in EFS distribution between treated and control groups.
Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (gray) activity.

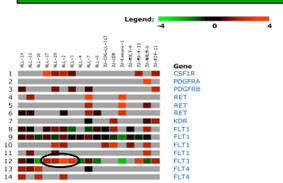
Examples of Sunitinb *In Vivo* Activity





The 14 xenografts for which tumor growth was controlled during the 28 days of sunitinib treatment were followed for an additional 14 days after treatment was stopped. Following cessation of treatment, the median tumor growth rate increased to rates that were similar to those observed for the comparable control groups

Gene Expression for ALL Xenografts & Cell Lines



- Gene expression data (Affymetrix U133 Plus 2.0) for the ALL xenografts and cell lines were evaluated for expression levels of sunitinib receptor tyrosine kinase targets.
- Sunitinib activity against the ALL xenografts matched the expression pattern for FLT3, with ALL-2 having the highest FLT3 expression and the greatest response to sunitinib (PR).
- The 3 ALL xenografts that had PD2 responses (ALL-3, ALL-17, and ALL-19) also had elevated FLT3 expression.
- RS4;11 (*in vitro* panel), an ALL cell line with an MLL gene rearrangement, had high expression of FLT3, but was not sensitive to sunitinib. RS4;11 is known from previous reports to not respond to small molecule FLT3 inhibitors.
- Neither RS4;11 nor ALL-2 have FLT3 ITD mutations.

CONCLUSIONS

- Sunitinib showed growth inhibitory activity against a broad range of the PPTP's solid tumor xenografts, as illustrated by intermediate activity for the EFS T/C activity measure for 4 of 5 Ewing sarcomas and 4 of 6 rhabdomyosarcomas. Less growth inhibitory activity was observed for the neuroblastoma panel.
- A single xenograft, KT-16 (rhabdoid tumor), showed an objective response to sunitinib.
- Sunitinib demonstrated limited activity against the PPTP ALL xenografts, with one of eight xenografts (ALL-2) showing a partial response. Response to sunitinib was associated with the pattern of FLT3 expression for the ALL xenografts.
- The pattern of *in vivo* antitumor activity exhibited by sunitinib against the solid tumor panels (predominantly tumor growth delay) combined with the limited effect of sunitinib on the cell lines of the PPTP *in vitro* panel suggest that sunitinib is exerting its antitumor effect primarily through an anti-angiogenic mechanism of action for the pediatric solid tumor models evaluated.
- Sunitinib is currently in phase 1 trials for children with cancer using the "4 weeks on/ 2 weeks off" treatment schedule. The observation of return to control growth rates for most responding xenografts upon cessation of sunitinib treatment suggests that it may be advantageous to explore continuous sunitinib administration schedules in future clinical evaluations of sunitinib for children with cancer.

Supported by NCI NO1CM42216