322 Pediatric Preclinical Testing Program (PPTP) Evaluation of the Src-Abl Inhibitor Dasatinib (BMS-354825)

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Abstract*

Background: Dasatinib is a dual Src/Bcr-Abl kinase inhibitor that maintains activity against most imatinib-resistant Bcr-Abl mutants and that is active in many patients with imatinib-resistant Bcr-Abl leukemias. Dasatinib induced complete regressions in Bcr-Abl preclinical models and primarily caused tumor growth delay in adult solid tumor preclinical models. The COG Phase 1 Consortium has initiated a phase 1 trial of dasatinib for children with refractory solid tumors and Bcr-Abl leukemias.

Methods: The PPTP includes an *in vitro* panel (23 lines) as well as panels of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. Dasatinib was administered orally twice-daily (solid tumor panels) or once daily (ALL panel) for four weeks (5-days on, 2-days off) at 50 mg/kg/dose . 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 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: Dasatinib induced significant tumor growth delay in 22% of the 27 evaluable solid tumor xenografts tested. Using the time to event measure of efficacy, dasatinib had intermediate activity against 1 (rhabdoid tumor) of 25 (4%) of the solid tumor xenografts evaluable for this activity measure. Dasatinib did not induce objective responses against any of the solid tumor xenografts, but induced a CR against the PPTP's Bcr-Abl ALL xenograft. One of 4 evaluable non-Bcr-Abl ALL xenografts achieved a PR and 2 of 4 showed significant growth delay. Seven of 22 tested cell lines in the *in vitro* panel had EC50 < 1 μM. Kasumi-1, the most sensitive line with an EC50 of 11 nM, is known to have a gain-of-function KITAsn822Lys mutation. Other cell lines with EC50 values below 100 nM were the rhabdoid tumor line CHLA-266 (15 nM) and the T-cell ALL line MOLT-4 (45 nM).

Conclusions: Dasatinib was highly active against the PPTP's Bcr-Abl ALL xenograft, but had limited activity against the xenografts of the PPTP's solid tumor panels. Several cell lines in the *in vitro* panel were particularly sensitive to dasatinib (EC50 < 50 nM), and further work is needed to define the molecular basis of this activity.

* Updated from initial submission.

PPTP *In Vitro* Testing Methods

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 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.

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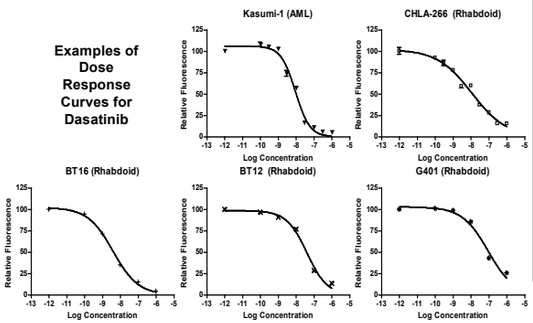
In Vitro Test Results for Dasatinib

- Dasatinib was active against 7 of 22 of the PPTP *in vitro* panel cell lines when tested at concentrations from 0.1 nm to 1μM.
- The most sensitive line was Kasumi-1 (EC50 = 9 nM), an AML cell line that is known to have a gain-of-function c-KIT mutation (Asn822Lys).
- The two rhabdoid tumor cell lines in the PPTP *in vitro* panel had EC50 values < 1 μM (CHLA-266 = 11 nM and BT-12 = 247 nM).
- Because of the sensitivity of these two lines to dasatinib, additional rhabdoid tumor lines were studied. These lines were also sensitive to dasatinib, with EC50 values ranging from 4 nM (BT-16) to 90 nM (G401) (see dose-response curves below).
- MOLT-4 (T-cell ALL) was the only ALL line with an EC50 value < 1 μM.

Line	Diagnosis	EC50	HILL SLOPE	R2	Ratio EC50 to Median EC50
RD	EMB RMS	> 1000	NA	NA	1
Rh41	ALV RMS	458	-0.86	0.79	2.4
Rh18	EMB RMS	> 1000	NA	NA	1
Rh30	ALV RMS	> 1000	NA	NA	1
BT-12	Rhabdoid	247	-0.5	0.74	4.5
CHLA-266	Rhabdoid	11	-0.45	0.96	98.4
TC-71	Ewing sarcoma	> 1000	NA	NA	1
CHLA-9	Ewing sarcoma	> 1000	NA	NA	1
CHLA-10	Ewing sarcoma	794	-0.58	0.8	1.4
CHLA-258	Ewing sarcoma	> 1000	NA	NA	1
SJ-GBM2	Ewing sarcoma	> 1000	NA	NA	1
NB-1643	Glioblastoma	> 1000	NA	NA	1
NB-EBc1	Neuroblastoma	> 1000	NA	NA	1
CHLA-90	Neuroblastoma	> 1000	NA	NA	1
CHLA-136	Neuroblastoma	360	-0.59	0.62	3.1
NALM-6	ALL	> 1000	NA	NA	1
RS4;11	ALL	> 1000	NA	NA	1
MOLT-4	ALL	36	-0.52	0.77	30.7
CCRF-CEM	ALL	> 1000	NA	NA	1
KASUMI-1	AML	9	-1.08	0.98	119.9
KARPAS-299	ALCL	> 1000	NA	NA	1
RAMOS	NHL	> 1000	NA	NA	1

* Red shading indicates lines with EC50 < median.

Examples of Dose Response Curves for Dasatinib



PPTP *In Vivo* Testing Methods

- 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 ($\pi/6$)×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.
- **Dasatinib** was provided by Bristol-Myers Squibb through the Cancer Therapy Evaluation Program (NCI). Dasatinib was dissolved in 80mM Citrate Buffer (pH 2.1) and administered daily X 5 (2 days off) for 4 weeks. Dasatinib was provided to each consortium investigator in coded vials for blinded testing according the PPTP program standard operating procedures.
- **Solid Tumor Response Criteria:**

Response		Definition	Score
PD1	Progressive Disease 1	<50% regression at all measurements and >25% increase in tumor volume at the end of the study period, TGD' value of ≤ 1.5	0
PD2	Progressive Disease 2	<50% regression at all measurements and >25% increase in tumor volume at the end of the study period, TGD value of >1.5	2
SD	Stable Disease	<50% regression at all measurements and ≤25% increase at the end of the study	4
PR	Partial Response	≥50% regression but with tumor volume ≥0.1 cm^3 during study	6
CR	Complete Response	Tumor volume <0.1 cm^3 for at least one study measurement	8
MCR	Maintained Complete Response	Tumor volume <0.1 cm^3 at the end of study	10

- **Leukemia Response Criteria:**

Response		Definition	Score
PD1	Progressive Disease 1	CD45% never dr ops below 1%, event s before end of study, TGD' value of ≤1.5	0
PD2	Progressive Disease 2	CD45% never dr ops below 1%, eve nts before end of study, TGD value of >1.5	2
SD	Stable Disease	CD45% never drops below 1%, n o events before end of study	4
PR	Partial Response	CD45% drops below 1% for only 1 week	6
CR	Complete Response	CD45% drops be low 1% for 2 consecut ive weeks	8
MCR	Maintained Complete Response	CD45% drops be low 1% for last 3 consecutive measurements of the study	10

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

If Average Score (AS) from (1):	Overall Group Response
0 ≤ AS ≤1	PD1
1 < AS ≤3	PD2
3 < AS ≤5	SD
5 < AS ≤7	PR
7 < AS ≤9	CR
9 < AS	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. Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The mean relative tumor volumes for control and treatment mice for each study were then calculated and the T/C value was the mean RTV for the treatment group divided by the mean RTV for the control group.

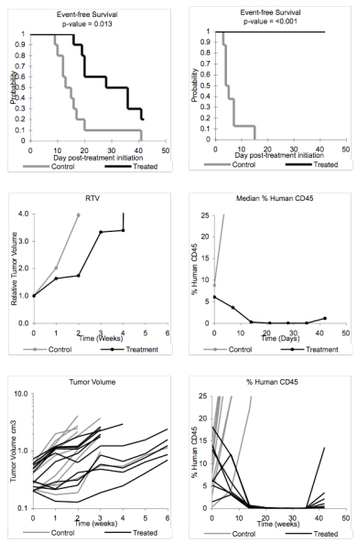
In Vivo Test Results for Dasatinib

- Dasatinib induced complete remission against the Bcr-Abl expressing leukemia xenograft ALL-4 (see growth curves below), and dasatinib produced intermediate activity against 2 additional ALL xenografts as assessed by the PPTP time to event measure of activity. The results for the leukemia panel were obtained with a daily dose of dasatinib and are being repeated with the twice-daily dosing schedule that was employed for the solid tumor panels.
- Dasatinib induced significant differences in EFS distribution in 6 of 27 (22%) of the solid tumor xenografts (highlighted in red in the table below). Tumor regressions were not observed for dasatinib among the solid tumor xenografts evaluated.
- Dasatinib demonstrated intermediate activity against the rhabdoid tumor xenograft KT-16 using the PPTP time to event measure of activity (EFS T/C) (highlighted in light blue in the table below and shown in the growth curves below).

Line	Tumor Type	P-value	EFS T/C	Median Final RTV	T/C	Overall Group Response
BT-29	Rhabdoid	0.663	1.00	>4	0.84	PD1
KT-16	Rhabdoid	0.013	2.30	>4	0.46	PD2
KT-14	Rhabdoid	0.358	1.60	>4	0.77	PD2
KT-10	Wilms	0.465	1.00	>4	0.89	PD1
KT-13	Wilms	0.773	1.00	>4	0.55	PD1
SKNEP	Ewings	0.819	0.90	>4	0.74	PD1
EW5	Ewings	0.856	1.00	>4	1.17	PD1
EW8	Ewings	0.634	1.10	>4	0.99	PD1
Rh10	ALV RMS	0.758	0.90	>4	1.17	PD1
Rh28	ALV RMS	0.284	0.90	>4	1.06	PD1
Rh30	ALV RMS	0.009	1.20	>4	0.67	PD1
Rh30R	ALV RMS	0.020	1.20	>4	0.80	PD1
Rh41	ALV RMS	0.003	0.80	>4	1.64	PD1
Rh18	EMB RMS	0.201	2.10	>4	0.67	PD2
BT-28	Medulloblastoma	0.143	0.80	>4	1.19	PD1
BT-45	Medulloblastoma	0.373	.	3.2	1.09	PD2
SJ-BT39	Glioblastoma	0.009	1.30	>4	1.02	PD1
D645	Glioblastoma	0.011	1.70	>4	0.60	PD2
NB-SD	Neuroblastoma	0.096	1.10	>4	0.78	PD1
NB-1771	Neuroblastoma	0.893	1.00	>4	1.04	PD1
NB-1691	Neuroblastoma	0.423	1.20	>4	0.70	PD1
NB-EBc1	Neuroblastoma	0.985	1.00	>4	1.02	PD1
CHLA-79	Neuroblastoma	0.384	0.90	>4	1.04	PD1
NB-1643	Neuroblastoma	0.529	0.90	>4	1.04	PD1
OS-1	Osteosarcoma	0.005	> 1.5	>4	0.70	PD2
OS-2	Osteosarcoma	0.222	0.70	>4	1.57	PD1
OS-17	Osteosarcoma	0.245	1.30	>4	0.68	PD1
ALL-2	ALL	0.428	1.00	>25	.	PD1
ALL-3	ALL	0.041	44.80	>25	.	PR
ALL-4	ALL	<0.001	> 8.9	1.1	.	CR
ALL-17	ALL	0.509	1.20	>25	.	PD1
ALL-19	ALL	0.009	7.80	>25	.	PD2

* Red shading indicates a significant difference in EFS distribution (treatment vs control), blue shading indicates either high (dark blue) or intermediate (light blue) activity using the time to event (EFS T/C) measure of activity.

KT-16 **ALL-4**



CONCLUSIONS

- Dasatinib demonstrated activity against the AML cell line Kasumi-1 (EC50 = 9 nM), confirming previous reports of dasatinib activity against cell lines with the gain-of-function c-KIT mutation, Asn822Lys.
- Dasatinib was active *in vitro* against multiple rhabdoid tumor cell lines. Tumor regressions were not observed for the rhabdoid tumor xenografts studied, although dasatinib did significantly inhibit the growth of one rhabdoid tumor xenograft. Further work is needed to understand the molecular basis for the *in vitro* sensitivity of the rhabdoid tumor lines to dasatinib.
- Dasatinib induced complete remission in a Bcr-Abl ALL xenograft and demonstrated anti-leukemia activity against other ALL xenografts. Dasatinib induced significant growth inhibition in a subset of the xenografts in the PPTP's solid tumor panels, but did not induce regressions for any xenografts in these panels. Based on the role of Src family kinases in metastasis, evaluations of dasatinib in pediatric preclinical models of metastatic disease are warranted.
- A phase 1 trial of dasatinib for children with refractory solid tumors and Bcr-Abl expressing leukemias has been initiated.