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Initial Testing (Stage 1) of the Multi-targeted Kinase Inhibitor Sorafenib by the Pediatric Preclinical Testing Program

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Abstract

Background—Sorafenib is an inhibitor of multiple kinases (e.g., VEGF receptors, PDGFR, FLT3, RET, BRAF, KIT) and is approved by FDA for treatment of two adult cancers. The activity of sorafenib was evaluated against the PPTP's *in vitro* and *in vivo* panels.

Procedures—Sorafenib was evaluated against the PPTP *in vitro* panel using 96 hour exposure at concentrations ranging from 1.0 nM to 10.0 μ M. It was tested against the PPTP *in vivo* panels at a dose of 60 mg/kg administered by oral gavage daily for 5 days per week, repeated for 6 weeks.

Results—*In vitro* sorafenib demonstrated cytotoxic activity, with a median IC₅₀ value of 4.3 μ M. Twenty of 23 cell lines had IC₅₀ values between 1.0 and 10.0 μ M. A single cell line (Kasumi-1) with an activating KIT mutation had an IC₅₀ value < 1.0 μ M (IC₅₀ = 0.02 μ M). *In vivo* sorafenib induced significant differences in EFS distribution compared to control in 27 of 36 (75%) of the evaluable solid tumor xenografts and in 1 of 8 (12.5%) of the evaluable ALL xenografts. Sorafenib induced tumor growth inhibition meeting criteria for intermediate activity (EFS T/C) in 15 of 34 (44%) evaluable solid tumor xenografts. No xenografts achieved an objective response.

Conclusions—The primary *in vitro* activity of sorafenib was noted at concentrations above 1 μ M, with the exception of a more sensitive cell line with an activating KIT mutation. The primary *in vivo* effect for sorafenib was tumor growth inhibition, which was observed across multiple histotypes.

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Keywords

Preclinical Testing; Developmental Therapeutics; tyrosine kinases

INTRODUCTION

Sorafenib is a small molecule inhibitor of multiple kinases that control tumor cell proliferation and angiogenesis. Sorafenib was originally developed to inhibit tumor cell growth and angiogenesis by specifically targeting Raf-1 (CRAF) serine/threonine kinase, a member of the RAF-MEK-ERK signaling pathway [1], and it is a potent *in vitro* inhibitor of this kinase [2]. Sorafenib has also been found to inhibit at low nanomolar concentrations vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor receptors (PDGFR), RET, FLT3, and KIT [3]. Preclinical studies of human melanoma, renal, colon, pancreatic, hepatocellular, thyroid, and ovarian and non-small cell lung carcinomas (NSCLCs) document the ability of sorafenib to inhibit tumor growth against a variety of cancers and in selected cases to induce tumor regression [4]. Furthermore, combination studies with other drugs (gefitinib, vinorelbine, gemcitabine, and irinotecan) indicate that sorafenib has a tolerability profile that is conducive to be combined with other agents [5].

Sorafenib was approved by FDA for the treatment of renal cell carcinoma (RCC) in 2005 and for hepatocellular cancer (HCC) in 2007. The approval for advanced RCC was based on an improvement in progression-free survival (PFS) from 2.8 months for patients assigned to placebo to 5.5 months for patients receiving sorafenib [6]. Partial responses were observed in 10% of patients, suggesting that the primary benefit for sorafenib resulted from tumor growth inhibition. For advanced HCC, sorafenib significantly increased median overall survival (10.7 months for sorafenib versus 7.9 months for placebo) and median time to radiologic progression (5.5 months for sorafenib versus 2.8 months for placebo). Tumor regression was uncommon, indicating that sorafenib is effective against HCC primarily by slowing the rate of disease progression [7]. Of direct relevance in the pediatric setting, sorafenib is also being evaluated for acute myeloid leukemia (AML) in adults in combination with standard anti-leukemia agents, given its potent activity against FLT3 and KIT [8]. On the strength of the clinical results for sorafenib and its interesting pattern of kinase inhibition, the PPTP evaluated this agent to gain insight into its utility against pediatric tumors.

MATERIALS AND METHODS

In vitro testing

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 [9]. Cells were incubated in the presence of sorafenib for 96 hours at concentrations from 1.0 nM to 10.0 μ M and analyzed as previously described [10].

In vivo tumor growth inhibition studies

CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [11]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-}

mice as described previously [12]. Female mice were used irrespective of the patient gender from which the original tumor was derived. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Ten mice (solid tumors) or 8 mice (leukemia models) were used in each control or treatment group. Tumor volumes (cm^3) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [13] and responses were determined using three activity measures as previously described [13]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies. The Mann-Whitney test was used to test the difference between VEGFA expression level between groups of xenografts with greater versus lesser tumor growth inhibition (EFS T/C ≥ 2 versus < 2).

Drugs and Formulation

Sorafenib was provided to the PPTP by Bayer HealthCare Pharmaceuticals Inc., Wayne, NJ, through the Cancer Therapy Evaluation Program (NCI). Sorafenib (as the tosylate salt) was formulated in CremophorEL/ethanol/water (12.5/12.5/75), warmed and sonicated (20-30 min), and the suspension was administered at a dose of 60 mg/kg by oral gavage daily for 5 days per week, repeated for 6 consecutive weeks.

RESULTS

Sorafenib in vitro testing

Sorafenib demonstrated an activity pattern consistent with a cytotoxic effect, with T/C% values approaching 0% for many cell lines at the highest concentration tested. Twenty of 23 cell lines of the PPTP showed IC_{50} values between 1.0 and 10.0 μM , Table I. The ratio of the median IC_{50} of the entire panel to that of each cell line is presented in Table I and Figure 1A. Higher ratios are indicative of greater sensitivity to sorafenib and are shown by bars to the right of the midpoint line. For most of the cell lines there was little variation in their *in vitro* response to sorafenib, with 20 of the 23 cell lines having IC_{50} values within 50% (above or below) the median for the entire panel (4.3 μM). The cell line with the lowest IC_{50} value, Kasumi-1, was derived from a patient with core binding factor AML with t(8;21) and is known to have a gain-of-function KIT mutation (Asn822Lys) [14]. The IC_{50} value for Kasumi-1 (0.02 μM , Figure 1B) was more than 200-fold lower than the median IC_{50} for the PPTP *in vitro* panel, and is contrasted with BT-12 a rhabdoid cell line with an IC_{50} of 3.5 μM (Figure 1C).

Sorafenib in vivo testing

Sorafenib was evaluated in 44 xenograft models. Nine of 840 mice died during the study (1.1%), with 4 of 419 in the control arm (1.0%) and 5 of 421 in the sorafenib treatment arm (1.2%). No tumor lines were excluded from analysis due to toxicity greater than 25 percent. A complete summary of results is provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

Sorafenib induced significant differences in event-free survival (EFS) distribution compared to control in 27 of 36 (75%) of the solid tumor xenografts and in 1 of 8 (12.5%) of the ALL xenografts. For those xenografts with a significant difference in EFS distribution between treated and control groups, the EFS T/C activity measure additionally requires an EFS T/C value of > 2.0 for intermediate activity and indicates a substantial agent effect in slowing tumor growth. High activity further requires a reduction in final tumor volume compared to the starting tumor volume. Sorafenib induced tumor growth inhibition meeting criteria for intermediate activity in 15 of 34 (44%) evaluable solid tumor xenografts, but no solid tumor xenografts met criteria for high EFS T/C activity. Intermediate activity for the EFS T/C metric occurred most frequently in the osteosarcoma (4 of 5), neuroblastoma (4 of 5), and glioblastoma (3 of 4) panels. Intermediate EFS T/C activity was not observed for any rhabdomyosarcoma xenografts (0 of 6) and was observed in only a single ALL xenograft (1 of 8). Examples of tumors demonstrating criteria for intermediate activity are shown in Figure 2.

No xenografts achieved an objective response, with the best response in the solid tumor panel being PD2 (progressive disease with growth delay), which was observed in 21 of 36 (56%) xenografts. A single ALL xenograft showed a PD2 response. The *in vivo* testing results for the objective response measure of activity are presented in Figure 3 in a 'heat-map' format as well as a 'COMPARE'-like format, based on the scoring criteria described the Supplemental Response Definitions section. The latter analysis demonstrates relative tumor sensitivities around the midpoint score of 5 (stable disease).

The heat map representation of VEGFA expression as measured by Affymetrix U133 Plus 2.0 arrays for the PPTP xenografts and cell lines shows that highest VEGFA expression occurs for selected xenografts in the osteosarcoma panel and for xenografts in the brain tumor panels (ependymoma and glioblastoma) (Figure 4). By comparison, the ALL xenografts and cell lines generally have very low VEGFA expression. There was a trend for xenografts with greater growth inhibition (EFS T/C ≥ 2 versus < 2) to have higher baseline VEGFA expression levels. This trend was noted when using combined VEGFA probe set expression data as well as when using the expression data for the most 3 probe set (201512_s_at) ($p=0.08$ and 0.06 , respectively).

DISCUSSION

The *in vitro* activity pattern observed for the PPTP cell lines is similar to that described for sorafenib in previous reports. Cell lines that have activating mutations or translocations involving selected receptor tyrosine kinases show IC_{50} values in the low nanomolar range, including cell lines with FLT3 [3,15-17], KIT [18], RET [19,20], and PDGFR [3,21] alterations. These genes are not known to show activating mutations at clinically relevant frequencies in common childhood cancers, with the exception of FLT3 and KIT for pediatric AML [22,23]. Sorafenib also inhibits VEGFR2 signaling, with IC_{50} values below 100 nM [24]. The PPTP *in vitro* panel contained a single cell line, Kasumi-1, with IC_{50} values in the low nM range, and this cell line has an activating KIT mutation [14].

At low micromolar concentrations, sorafenib has broad activity against the PPTP cell lines. Similar results have been described for a range of adult and pediatric cancer cell lines, including those for hepatocellular carcinoma [25], papillary thyroid cancer with BRAF mutations [19], breast cancer [26], lung carcinoma [26], medulloblastoma [27], and various types of leukemias [28]. This broader activity at micromolar concentrations has been associated with inhibition of a range of cellular processes, including reduced phosphorylation levels of eIF4E [28,29], reduced levels of Mcl-1 [26,28,30], STAT3 activation [27], and reduced levels of MAP kinase signaling as measured by reduced

phospho-ERK levels [24]. The effect of sorafenib at these higher concentrations is clearly cytotoxic for many of the PPTP cell lines, consistent with data from adult cancer cell lines, as the T/C% values approach 0% for many cell lines. There is no histotype specificity in the activity of sorafenib at micromolar concentrations, as almost all cell lines, regardless of diagnosis, are sensitive. This micromolar level activity is unlikely to have clinical relevance, as sorafenib shows high protein binding (99.5%), resulting in more than 100-fold higher concentrations being required to achieve IC₅₀ concentrations in plasma compared to the concentration required using standard low-serum *in vitro* testing conditions [31]. The steady state sorafenib blood levels observed in patients are only in the low micromolar range and are therefore unlikely to be sufficient to inhibit tumors with sensitivity to sorafenib that is comparable to those cell lines showing micromolar level IC₅₀ values under standard *in vitro* testing conditions. Furthermore, *in vitro* micromolar concentrations exceed by 100-fold or greater those at which sorafenib inhibits cell lines with activating alterations in RET, KIT, PDGFR, and FLT3, and this activity at high concentrations likely reflects non-specific effects on multiple other kinases [17].

The *in vivo* antitumor activity observed for sorafenib against the PPTP childhood cancer models consisted exclusively of tumor growth inhibition. The growth inhibitory effect was particularly notable for two of the four tested glioblastoma xenografts and for four of the five osteosarcoma xenografts. In selected adult cancer preclinical models, sorafenib induces complete growth inhibition and in some cases regressions. For example, in the renal cell carcinoma xenograft 788-O, near complete growth inhibition was observed at a sorafenib dose of 60 mg/kg administered daily [32]. Tumor growth inhibition in renal cancer models has been associated with a profound reduction in microvessel density at higher sorafenib doses. Sorafenib induced partial tumor regressions against the hepatocellular carcinoma xenograft PLC/PRF/5 at doses of 30 mg/kg and 100 mg/kg administered daily [25]. MAPK pathway activation is a common feature of hepatocellular carcinoma. Mutant FLT3 AML preclinical models are responsive to sorafenib *in vivo* at doses as low as 3 mg/kg, indicating that these doses achieve sufficient levels to have a strong anti-leukemia effect against cells that have low nanomolar IC₅₀ values [15]. Papillary thyroid carcinoma xenografts harboring a RET translocation are more responsive to sorafenib than are xenografts with BRAF mutations [19], and a dose of 60 mg/kg administered daily is able to induce regressions in xenografts with an activating RET mutation [20]. In a number of other adult cancer xenograft models, the *in vivo* activity is more similar to the activity pattern observed for the PPTP xenografts (i.e., tumor growth inhibition), and convincing evidence for sorafenib *in vivo* anti-angiogenic activity has been presented for these models [24,25,33]. The pattern of tumor growth inhibitory activity observed by the PPTP for sorafenib is consistent with an antiangiogenic mechanism of action for this agent against selected PPTP solid tumor xenografts, and it is similar to the pattern of activity previously described by the PPTP for sunitinib and for cediranib, both of which also inhibit VEGFR2 signaling [34,35]. The relevance of VEGFR2 inhibition to sorafenib's activity against PPTP xenografts is supported by the trend for an association between greater tumor growth inhibition and higher baseline VEGFA expression (Figure 4).

Activating mutations of RAS genes and BRAF are not commonly found in most pediatric cancers [27,28,30,36-38]. Pilocytic astrocytomas have MAPK pathway activation through BRAF activating point mutations and through a tandem duplication that results in an in-frame fusion between the 5' end of the KIAA1549 gene and the 3' end of the BRAF gene producing an oncogenic fusion protein [39-41]. However, none of the cell lines in the PPTP *in vitro* panel or the *in vivo* panel against which sorafenib was tested are known to have activating BRAF mutations. Sorafenib appears to show greater *in vitro* and *in vivo* activity against preclinical models with activated receptor tyrosine kinases (e.g., RET) compared to models with activating BRAF mutations [19], and it shows no preferential activity against

BRAF mutant cell lines [42]. Clinically, sorafenib has shown limited tumor regressing activity against melanoma (a tumor with a high proportion of BRAF mutations) [43]. Thus, it is not clear that sorafenib should be prioritized for evaluation against BRAF-mutated pediatric tumors.

Plans for the clinical development of sorafenib in the pediatric population are under discussion, and the pediatric phase 1 trial of sorafenib has been completed [44]. The results presented here suggest that sorafenib will primarily have tumor growth inhibitory activity rather than tumor regression inducing activity against tumors lacking those mutation-activated receptor tyrosine kinases against which sorafenib shows potent inhibitory activity. Studies of sorafenib in the pediatric AML setting for children with FLT3 internal tandem duplication (ITD) mutations are of particular interest given the potent activity of sorafenib against AML cell lines with FLT3-IT, the *in vivo* regression-inducing activity observed for sorafenib at low doses against FLT3-ITD xenografts, and the clinical activity of sorafenib in AML patients with FLT3-ITD [15-17,45,46]. Sorafenib, like several other VEGFR inhibitors may have relatively little single agent activity against most solid tumors, but may have value when combined with cytotoxic agents or other signaling inhibitors. Thus, this agent may have value in treatment of pediatric cancers, and future preclinical studies should include evaluation of sorafenib with standard cytotoxic agents and with other cell signaling inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 2. Sorafenib activity against individual solid tumor xenografts. Kaplan-Meier curves for EFS, median relative tumor volume graphs, and individual tumor volume graphs are shown for selected lines: (A) OS17, and (B) OS33, osteosarcomas, (C) BT28 medulloblastoma and (D) TC-71 Ewing sarcoma xenografts. Controls (gray lines); Treated (black lines).

Figure 3. Sorafenib *in vivo* objective response activity, left: The colored heat map depicts group response scores. A high level of activity is indicated by a score of 6 or more, intermediate activity by a score of 2 but < 6, and low activity by a score of < 2. Right: representation of tumor sensitivity based on the difference of individual tumor lines from the midpoint response (stable disease). Bars to the right of the median represent lines that are more sensitive, and to the left are tumor models that are less sensitive. Red bars indicate lines with a significant difference in EFS distribution between treatment and control groups, while blue bars indicate lines for which the EFS distributions were not significantly different.

Figure 4. VEGFA gene expression (Affymetrix U133 Plus 2.0) in PPTP cell lines and xenografts as visualized using GeneSifter software (VizX Labs, Seattle, WA). Gray indicates an absent call from Affymetrix quality control. Gene expression analysis methods are as previously described [47].

Table IActivity of sorafenib against the PPTP *in vitro* panel.

Cell Line	Histology	IC ₅₀ (μM)	Median IC ₅₀ Ratio	Ymin (T/C%)
RD	Rhabdomyosarcoma	5.4	0.80	21.0
Rh41	Rhabdomyosarcoma	4.5	0.96	7.5
Rh18	Rhabdomyosarcoma	>10.0	0.43	73.4
Rh30	Rhabdomyosarcoma	4.3	1.00	19.0
BT-12	Rhabdoid	3.5	1.24	3.8
CHLA-266	Rhabdoid	4.3	1.00	2.5
TC-71	Ewing sarcoma	5.9	0.73	16.7
CHLA-9	Ewing sarcoma	4.8	0.91	14.3
CHLA-10	Ewing sarcoma	3.2	1.36	4.5
CHLA-258	Ewing sarcoma	7.6	0.56	29.7
GBM2	Glioblastoma	4.7	0.91	2.5
NB-1643	Neuroblastoma	5.7	0.75	26.0
NB-EBc1	Neuroblastoma	3.7	1.17	0.3
CHLA-90	Neuroblastoma	10.0	0.43	53.5
CHLA-136	Neuroblastoma	3.7	1.16	0.3
NALM-6	ALL	4.1	1.06	9.7
COG-LL-317	ALL	3.6	1.19	0.2
RS4;11	ALL	6.8	0.64	32.9
MOLT-4	ALL	3.8	1.12	3.4
CCRF-CEM	ALL	4.5	0.97	22.9
Kasumi-1	AML	0.02	219.91	2.2
Karpas-299	ALCL	3.9	1.12	1.4
Ramos-RA1	NHL	3.7	1.16	0.0
Median		4.3	1.00	7.5
Minimum		0.02	0.43	0.0
Maximum		>10.0	219.91	73.4

Table II

Activity of sorafenib against the PPTP *in vivo* panel.

Xenograft Line	Histology	Time to Event ¹	P-value	EFS T/C ²	Median Final RTV ³	Tumor Volume T/C ⁴	T/C Activity ⁵	EFS Activity ⁵	Response Activity ⁵
BT-29	Rhabdoid	> EP	<0.001	> 2.0	2.6	0.67	Low	NE	Int
KT-14	Rhabdoid	23.6	0.066	1.2	>	0.81	Low	Low	Low
KT-12	Rhabdoid	27	<0.001	3	>	0.62	Low	Int	Int
KT-10	Wilms	10.7	<0.001	1.7	>	0.56	Low	Low	Low
KT-11	Wilms	13.4	0.01	1.2	>	0.73	Low	Low	Low
KT-13	Wilms	8.4	0.013	1.4	>	0.72	Low	Low	Low
SK-NEP-1	Ewing	19.8	<0.001	2.6	>	0.56	Low	Int	Int
EW5	Ewing	5.9	0.02	1.2	>	0.69	Low	Low	Low
EW8	Ewing	19.8	0.002	1.6	>	0.65	Low	Low	Int
TC-71	Ewing	22.1	<0.001	2.9	>	0.41	Int	Int	Int
CHLA258	Ewing	18.8	<0.001	1.5	>	0.46	Low	Low	Low
Rh10	ALV RMS	13.7	0.255	1.1	>	0.73	Low	Low	Low
Rh28	ALV RMS	24	0.043	1.5	>	0.76	Low	Low	Low
Rh30	ALV RMS	13.4	0.983	1	>	0.97	Low	Low	Low
Rh30R	ALV RMS	25.2	0.4	2.1	>	0.8	Low	Low	Int
Rh41	ALV RMS	20.5	0.24	1.5	>	0.7	Low	Low	Low
Rh18	EMB RMS	22.4	<0.001	1.8	>	0.64	Low	Low	Int
BT-28	Medulloblastoma	17.3	<0.001	3.5	>	0.1	High	Int	Int
BT-45	Medulloblastoma	18.4	0.005	1.9	>	0.34	Int	Low	Int
BT-50	Medulloblastoma	21	0.17	0.7	>	1.42	Low	Low	Low
BT-41	Ependymoma	> EP	0.424	.	2.8	0.6	Low	NE	Int
BT-44	Ependymoma	19.3	0.4	1	>	0.98	Low	Low	Low
GBM2	Glioblastoma	34.1	0.001	3.6	>	0.65	Low	Int	Int
BT-39	Glioblastoma	19.8	0.028	1.3	>	0.74	Low	Low	Low
D645	Glioblastoma	27.5	<0.001	5.8	>	0.28	Int	Int	Int

Xenograft Line	Histology	Time to Event ¹	P-value	EFS T/C ²	Median Final RTV ³	Tumor Volume T/C ⁴	T/C Activity ⁵	EFS Activity ⁵	Response Activity ⁵
D456	Glioblastoma	13.7	<0.001	2.7	>4	0.4	Int	Int	Int
NB-SD	Neuroblastoma	31.2	<0.001	2.8	>4	0.16	Int	Int	Int
NB-1771	Neuroblastoma	8.9	<0.001	2	>4	0.39	Int	Int	Int
NB-1691	Neuroblastoma	17.9	0.066	1.4	>4	1	Low	Low	Low
NB-EBc1	Neuroblastoma	14	<0.001	2.4	>4	0.38	Int	Int	Int
NB-1643	Neuroblastoma	20	<0.001	3.3	>4	0.53	Low	Int	Int
OS-1	Osteosarcoma	> EP	<0.001	> 2.3	3.5	0.42	Int	Int	Int
OS-2	Osteosarcoma	20.9	0.011	1.1	>4	0.8	Low	Low	Low
OS-17	Osteosarcoma	> EP	<0.001	> 2.8	2	0.27	Int	Int	Int
OS-9	Osteosarcoma	> EP	<0.001	> 2.4	2.8	0.43	Int	Int	Int
OS-33	Osteosarcoma	> EP	<0.001	> 2.5	1.7	0.36	Int	Int	Int
ALL-2	ALL B-precursor	25.7	<0.001	2.3	>25	.		Int	Int
ALL-3	ALL B-precursor	5.6	0.679	0.7	>25	.		Low	Low
ALL-4	ALL B-precursor	2	1	1	>25	.		Low	Low
ALL-7	ALL B-precursor	2	0.437	1	>25	.		Low	Low
ALL-8	ALL T-cell	4.4	0.216	1.3	>25	.		Low	Low
ALL-16	ALL T-cell	13.2	0.918	1.1	>25	.		Low	Low
ALL-17	ALL B-precursor	13.5	0.497	1.1	>25	.		Low	Low
ALL-19	ALL B-precursor	7.3	0.531	0.7	>25	.		Low	Low

¹ Kaplan-Meier estimate of the median time to event for the treatment group

² EFS T/C value = ratio of the median time to event of the treatment group and of the respective control group

³ RTV (Relative tumor volume) = ratio of tumor volume at specified time post-treatment to pretreatment tumor volume

⁴ Tumor Volume T/C = ratio of RTV for treatment and control groups calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days).

⁵ See Supplemental Response Definitions.