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Testing of the Topoisomerase 1 Inhibitor Genz644282 by the Pediatric Preclinical Testing Program

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Abstract

Background—Genz644282 is a novel non-camptothecin topoisomerase I poison that is in clinical development.

Procedures—Genz644282 was tested against the PPTP *in vitro* panel (0.1 nM–1 μM), and *in vivo* using three times per week × 2 schedule repeated at day 21 at its maximum tolerated dose (MTD) of 4 mg/kg. Subsequently Genz644282 was tested at 4, 3, 2 and 1 mg/kg in 3 models to assess the dose response relationship. mRNA gene signatures predictive for Genz644282 response *in vitro* were applied to select 15 tumor models that were evaluated prospectively.

Results—*In vitro*, Genz644282 demonstrated potent cytotoxic activity with a median IC_{50} of 1.2 nM (range 0.2–21.9 nM). *In vivo*, Genz644282 at its MTD (4 mg/kg) induced maintained complete responses (MCR) in 6/6 evaluable solid tumor models. At 2 mg/kg Genz644282 induced CR or MCR in 3/3 tumor models relatively insensitive to topotecan, but there were no objective responses at 1 mg/kg. Further testing at 2 mg/kg showed that Genz644282 induced objective

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CONFLICT OF INTEREST STATEMENT: Dr. Madden and Ms. Zhang is an employee of Genzyme Corporation, and Dr. Teicher a former employee. Dr. Teicher's current address is Developmental Therapeutics Program, National Cancer Institute, 6130 Executive Boulevard, Rockville, MD 20852. The other authors consider that there are no actual or perceived conflicts of interest.

regressions in 7 of 17 (41%) models. There was a significant correlation between predictive response scores based on Affymetrix U133Plus2 baseline tumor expression profiles and the observed *in vivo* responses to Genz644282.

Conclusions—Genz644282 was highly active within a narrow dose range (2–4 mg/kg), typical of other topoisomerase I poisons.. As with other topoisomerase I poisons, how accurately these data will translate to clinical activity will depend upon the drug exposures that can be achieved in children treated with this agent.

Keywords

Preclinical Testing; Developmental Therapeutics; Genz644282

INTRODUCTION

The camptothecin derivatives topotecan (Hycamtin) and irinotecan (Camptosar) have demonstrated significant antitumor activity in preclinical models of childhood cancer [1–5], showed activity in both phase I and –II clinical trials [6–8], and are now established agents in the treatment of childhood solid tumors. Camptothecins target the enzyme DNA topoisomerase I, acting as poisons that convert the enzyme into a cellular toxin. Camptothecins form a ternary complex with DNA, and topoisomerase I after the enzyme has made a single strand cut, stabilizing the intermediate cleavable complex. As a consequence of drug binding, the rate of religation of DNA ends is retarded increasing the probability for a collision between an advancing replication fork and the complex that leads to a double strand DNA break [9]. More recent single molecule data suggests that formation of the ternary complex leads to progressive overwinding of DNA during replication fork progression, leading to replication fork stalling, DNA damage and induction of apoptosis [10,11].

Despite the similarity in mechanism of action, topotecan and irinotecan exhibit different toxicities, and have a distinct spectra of tumors that are responsive to treatment. In children toptecan is primarily myelosuppressive [12]. In contrast, the primary toxicity of irinotecan is schedule-dependent with diarrhea as the limiting toxicity using protracted schedules of administration [8], whereas neutropenia was limiting when irinotecan was administered at high doses every three weeks [6]. This difference probably relates to the route of drug clearance which for topotecan is mainly renal whereas irinotecan and its active metabolite SN-38, or SN-38-glucuronide, are eliminated in the bile. Deconjugation of SN-38-glucuronide to the active SN-38 appears to be through intestinal bacterial β-glucuronidase [13] leading to direct mucosal damage. These agents also show differences in the spectrum of malignancies that are responsive, for example the activity of irinotecan is established in treatment of colorectal cancer [14] whereas topotecan has limited activity [15,16]. In part this may reflect the distribution of ABC transporters that act as drug efflux pumps in different malignancies, and that subtle chemical differences in camptothecin analogs alter their ability to be transported by these pumps. From a pharmaceutical perspective, camptothecins are intrinsically unstable at physiologic pH, where they exist in equilibrium with the inactive open form of the E-ring lactone. Further, some camptothecin analogs are highly protein bound. Thus, although there is ample data to support topoisomerase I as a valid target for drug development, current drugs are not optimal.

Genz644282, a non-camptothecin topoisomerase I poison, was developed from a lead compound 8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxy-5-*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (ARC-111, topovale) [17]. In non-clinical testing ARC-111 had equivalent or superior activity to irinotecan in pediatric and adult xenografts and induced regression in a topotecan insensitive tumor xenograft [17,18]. ARC-111 also

differentiated itself from other camptothecin derivatives in that it was not a substrate for the ABCG2 drug transporter. Because topotecan had been evaluated against the PPTP models, a step-wise approach to testing Genz644282 was taken; initially it was evaluated against *in vivo* models that demonstrated only intermediate sensitivity to topotecan, followed by defining the dose-response relationship. In the third *in vivo* component we tested prospectively a gene expression signature considered to predict sensitivity or resistance to Genz644282 against 15 solid tumor models.

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 [19]. Cells were incubated in the presence of Genz644282 for 96 hours under aerobic conditions at concentrations from 0.1 nM to 1 μM and analyzed as previously described [20]. Absolute IC_{50} values represent the concentration of Genz644282 that reduces cell survival to 50% of the control value, while relative IC_{50} values represent the Genz644282 concentration that reduces cell survival by 50% of the maximum effect [21]. Relative In/Out (I/O)% values represent the percentage difference between the Y_{min} value and the estimated starting cell number and either the control cell number (for agents with Y_{min} > starting cell number) or 0 (for agents with Y_{min} < estimated starting cell number). Relative I/O% values range between 100% (no treatment effect) to −100% (complete cytotoxic effect), with a Relative I/O% value of 0 being observed for a completely effective cytostatic agent.

In Vivo Tumor Growth Inhibition Studies

CB17SC *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 [20,22–24]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*$^{-/-}$ mice as described previously [25]. 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 eight mice (leukemias) 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 [25] and responses were determined using three activity measures as previously described [26].

Determination of response

Responses were determined using three activity measures as previously described [26]. For individual mice, progressive disease (PD) was defined as < 50% regression from initial volume during the study period and > 25% increase in initial volume at the end of study period. Stable disease (SD) was defined as < 50% regression from initial volume during the study period and ≤ 25% increase in initial volume at the end of the study. Partial response (PR) was defined as a tumor volume regression ≥50% for at least one time point but with measurable tumor (≥ 0.10 cm^3). Complete response (CR) was defined as a disappearance of measurable tumor mass (< 0.10 cm^3) for at least one time point. A complete response was considered maintained (MCR) if the tumor volume was <0.10 cm^3 at the end of the study period. For treatment groups only, if the tumor response was PD, then PD was further classified into PD1 or PD2 based on the tumor growth delay (TGD) value. TGD values were

calculated based on the numbers of days to event. For each individual mouse that had PD and had an event in the treatment groups, a TGD value was calculated by dividing the time to event for that mouse by the median time to event in the respective control group. Median times to event were estimated based on the Kaplan-Meier event-free survival distribution. If a mouse had a TGD value ≤ 1.5, that mouse was considered PD1. If the TGD value was > 1.5, the mouse was considered PD2. Mice that had PD but did not have an event at the end of the study were coded as PD2.

Event-free survival

An event in the solid tumor xenograft models was defined as a quadrupling of tumor volume from the initial tumor volume. Event-free survival was defined as the time interval from initiation of study to the first event or to the end of the study period for tumors that did not quadruple in volume. The time to event was determined using interpolation based on the formula:

$$t_x = t_1 + (t_2 - t_1)\ln(V_e/V_1)/\ln(V_2/V_1),$$

where t_x is the lower observation day bracketing the event, t_1 is the interpolated day to event, t_2 is the upper observation day bracketing the event, V_1 is the tumor volume on day t_1, V_2 is the tumor volume on day t_2 and V_e is the event threshold (4 times initial tumor volume for solid tumor xenografts).

Response and Event Definitions for Acute Lymphoblastic Leukemia (ALL) Xenograft Models—Individual mice were categorized as PD if their percentage of hCD45 cells never dropped below 1% and they had an event before the end of the study period. An event is defined as hCD45 cells above 25% in the peripheral blood with times to event calculated as above. Individual mice were classified as SD if their percentage of hCD45 cells never dropped below 1% and no event occurred before the end of the study. PR was assigned if the percentage of cells dropped below 1% for any one time point regardless of whether the percentage eventually reached 25%. A CR was assigned if the percentage of hCD45 cells dropped below 1% for 2 consecutive weeks of the study and regardless of whether the percentage reached 25% or not. A CR was considered maintained if the percentage of hCD45 was less than 1% for the last three measurements of the study. For treatment groups, PD was further classified into PD1 and PD2 according to the TGD value.

The time to event was determined using interpolation based on the formula:

$$t_x = t_1 + (t_2 - t_1)\ln(V_e/V_1)/\ln(V_2/V_1),$$

where t_x is the interpolated day to event, t_1 is the lower observation day bracketing the event, t_2 is the upper observation day bracketing the event, V_1 is the hCD45 percentage on day t_1, V_2 is the tumor volume (or hCD45 percentage) on day t_2 and V_e is the event threshold (25% for ALL xenografts).

Summary statistics and analysis methods

Overall Group Response: Each individual mouse was assigned a score from 0 to 10 based on their response: PD1=0, PD2=2, SD=4, PR=6, CR=8, and MCR=10, and the median for the group determined the overall response. Studies in which toxicity was greater than 25% or in which the control group was not at least SD, were considered inevaluable and were excluded from analysis. Treatment groups with PR, CR, or MCR are considered to have had

an objective response. Agents inducing objective responses are considered highly active against the tested line, while agents inducing stable disease or PD2 are considered to have intermediate activity, and agents producing PD1 are considered to have a low level of activity against the tested line.

Tumor Volume T/C value: 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. For the tumor volume T/C response measure, agents producing a T/C of ≤ 15% are considered highly active, those with a mean tumor volume T/C of ≤ 45% but > 15% are considered to have intermediate activity, and those with mean T/C values > 45% are considered to have low levels of activity [27]

EFS T/C value: An EFS T/C value was 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. If the treatment group did not have a median time to event, then EFS T/C was defined as greater than the ratio of the last day of the study for the treatment group divided by the median time to event for the control group. For the EFS T/C measure, agents are considered highly active if they meet three criteria: a) an EFS T/C > 2; b) a significant difference in EFS distributions ($p \leq 0.050$), and c) a net reduction in median tumor volume for animals in the treated group at the end of treatment as compared to at treatment initiation. Agents meeting the first two criteria, but not having a net reduction in median tumor volume for treated animals at the end of the study are considered to have intermediate activity. Agents with an EFS T/C < 2 are considered to have low levels of activity. Xenografts in which the median EFS for the control line was greater than one-half of the study period or in which the median EFS for the control line did not exist are considered not evaluable for the EFS T/C measure of activity.

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 of medians of IC_{50} values between cell lines of a given histotype compared to the remaining cell lines of the panel.

Developing and Applying a Predictive Gene Expression Signature: The sensitivity of 79 cancer cell lines to Genz644283 was measured with a cell proliferation assay. Cells were plated in a 96-well plate. After incubation overnight, Genz644283 was added in the dose range of 0.3nM to 3uM, and cell viability was measured using CellTiter-Glo Luminescent Cell Viability Assay (Promega). GI50, TGI and LC50 were calculated (GraphPad Prism 5.0, GraphPad Software) as described [28]. Twenty of the most resistant and 16 of the most sensitive cell lines were identified and used as a training set in the predictive models. Total RNA was extracted using AllPrep DNA/RNA Mini Kit (Qiagen). Whole genome expression profiling was performed on Affymetrix Human Genome U133 Plus 2.0 Arrays (Affymetrix).

Raw intensity was normalized using the robust multiarray average (RMA) [29]. Probe sets with low signal intensity were excluded in the analysis. Genes that are most discriminative for resistant and sensitive cell lines were first identified by t-test (JMP Genomics 4, SAS). Baysian fitting of binary probit regression models was applied to identify predictive signatures as described [30,31]. Leave-one-out cross-validation was performed to assess the predictive accuracy. The final predictive signature was comprised of 250 probe sets.

Whole genome expression profiles of PPTP xenograft models were collected on Affymetrix U133A gene chips [29,32]. Raw intensity files were pre-processed by RMA. Common probes between the two array platforms were identified and used to generate a probability score of response of PPTP xenograft models to Genz644282. Prior to predictive modeling, quantile normalization was performed to minimize batch effect between the training and test sets. Mann-Whitney U test was performed to examine the correlation between the predicted probability and experimental observed response (GraphPad Prism 5.0, GraphPad Software).

Compound and Formulation: Genz644282 was provided to the Pediatric Preclinical Testing Program by Genzyme Corporation, through the Cancer Therapy Evaluation Program (NCI). For *in vitro* testing, stock solutions of Genz644282 was prepared in DMSO, with dilutions in culture media. For *in vivo* testing, Genz644282 was dissolved in sterile water, diluted in sterile saline, and administered i.p., three times weekly for 2 weeks at a dose of 4 mg/kg. For dose response determination Genz644282 was administered at 4, 3, 2, or 1 mg/kg using the same schedule but for only one cycle. For prospective testing, Genz644282 was administered at 2 mg/kg using the same schedule as for the dose response study. Genz644282 was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In vitro testing

Genz644282 was tested against the PPTP *in vitro* panel at concentrations ranging from 0.1 nM to 1 μM. The median relative IC_{50} was 1.2 nM (range 0.2–21.9 nM) (Table I). The Y_{min} T/C% values approached zero (median = 0.1%) and the Relative I/O% values approached −100%, indicative of potent cytotoxic activity. The ALL cell lines had lower relative IC_{50} values than the remaining cell lines (median of 0.4 nM versus 1.3 nM, p=0.034), while the rhabdomyosarcoma cell lines had higher relative IC_{50} values than the other PPTP cell lines (median of 2.5 nM versus 1.0 nM, p=0.047). The relative sensitivity of the PPTP cell lines is illustrated in the median IC_{50} ratio graph in which bars to the right represent lines with higher sensitivity (Figure 1).

In vivo testing

The PPTP previously tested another topoisomerase I poison, topotecan [1], and hence initially limited testing of Genz644282 was performed using 8 tumor models both sensitive and resistant to topotecan. There were 11 of 160 deaths (1/80 (1%) controls and 10/80 (12.5%) treated), two tumor lines were excluded because of excessive toxicity (Supplemental Table I). Genz6244 caused maintained complete responses (MCR) in the remaining 6 tumor lines (Table II). MCR was achieved during cycle 1 in all tumors including those excluded for toxicity, which occurred during therapy cycle two. Results for tumor models with only moderate sensitivity to topotecan (KT-11, SK-NEP1) as well as topotecan-sensitive models (KT-13, Rh28) treated with Genz644282 at the MTD are shown in Figure 2.

The dose response relationship was determined against 3 tumor models that have the least sensitivity to topotecan (SK-NEP1, Rh18, Rh30) at dose levels of 4, 3, 2, and 1 mg/kg administered 3 times per week for 2 weeks only. Genz644282 induced CR or MCR in each model at 2 mg/kg and above. However, at 1 mg/kg there was progressive growth for each model (Figure 3, Supplemental Table II), suggesting a narrow dose response range, typical of cytotoxic agents that target topoisomerase I.

A gene expression signature for predicting response to Genz644282 based on an independent dataset using gene expression and *in vitro* response data from 79 cancer cell

lines was then applied to the PPTP *in vivo* panel using the PPTP Affymetrix expression data set (http://pptp.nchresearch.org/comparison.html), and predictive scores were developed for each PPTP model. Fifteen tumor models were identified that exhibited gene signatures producing a range of predictive scores and hence a range of predicted sensitivity to Genz644282 (Supplemental Table 3). These models (along with 2 additional models, D645 and BT-45) were tested using the 2 mg/kg dose administered thrice weekly for two weeks to determine whether the gene expression derived predictive scores correlated with actual response to Genz644282. Genz644282 was very well tolerated at this dose and schedule, with only 1.2% toxicity (see Supplemental Table 4 for complete testing results at 2 mg/kg). Eleven of 17 (65%) xenografts showed significant differences in EFS distribution between treated and control animals, while 8 of 15 (53%) evaluable models showed EFS T/C values > 2 (Table II). Seven of the 17 xenografts (41%) evaluated showed objective responses, with 4 xenografts showing MCRs. Four of 5 osteosarcoma xenografts compared to 0 of 4 neuroblastoma xenografts showed objective responses. Results for four of the osteosarcoma xenografts are shown in Figure 4.

For testing the predictive signature, three responsive classes were categorized. The sensitive class responded effectively to the treatment, and the tumor regression was maintained at the end of study (i.e., MCR). The resistant class was characterized by continuing tumor growth during the study (i.e., PD1 or PD2). The intermediate class all presented tumor regression at one point, however, the tumor growth resumed later in the course of the study (Supplemental Table III). The relationship between sensitivity (i.e., sensitive versus resistant) and the predictive score is shown in Figure 5 and Supplemental Table III. Significant correlation was observed between the predictive score and the actual response of these tumor models ($p<0.02$).

DISCUSSION

Genz644282 potently inhibited proliferation and induced cytotoxicity *in vitro*. The relative IC_{50} value for Genz644282 against the 23 cell lines was highly correlated with that of another topoisomerase poison topotecan ($R^2=0.950$), tested previously against the same cell line panel [1]. The rhabdomyosarcoma cell line Rh18 was most resistant to both agents, and after excluding this line, the range for IC_{50} concentrations was relatively small for Genz644282 (~ 25-fold; 0.19–4.81 nM). For both topotecan and for Genz644282, the ALL cell lines had the lowest IC_{50} values. The median relative IC_{50} concentration for Genz644282 was approximately 6.8-fold lower than that for topotecan.

Dose scheduling studies conducted at Genzyme showed MWF × 2 weeks provided the optimal efficacy with the least toxicity. Weekly dosing was not as effective and daily was more toxic than efficacious. *In vivo*, Genz644282 was initially evaluated against a small panel of xenograft models that had shown a spectrum of sensitivity to topotecan such that any differential activity of this non-camptothecin topoisomerase I poison would be revealed. In agreement with previous data showing that ARC-111, a precursor molecule to Genz644282, had activity against topotecan-insensitive SK-NEP-1 [17], Genz644282 was highly active. Indeed, at the MTD (4 mg/kg), all 6 evaluable tumor models were classified as MCR. However, relative to humans, mice are highly tolerant of topoisomerase I poisons [33]. For topotecan a dose of 0.6 mg/kg (approximately 0.3MTD) gives an equivalent plasma exposure to that in children receiving ~2.7 mg/m^2 [7]. This differential is even greater for irinotecan as mice activate this agent far more efficiently than humans [8]. The large differential sensitivity of 10- to 100-fold between mouse and human bone marrow to camptothecin topoisomerase I poisons may explain, in part, why curative doses/blood levels of topotecan and irinotecan/SN-38 in mice with human tumor xenografts are not achievable in patients [34,35]. Compounds with little differential in bone marrow sensitivity across

species may have greater potential for reaching similar blood levels in patients as in mice. The differential between mouse and human bone marrow to Genz-644282 are found to be 4- to 6-fold, providing support for Genz-644282 as a development candidate [36,37]. Although the detailed organ toxicity was not conducted in our study, rodent toxicity studies conducted at Genzyme show that target organs are bone marrow and gastrointestinal tract, with the latter toxicity occurring at doses greater than the MTD. However, it is important to place the very high level of activity observed at the MTD for Genz644282 into perspective by defining the effective dose range for this agent. As shown in Figure 3, Genz644282 induced tumor regressions at 0.5MTD (2 mg/kg) after one cycle of treatment, but lost activity at 1 mg/kg. These data are reminiscent of topotecan which also shows an acute drop-off in activity (i.e. regression to progressive disease) over a relatively narrow dose range [3].

Pharmacokinetic studies in mice, conducted by Genzyme, indicated a half-life approaching 18 hours and drug associated with the tumor was greater than seen in plasma. However, in the absence of human pharmacokinetic data for Genz644282, we chose 2 mg/kg as a representative dose to test whether a predictive gene signature developed at Genzyme Corporation could prospectively identify tumor models that were sensitive or intrinsically resistant to treatment with Genz644282. Significant correlation between the observed response to Genz644282 (MCR versus PD1/PD2) and the predictive score was observed (P=0.0359). While this level of prediction is not adequate for clinical application, the significant correlation suggests that with refinements a clinically useful signature might be developed.

At the 2 mg/kg dose level administered for one cycle of treatment, Genz644282 demonstrated a high level of activity inducing regressions in 7 of 17 models evaluated (41%), including models that are intrinsically insensitive to topotecan. As with other topoisomerase I poisons, how accurately these data will translate to clinical activity will depend upon the drug exposures that can be achieved in children treated with this agent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Reference List

1. Carol H, Houghton PJ, Morton CL, et al. Initial testing of topotecan by the pediatric preclinical testing program. Pediatr Blood Cancer. 2010; 54(5):707–715. [PubMed: 20017204]
2. Houghton PJ, Cheshire PJ, Hallman JD 2nd, et al. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol. 1995; 36(5):393–403. [PubMed: 7634381]
3. Houghton PJ, Cheshire PJ, Myers L, et al. Evaluation of 9-dimethylaminomethyl-10-hydroxycamptothecin against xenografts derived from adult and childhood solid tumors. Cancer Chemother Pharmacol. 1992; 31(3):229–239. [PubMed: 1464161]

4. Thompson J, Zamboni WC, Cheshire PJ, et al. Efficacy of systemic administration of irinotecan against neuroblastoma xenografts. Clin Cancer Res. 1997; 3(3):423–431. [PubMed: 9815701]
5. Vassal G, Boland I, Santos A, et al. Potent therapeutic activity of irinotecan (CPT-11) and its schedule dependency in medulloblastoma xenografts in nude mice. Int J Cancer. 1997; 73(1):156–163. [PubMed: 9334824]
6. Vassal G, Couanet D, Stockdale E, et al. Phase II trial of irinotecan in children with relapsed or refractory rhabdomyosarcoma: a joint study of the French Society of Pediatric Oncology and the United Kingdom Children's Cancer Study Group. J Clin Oncol. 2007; 25(4):356–361. [PubMed: 17264330]
7. Santana VM, Furman WL, Billups CA, et al. Improved response in high-risk neuroblastoma with protracted topotecan administration using a pharmacokinetically guided dosing approach. J Clin Oncol. 2005; 23(18):4039–4047. [PubMed: 15961757]
8. Furman WL, Stewart CF, Poquette CA, et al. Direct translation of a protracted irinotecan schedule from a xenograft model to a phase I trial in children. J Clin Oncol. 1999; 17(6):1815–1824. [PubMed: 10561220]
9. Hsiang YH, Lihou MG, Liu LF. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. Cancer Res. 1989; 49(18): 5077–5082. [PubMed: 2548710]
10. Koster DA, Crut A, Shuman S, et al. Cellular strategies for regulating DNA supercoiling: a single-molecule perspective. Cell. 2010; 142(4):519–530. [PubMed: 20723754]
11. Koster DA, Palle K, Bot ES, et al. Antitumour drugs impede DNA uncoiling by topoisomerase I. Nature. 2007; 448(7150):213–217. [PubMed: 17589503]
12. Tubergen DG, Stewart CF, Pratt CB, et al. Phase I trial and pharmacokinetic (PK) and pharmacodynamics (PD) study of topotecan using a five-day course in children with refractory solid tumors: a pediatric oncology group study. J Pediatr Hematol Oncol. 1996; 18(4):352–361. [PubMed: 8888741]
13. Furman WL, Crews KR, Billups C, et al. Cefixime allows greater dose escalation of oral irinotecan: a phase I study in pediatric patients with refractory solid tumors. J Clin Oncol. 2006; 24(4):563–570. [PubMed: 16446328]
14. Takimoto CH, Kieffer LV, Arbuck SG. DNA topoisomerase I inhibitors. Cancer Chemother Biol Response Modif. 1997; 17:80–113. [PubMed: 9551210]
15. Takimoto CH, Arbuck SG. Clinical status and optimal use of topotecan. Oncology (Williston Park). 1997; 11(11):1635–1646. discussion 1649–1651, 1655–1637. [PubMed: 9394364]
16. Creemers GJ, Wanders J, Gamucci T, et al. Topotecan in colorectal cancer: a phase II study of the EORTC early clinical trials group. Ann Oncol. 1995; 6(8):844–846. [PubMed: 8589027]
17. Li TK, Houghton PJ, Desai SD, et al. Characterization of ARC-111 as a novel topoisomerase I-targeting anticancer drug. Cancer Res. 2003; 63(23):8400–8407. [PubMed: 14679002]
18. Kurtzberg LS, Battle T, Rouleau C, et al. Bone marrow and tumor cell colony-forming units and human tumor xenograft efficacy of noncamptothecin and camptothecin topoisomerase I inhibitors. Mol Cancer Ther. 2008; 7(10):3212–3222. [PubMed: 18852125]
19. Frgala T, Kalous O, Proffitt RT, et al. A fluorescence microplate cytotoxicity assay with a 4-log dynamic range that identifies synergistic drug combinations. Mol Cancer Ther. 2007; 6(3):886–897. [PubMed: 17363483]
20. Houghton PJ, Morton CL, Kolb EA, et al. Initial testing (stage 1) of the proteasome inhibitor bortezomib by the pediatric preclinical testing program. Pediatr Blood Cancer. 2008; 50(1):37–45. [PubMed: 17420992]
21. Sebaugh JL. Guidelines for accurate EC50/IC50 estimation. Pharmaceut Statist. 201010.1002/pst. 426
22. Friedman HS, Colvin OM, Skapek SX, et al. Experimental chemotherapy of human medulloblastoma cell lines and transplantable xenografts with bifunctional alkylating agents. Cancer Res. 1988; 48(15):4189–4195. [PubMed: 3390813]
23. Graham C, Tucker C, Creech J, et al. Evaluation of the antitumor efficacy, pharmacokinetics, and pharmacodynamics of the histone deacetylase inhibitor depsipeptide in childhood cancer models in vivo. Clin Cancer Res. 2006; 12(1):223–234. [PubMed: 16397046]

24. Peterson JK, Tucker C, Favours E, et al. In vivo evaluation of ixabepilone (BMS247550), a novel epothilone B derivative, against pediatric cancer models. Clin Cancer Res. 2005; 11(19 Pt 1): 6950–6958. [PubMed: 16203787]
25. Liem NL, Papa RA, Milross CG, et al. Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. Blood. 2004; 103(10):3905–3914. [PubMed: 14764536]
26. Houghton PJ, Morton CL, Tucker C, et al. The Pediatric Preclinical Testing Program: description of models and early testing results. Pediatr Blood Cancer. 2007; 49(7):928–940. [PubMed: 17066459]
27. Plowman, JCR.; Alley, M.; Sausville, E.; Schepartz, S. US-NCI testing procedures. In: Feibig, HHBA., editor. Relevance of tumor models for anticancer drug development. Basel, Karger: 1999. p. 121-135.
28. Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics. 2003; 4(2):249–264. [PubMed: 12925520]
29. Bolstad BM, Irizarry RA, Astrand M, et al. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics. 2003; 19(2):185–193. [PubMed: 12538238]
30. West M, Blanchette C, Dressman H, et al. Predicting the clinical status of human breast cancer by using gene expression profiles. Proc Natl Acad Sci U S A. 2001; 98(20):11462–11467. [PubMed: 11562467]
31. Bild AH, Yao G, Chang JT, et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature. 2006; 439(7074):353–357. [PubMed: 16273092]
32. Neale G, Su X, Morton CL, et al. Molecular characterization of the pediatric preclinical testing panel. Clin Cancer Res. 2008; 14(14):4572–4583. [PubMed: 18628472]
33. Peterson JK, Houghton PJ. Integrating pharmacology and in vivo cancer models in preclinical and clinical drug development. Eur J Cancer. 2004; 40(6):837–844. [PubMed: 15120039]
34. Masubuchi N, May RD, Atsumi R. A predictive model of human myelotoxicity using five camptothecin derivatives and the in vitro colony-forming unit granulocyte/macrophage assay. Clin Cancer Res. 2004; 10(19):6722–6731. [PubMed: 15475463]
35. Erickson-Miller CL, May RD, Tomaszewski J, et al. Differential toxicity of camptothecin, topotecan and 9-aminocamptothecin to human, canine, and murine myeloid progenitors (CFU-GM) in vitro. Cancer Chemother Pharmacol. 1997; 39(5):467–472. [PubMed: 9054963]
36. Kurtzberg L, Yao M, Rouleau C, et al. Genz-644282 a novel non-camptothecin topoisomerase I inhibitor for cancer treatment. Mol Cancer Ther. 2009; (Suppl) (AACR-NCI-EORTC Molecular Targets Conference):Abstr #C217.
37. Kurtzberg LS, Roth S, Krumbholz R, et al. Genz-644282, a novel non-camptothecin topoisomerase I inhibitor for cancer treatment. Clin Cancer Res. 2010 In press.

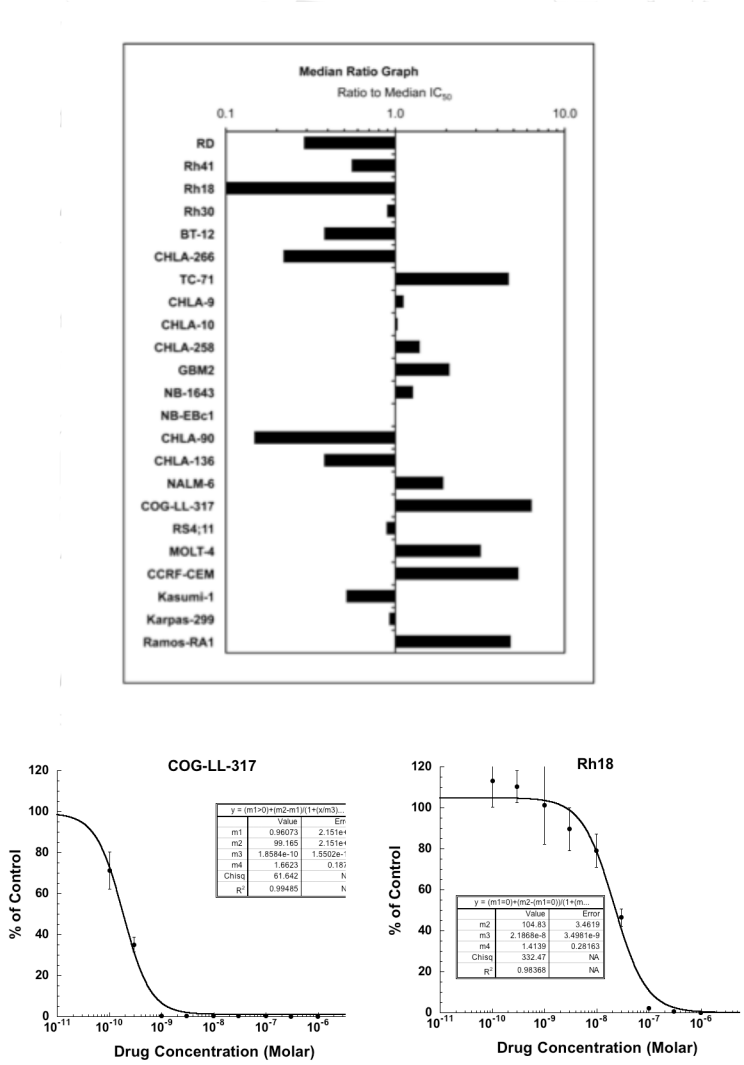

Figure 1.

Genz644282 *in vitro* activity. *Top panel:* The median IC_{50} ratio graph shows the relative IC_{50} values for the cell lines of the PPTP panel. Each bar represents the ratio of the panel IC_{50} to the IC_{50} value of the indicated cell line. Bars to the right represent cell lines with higher sensitivity, while bars to the left indicate cell lines with lesser sensitivity. The median relative IC_{50} was 1.2 nM (range 0.2–21.9 nM). *Bottom panels:* Representative dose response curves for the most sensitive (COG-LL-317) and least sensitive (Rh18) cell lines.

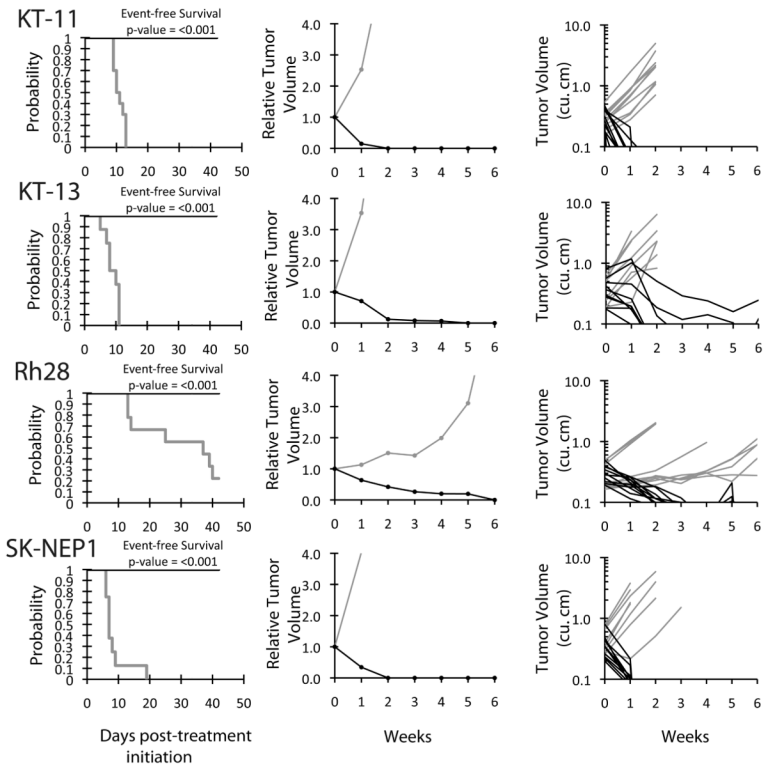

Figure 2.

Genz644282 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: (KT-11, KT-13 (Wilms tumors), Rh28 (alveolar RMS); SK-NEP (Ewing sarcoma of kidney). Mice received Genz644282 administered 3 times weekly (M-W-F) for 2 weeks. The cycle was repeated at day 21.

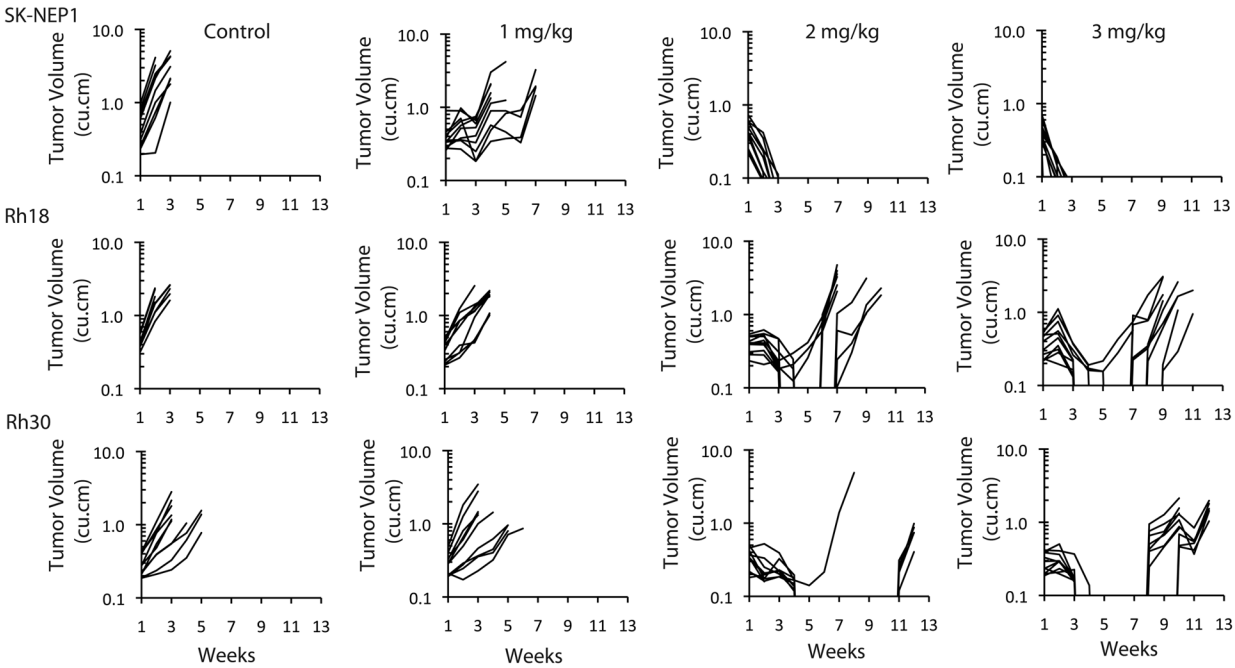

Figure 3.

Dose response data for Genz644282. Mice received Genz644282 administered 3 times weekly for 2 weeks only at 1, 2, 3 mg/kg. Graphs show growth curves for individual tumors.

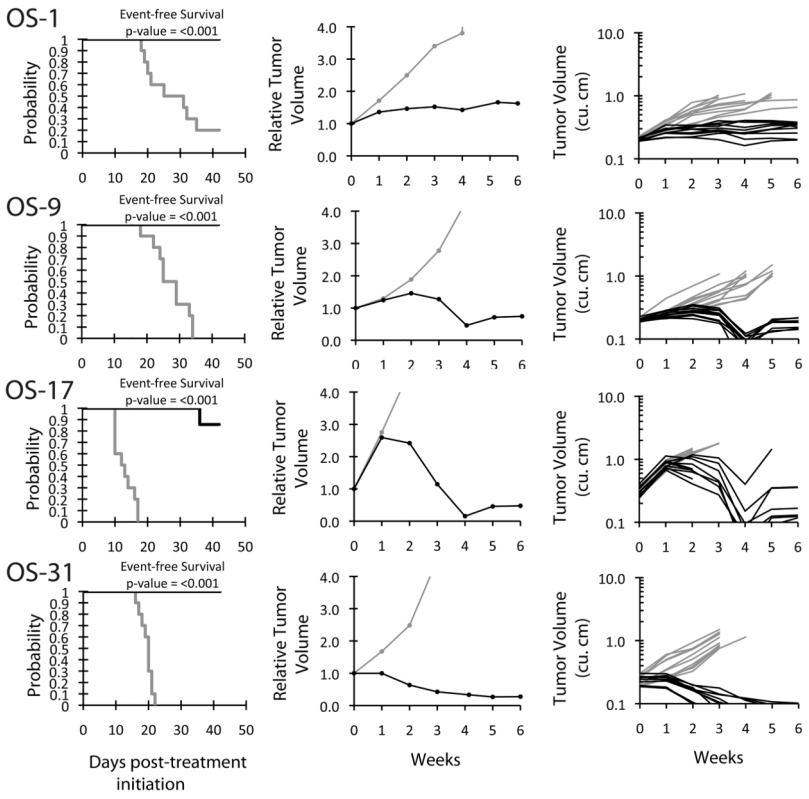

Figure 4.

Prediction of activity from expression profiling. The activity of Genz644282 was evaluated against a panel of xenografts to test the predictions derived from expression profiling. Kaplan-Meier curves for EFS, median relative tumor volume graphs, and individual tumor volume graphs are shown for osteosarcoma lines: OS-1, OS-9, OS-17, OS-31. Mice received Genz644282 administered 3 times weekly for 2 weeks.

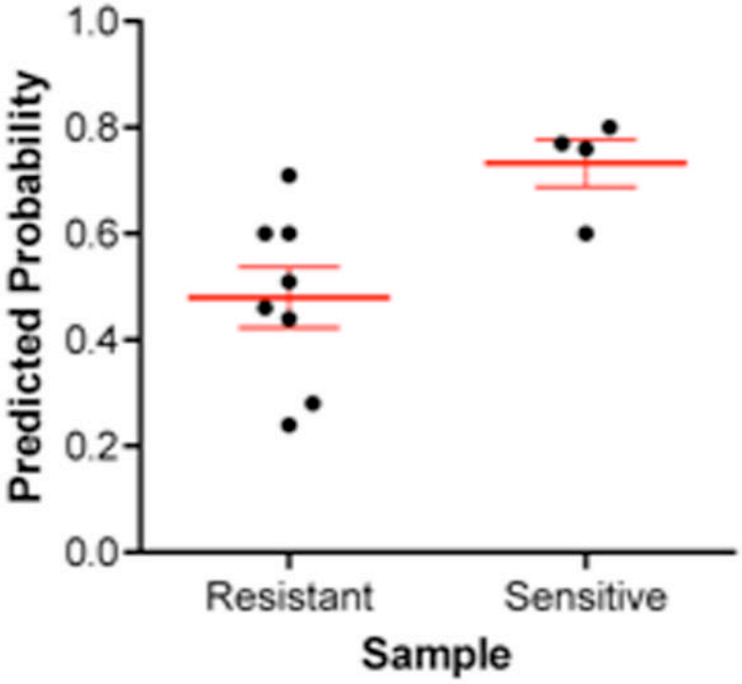

Figure 5.
Correlation of the predicted response of xenografts to the measured response. X-axis represents classes of xenografts based on measured response, Y-axis the predicted probability of Genz644282 sensitivity. (Mann-Whitney U test $p<0.02$)

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Table I

In vitro sensitivity of PPTP cell lines to Genz-644282

Cell Line	Histology	Relative IC_{50} (nM)	Absolute IC_{50} (nM)	Ymin	R^2	Median Relative IC_{50} Ratio	Relative I/O
RD	Rhabdomyosarcoma	3.0	3.0	0.0	0.941	0.39	−99.7%
Rh41	Rhabdomyosarcoma	1.9	2.2	0.0	0.991	0.63	−100.0%
Rh18	Rhabdomyosarcoma	21.9	21.9	0.1	0.984	0.05	−99.7%
Rh30	Rhabdomyosarcoma	1.4	1.3	0.0	0.994	0.86	−100.0%
BT-12	Rhabdoid	3.1	3.1	0.0	0.997	0.38	−100.0%
CHLA-266	Rhabdoid	4.8	5.4	0.1	0.995	0.25	−99.8%
TC-71	Ewing sarcoma	0.2	0.3	0.0	0.985	4.82	−99.9%
CHLA-9	Ewing sarcoma	1.0	1.1	0.0	0.996	1.14	−99.6%
CHLA-10	Ewing sarcoma	1.1	1.2	0.0	0.999	1.04	−100.0%
CHLA-258	Ewing sarcoma	0.8	0.9	0.0	0.989	1.48	−100.0%
GBM2	Glioblastoma	0.6	0.6	0.0	1.000	2.10	−99.9%
NB-1643	Neuroblastoma	0.9	0.9	0.0	0.998	1.32	−99.9%
NB-EBc1	Neuroblastoma	1.2	1.2	0.0	0.998	1.00	−100.0%
CHLA-90	Neuroblastoma	4.8	8.1	10.8	0.978	0.25	−61.3%
CHLA-136	Neuroblastoma	2.9	3.1	0.1	0.991	0.40	−99.7%
NALM-6	ALL	0.6	0.6	0.0	0.999	1.94	−99.9%
COG-LL-317	ALL	0.2	0.2	0.0	0.995	6.37	−99.8%
RS4;11	ALL	1.3	1.3	0.0	0.996	0.88	−100.0%
MOLT-4	ALL	0.4	0.4	0.0	1.000	3.19	−99.9%
CCRF-CEM	ALL	0.3	0.3	0.0	0.998	3.69	−100.0%
Kasumi-1	AML	2.2	2.3	0.0	0.993	0.53	−100.0%
Karpas-299	ALCL	1.3	1.3	0.0	0.998	0.91	−100.0%
Ramos-RA1	NHL	0.3	0.2	0.0	0.985	4.55	−99.4%
Median		1.2	1.2	0.01	0.995	1.00	−99.9%
Minimum		0.2	0.2	0.00	0.941	0.05	−100.0%
Maximum		21.9	21.9	10.8	1.000	6.37	−61.3%

[1] Relative IC_{50} is the concentration of agent that gives a response half way between Bottom and Top;

[2] Absolute IC_{50} values represent the concentration at which the agent reduces cell survival to 50% of the control value;

[3] To compare activity between cell lines, the ratio of the median relative IC_{50} to individual cell line's relative IC_{50} value is used (larger values connote greater sensitivity);

[4] The lowest T/C% value is the Y_{min}.

[5] Relative In/Out (I/O)% values represent the percentage difference between the Y_{min} value and the estimated starting cell number and either the control cell number (for agents with Y_{min} > starting cell number) or 0 (for agents with Y_{min} < estimated starting cell number); Relative I/O% values range between 100% (no treatment effect) to −100% (complete cytotoxic effect), with a Relative I/O% value of 0 being observed for a completely effective cytostatic agent.

Table II

Summary of Anti-tumor activity of Genz-644282

Xenograft Line	Histology	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	P-value	T/C Volume Activity	EFS Activity	Response Activity
4 mg/kg										
KT-10	Wilms	> EP	<0.001	> 2.9	0	0.04	<0.001	High	High	High (MCR)
KT-11	Wilms	> EP	<0.001	> 4.1	0	0	<0.001	High	High	High (MCR)
KT-13	Wilms	> EP	<0.001	> 4.6	0	0.27	<0.001	Int	High	High (MCR)
SK-NEP-1	Ewings	> EP	<0.001	> 6.0	0	0.09	<0.001	High	High	High (MCR)
CHLA258	Ewings	> EP	<0.001	> 4.1	0	0.46	<0.001	Low	High	High (MCR)
Rh28	ALV Rhabdomyosarcoma	> EP	<0.001	> 1.1	0	0.18	<0.001	Int	NE	High (MCR)
Rh30	ALV Rhabdomyosarcoma	> EP	<0.001	> 3.6	0	0.05	<0.001	High	High	High (MCR)
Rh18	EMB Rhabdomyosarcoma	> EP	<0.001	> 6.2	0	0.12	0.002	High	High	High (MCR)
2 mg/kg[1]										
SK-NEP-1	Ewing	> EP	<0.001	> 4.4	0.0	0.02	<0.001	High	High	High (MCR)
Rh30	ALV Rhabdomyosarcoma	82.5	<0.001	6.2	>4	0.22	<0.001	Int	Int	High (CR)
Rh18	EMB Rhabdomyosarcoma	41.8	<0.001	5.5	>4	0.29	<0.001	Int	Int	High (CR)
2 mg/kg[2]										
BT-29	Rhabdoid	12.7	0.346	1.1	>4	0.81	0.089	Low	Low	Low (PD1)
SK-NEP-1	Ewing	> EP	<0.001	> 4.4	0.0	0.02	<0.001	High	High	High (MCR)
EW5	Ewing	11.3	<0.001	1.7	>4	0.60	0.011	Low	Low	Int (PD2)
EW8	Ewing	11.1	0.657	1.2	>4	1.02	0.912	Low	Low	Low (PD1)
Rh30R	ALV Rhabdomyosarcoma	15.1	0.088	1.8	>4	0.84	0.481	Low	Low	Int (PD2)
Rh18	EMB Rhabdomyosarcoma	> EP	<0.001	> 3.8	0.0	0.43	<0.001	Int	High	High (MCR)
BT-45	Medulloblastoma	6.1	0.084	1.1	>4	0.88	0.161	Low	Low	Low (PD1)
D645	Glioblastoma	40.8	<0.001	3.5	>4	0.11	<0.001	High	Int	High (PR)
NB-SD	Neuroblastoma	5.6	0.935	1.0	>4	0.93	0.971	Low	Low	Low(PD1)
NB-1771	Neuroblastoma	25.9	0.004	2.3	>4	0.65	0.043	Low	Int	Int (PD2)
CHLA-79	Neuroblastoma	26.0	0.164	1.0	>4	0.61	0.481	Low	Low	Int (PD2)
NB-1643	Neuroblastoma	19.9	<0.001	3.2	>4	0.51	0.002	Low	Int	Int (PD2)
OS-1	Osteosarcoma	> EP	<0.001	> 1.5	1.6	0.44	<0.001	Int	NE	Int (PD2)

Xenograft Line	Histology	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	P-value	T/C Volume Activity	EFS Activity	Response Activity
OS-17	Osteosarcoma	> EP	<0.001	> 3.5	1.0	0.53	<0.001	Low	High	High (PR)
OS-9	Osteosarcoma	> EP	<0.001	> 1.6	0.7	0.43	<0.001	Int	NE	High (CR)
OS-33	Osteosarcoma	> EP	<0.001	> 2.2	0.0	0.16	<0.001	Int	High	High (MCR)
OS-31	Osteosarcoma	> EP	<0.001	> 2.1	0.3	0.10	<0.001	High	High	High (MCR)

[1]Data from dose response studies;

[2]Data from ‘predictive’ studies.