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Initial Testing of the MDM2 Inhibitor RG7112 by the Pediatric Preclinical Testing Program

Hernan Carol, PhD¹, C. Patrick Reynolds, MD, PhD², Min H. Kang, PharmD², Stephen T. Keir, PhD³, John M. Maris, MD⁴, Richard Gorlick, MD⁵, E. Anders Kolb, MD⁶, Catherine A. Billups, MS⁷, Brian Geier, MS⁸, Raushan T. Kurmasheva, PhD⁸, Peter J. Houghton, PhD⁸, Malcolm A. Smith, MD, PhD⁹, and Richard B. Lock, PhD¹

¹Children's Cancer Institute Australia for Medical Research, University of New South Wales, Sydney, NSW, Australia.

²Texas Tech University Health Sciences Center, Lubbock, TX.

³Duke University Medical Center, Durham, NC.

⁴Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine and Abramson Family Cancer Research Institute, Philadelphia, PA.

⁵The Children's Hospital at Montefiore, Bronx, NY.

⁶A.I. duPont Hospital for Children, Wilmington, DE.

⁷St. Jude Children's Research Hospital, Memphis, TN.

⁸Nationwide Children's Hospital, Columbus, OH.

⁹Cancer Therapy Evaluation Program, NCI, Bethesda, MD.

Abstract

Background—RG7112 is a selective inhibitor of p53-MDM2 binding that frees p53 from negative control, activating the p53 pathway in cancer cells leading to cell cycle arrest and apoptosis. RG7112 was selected for evaluation by the Pediatric Preclinical Testing Program (PPTP) due to the relatively low incidence of p53 mutations in pediatric cancers compared with adult malignancies.

Procedures—RG7112 and its inactive enantiomer RG7112i were evaluated against the 23 cell lines of the PPTP in vitro panel using 96 hour exposure (1 nM to 10 μ M). It was tested against the PPTP in vivo panel focusing on p53 wild-type (WT) xenografts at a dose of 100 mg/kg daily for 14 days followed by 4 weeks of observation. Response outcomes were related to MDM2 and p53 expression datasets (<http://pptp.nchresearch.org/data.html>).

Results—RG7112 demonstrated cytotoxic activity with a lower median IC₅₀ for p53 WT versus p53 mutant cell lines (approximately 0.4 μ M versus > 10 μ M, respectively). RG7112 induced tumor growth inhibition meeting criteria for intermediate activity (EFS T/C > 2) in 10 of 26 (38%) solid tumor xenografts. Objective responses included medulloblastoma, alveolar rhabdomyosarcoma, Wilms, rhabdoid and Ewing sarcoma xenografts. For the ALL panel, there was 1 partial response, 5 complete responses and 1 maintained complete response. The ALL xenografts expressed the highest levels of p53 among the PPTP panels.

Corresponding Author: Richard B. Lock, PhD, Leukaemia Biology Program, Children's Cancer Institute Australia, Lowy Cancer Research Centre, High St., Randwick, NSW 2031, rlock@ccia.unsw.edu.au, Voice: 011-612-9385-2513.

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Conclusions—RG7112 induced tumor regressions in solid tumors from different histotype panels, and exhibited consistent high-level activity against ALL xenografts. This high level of activity supports prioritization of RG7112 for further evaluation.

Keywords

Preclinical Testing; Developmental Therapeutics; RG7112

INTRODUCTION

The transcription factor and tumor suppressor p53 is activated in response to a variety of cellular perturbations, in particular genotoxic stress, to prevent fixation of DNA damage and proliferation of cells with genetic defects (reviewed in [1]). Consequences of p53 activation include induction or repression of p53-responsive genes at the transcriptional level, resulting in cell cycle arrest, senescence, or apoptosis [2]. Due to this central function in preserving the genetic integrity of higher organisms p53 has been labeled the “guardian of the genome” [3]. Evasion of growth suppression is a hallmark of cancer [4], and disruption of the p53 pathway is considered to occur early during oncogenesis [5].

The activity of p53 is tightly regulated by post-translational modifications that affect its cellular localization and ability to bind DNA consensus sequences [6,7]. However, p53 is principally regulated by its high rate of turnover and degradation mediated by the E3 ubiquitin ligase, MDM2 (mouse double minute 2), which catalyzes p53 ubiquitination and subsequent degradation by the proteasome [7–9]. Evidence for the importance of this pathway is the embryonic lethality of *MDM2* knockout mice and their rescue by knocking out p53 [10,11]. Overexpression of MDM2 has also been frequently described in transformed cells that express wild-type p53 [12]. The feedback loop that regulates p53 degradation is based on p53-mediated transcriptional induction of *MDM2*, with an additional layer of regulation imposed by MDMX, a homolog of *MDM2* that represses p53-mediated gene transactivation [13].

p53 mutations are present in approximately 50% of all cancers [2]. Mutant forms of p53 not only inactivate the p53 pathway, since most are dominant negative, but have also been reported to confer additional tumorigenic properties through “gain of function”, such as p63 and p73 inactivation [14]. Furthermore, in those cancers that express only a wild-type p53 there is a high frequency of MDM2 overexpression [15], and of either upstream (p14^{ARF}) or downstream (p21^{Cip1/WAF1}, Puma) defects in the p53 pathway [5,16]. p53 mutations are less prevalent in pediatric compared with adult cancers [17], including glioma [18], neuroblastoma [19], sarcomas [8,20,21], Wilms tumor [22] and leukemia [23,24], suggesting that a larger proportion of pediatric patients may benefit from pharmacological unleashing of the wild-type p53 protein. However, p53 mutations are reported to occur at a higher frequency in relapsed patients with neuroblastoma [16,25] and acute lymphoblastic leukemia (ALL) [24,26], and where present have been associated with aggressive and chemo-refractory disease [24,26,27].

Reconstitution of a functional p53 pathway is an attractive anticancer strategy. However, because the interactions between p53 and its two principal regulatory molecules (MDM2/MDMX) involve a large protein-protein interface with numerous atomic interactions, it was for some time considered a difficult target for pharmacological intervention [28]. Only recently have molecules been identified that are able to effectively inhibit the MDM2-mediated degradation of p53 or inhibition of *MDM2* transcription [29]. Among these entities, compounds with quite diverse structures have been investigated, including peptides, chalcones [30], spiro-oxindoles [31], benzodiazepinediones [32], the compound RITA [33],

and cis-imidazolines (Nutlins) [29]. Most of these compounds exhibited *in vitro* activity, while the Nutlins have also demonstrated impressive activity *in vivo* with limited toxicity [29]. The cis-imidazoline, RG7112, is an orally available inhibitor of the MDM2-p53 interaction.

The Pediatric Preclinical Testing Program (PPTP) aims to identify new agents with significant activity against preclinical models of pediatric malignancies in order to prioritize new drugs for their advancement into clinical trials for childhood cancer [34]. RG7112 was selected for systematic testing by the PPTP based on its potential efficacy against p53 wild-type cancers. This report describes testing of RG7112 as a single agent against the PPTP's *in vitro* panel as well as against the *in vivo* tumor panel as a single agent at its maximum tolerated dose.

MATERIALS AND METHODS

In Vitro Testing

In vitro testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable cell numbers (using fluorescein diacetate [FDA]) in tissue culture multiwell plates [35]. Cells were incubated in the presence of RG7112 or its inactive enantiomer (RG7112i) for 96 hours at concentrations from 1 nM to 10 μ M and analyzed as previously described [36]. Absolute IC₅₀ values represent the concentration of RG7112 that reduces cell survival to 50% of the control value, while relative IC₅₀ (rIC₅₀) values represent the RG7112 concentration that reduces cell survival by 50% of the maximum RG7112 effect [37]. 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 [38–40]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [41]. 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. Female mice were used irrespective of the gender from which the tumor was derived. Tumor volumes (cm³) [solid tumor xenografts] or percentages of human CD45-positive cells in the peripheral blood [%hCD45] [ALL xenografts] were determined as previously described [34]. Responses were determined using three activity measures as previously described [34]. Ten mice (solid tumors) or 7–9 mice (leukemias) were used per group. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Genomic DNA Extraction and Sequencing of the p53 Gene

Genomic DNA was extracted using a Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN) and quantified spectrophotometrically. Aliquots (200 ng) of DNA were used for polymerase chain reaction (PCR) amplification of the entire p53 coding region (exons 2–11) using Human p53 Amplimer Panels (Clontech Laboratories, Palo Alto, CA),

according to the manufacturer's instructions, and sequenced using dye-terminator chemistry, as described previously [42]. For xenograft-specific p53 status see <http://pptp.nchresearch.org/documents.html>.

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. Fisher's exact test (either one or two tailed) was used for testing associations between groups and observed effects. The Mann-Whitney test was used to test the difference of medians of rIC₅₀ values between groups of cell lines based on p53 mutation status.

Prior to analysis for gene expression data, the mRNA features mapping to a particular gene were collapsed to a single value by taking the maximum intensity across mapped features. An unpaired t-test permutation was applied to gauge the local significance of each gene. The Storey q-value [43], a false discovery analogue of the p-value, was computed for each gene and was our criteria to determine significance when correcting for multiple hypothesis testing. Additionally, we computed the average fold change per gene, which is the ratio of the mean expression level of group A (drug sensitive) versus the mean expression level of group B (drug insensitive). The Spearman correlation confidence intervals were computed using the bias-corrected and accelerated (BCa) bootstrap percentile method.

Compound Information and Formulation

Roche Pharmaceuticals, Inc., through the Cancer Therapy Evaluation Program (NCI), provided RG7112 and RG7112i to the PPTP. RG7112 was pre-formulated and provided to each consortium investigator in coded vials for blinded testing against a placebo control consisting of vehicle only, and administered orally (P.O.) at a dose of 100 mg/kg daily for 14 days, as recommended by the drug supplier.

RESULTS

RG7112 *In Vitro* Testing

The median rIC₅₀ for RG7112 against the PPTP cell lines was approximately 1 μ M (Table I). The RG7112 median rIC₅₀ was significantly lower for p53 wild-type versus mutant cell lines [0.44 μ M versus >10 μ M, respectively ($p < 0.001$)]. RG7112 demonstrated potent cytotoxic activity against a subset of PPTP cell lines across a range of histotypes, with Ymin T/C% values approaching 0% and with Relative I/O% values approaching -100% at the highest concentration tested (Figure 1A and Table I). p53 status was related to the Relative I/O% values, with most p53 mutant cell lines showing values >0% (median 42%) and most wild-type cell lines showing values approaching -100% (median -98%). Specificity of RG7112 was further assessed by comparing the ratio of the rIC₅₀ of the inactive enantiomer (RG7112i) to the rIC₅₀ of RG7112. For p53 wild-type cell lines, the median value for the ratio of their RG7112i rIC₅₀ to their RG7112 rIC₅₀ was 12.75, compared to a median ratio value of 0.9 for p53 mutant cell lines ($p < 0.001$). (Table I and Figure 1B).

RG7112 *In Vivo* Testing

RG7112 was well tolerated *in vivo*. A total of 12 out of 739 mice died during the study (1.6%), with 3 of 365 in the control arms (0.8%) and 9 of 374 in the RG7112 treatment arms (2.4%). One xenograft line (ALL-2) was 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.

RG7112 induced statistically significant differences in EFS distribution compared to control in 15 of 26 (58%) evaluable p53 wild-type solid tumor xenografts, Table II. RG7112 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity (EFS T/C > 2) in 10 of 26 (38%) p53 wild-type solid tumor xenografts evaluable for this measure, including: 3/3 rhabdoid tumor, 1/1 Wilms tumor, 2/2 Ewing with WT p53, 3/5 rhabdomyosarcoma models with WT p53, 1/2 glioblastomas, but no neuroblastoma (n=5) or osteosarcoma (n=6) models. Objective responses were observed in 5 solid tumor xenografts: maintained complete response (MCR) or complete response (CR) for a medulloblastoma and an alveolar rhabdomyosarcoma, respectively, and partial responses (PRs) for a Wilms tumor, rhabdoid tumor, and Ewing tumor xenograft (Figure 2). The MCR for the medulloblastoma (BT-50) was not statistically significant from the controls, as the untreated tumors did not have an event during the 6 weeks observation due to slow growth rate of this model. Solid tumor xenografts with mutant p53 (Rh30R, Rh10 and EW5) showed no *in vivo* response to RG7112 as expected.

For the systemic-disease ALL panel, among 7 xenografts there were 5CRs, 1 MCR and 1 PR (four of them depicted in Figure 3). Among the ALL panel the most impressive response was elicited in the infant Mixed-Lineage Leukemia (*MLL*)-rearranged xenograft MLL-7. Two additional *MLL*-rearranged xenografts (MV4;11 and RS4;11), grown subcutaneously, were also tested, with the former showing MCR and the latter tumor growth delay (PD2). The *in vivo* testing results for the objective response measure of activity are presented in Figure 4 in a 'heat-map' format as well as a 'COMPARE'-like format, based on the scoring criteria described in the Material and Methods and the Supplemental Response Definitions section. The latter analysis demonstrates relative tumor sensitivities around the midpoint score of 5 (stable disease).

Relative expression of p53 and MDM2 for the *in vitro* cell lines (Supplemental Figure 1A) and *in vivo* xenografts (Supplemental Figure 1B) are shown together with p53 genotype and *in vivo* responsiveness to RG7112. The ALL xenografts expressed the highest levels of p53 and MDM2 among the PPTP panels and showed the most consistent responses to RG7112, Supplemental Figure 1B. Conversely, the osteosarcoma xenografts were p53 wild-type, but had very low p53 expression, and they failed to respond to RG7112. The significance between p53 or *MDM2* basal expression and RG7112 efficacy was assessed by a Spearman correlation with EFS activity. The basal expression of p53 is correlated with EFS activity, as supported by a 95% Spearman correlation confidence interval of 0.36–0.83, and the basal expression of *MDM2* is correlated with EFS activity, as supported by a 95% Spearman correlation confidence interval of 0.089–0.67. However, we determined from visual inspection that p53 expression and EFS activity are possibly correlated by the presence of osteosarcoma samples. Upon removing the osteosarcoma samples (n=6), the correlation with p53 expression is still significant but the confidence interval widens to 0.04–0.68. Additionally, the p53 basal expression is significantly expressed (q=0.0092, FC=2.4) in the comparison of SD, PR, CR, and MCR versus PD1 and PD2 samples within the test cohort but is not significant (q=0.04, FC=1.5) when removing osteosarcoma xenografts. Also, *MDM2* basal expression is not differentially expressed (q=0.20, FC=1.47) within the comparison of SD, PR, CR, and MCR versus PD1 and PD2 samples despite being correlated with EFS activity.

DISCUSSION

The p53:MDM2 interaction is a priority target for pediatric evaluation given the high prevalence of p53 wild-type cancers in the childhood cancer setting. Previous results have shown that the MDM2 inhibitor Nutlin is active against cell lines derived from a number of pediatric cancers, including cell lines for neuroblastoma [44,45], osteosarcoma [46], rhabdomyosarcoma [47], Ewing sarcoma [48], and ALL [49]. Our results extend previous *in vitro* findings for Nutlin to RG7112, and they provide insight into the *in vivo* pattern of activity of RG7112 against p53 wild-type childhood cancer preclinical models.

The pattern of *in vitro* activity of RG7112 against the cell line panel tested is consistent with p53-MDM2 interaction inhibition. Each of the p53 wild-type cell lines had rIC₅₀ values < 1 μM, while only one p53 mutant cell line (Ramos-RA1) had a rIC₅₀ value below 1 μM. The Ramos Burkitt lymphoma line is hemizygous for p53 and carries only a mutant allele [50]. This mutant p53 protein was unable to transactivate the appropriate reporter plasmids suggesting a lack of function [51]. The p53 wild-type cell lines showed approximately 10-fold greater sensitivity to RG7112 compared to RG7112i, whereas the p53 mutant cell lines showed similar sensitivity to both agents. The p53 wild-type cell lines, but not the p53 mutant cell lines, showed a clear cytotoxic response to RG7112 with Ymin value approaching 0% and Relative I/O% values approaching -100% for most p53 wild-type cell lines. The activity of RG7112 *in vitro* was not histotype restricted, as no cancer type among those tested showed either all or no response.

The dose and schedule of drug administration selected for *in vivo* treatment (100 mg/kg for 14 days) was well tolerated and only minor toxicity was observed across the xenograft panel. Only one of the xenograft models was excluded from evaluation because of excessive toxicity (ALL-2, 4 out of 9 mice), and overall only 9 of 372 (2.4%) died in the treated cohorts during the 42 days study window. It remains to be determined how pharmacokinetic parameters for this dose/schedule in mice compare to those achieved at tolerable doses in humans.

The *in vivo* efficacy results obtained are encouraging and provide confirmation of the specificity of the mode of action of RG7112, since three p53 mutant xenografts (EW5, Rh10 and Rh30R) did not show evidence of activity (non-significant growth delays). Among the p53 WT solid tumors RG7112 treatment induced an objective response in 5 out of 26 models while 10 out of 26 achieved intermediated EFS T/C activity and 16 out of 26 had significant growth delays. However, since the solid tumor panel tested included 26 p53 wild-type xenografts but resulted in objective responses in only five, our results suggest that factors other than p53 mutation status determine *in vivo* sensitivity to RG7112. The neuroblastoma and osteosarcoma panels were the only two panels that did not show any objective responses to RG7112 treatment. Understanding the cellular mechanisms responsible for limited activity in these p53 wild-type tumors is an important line for future investigations, as is exploring the utility of RG7112 given in combination with other agents in tumors with limited single agent response to RG7112.

All seven evaluable ALL xenografts showed leukemia regression, suggesting this type of cancer is particularly sensitive to reconstitution of the p53 pathway by means of blocking MDM2 driven p53 degradation. The relatively high basal gene expression levels of p53 in the ALL panel indicates that these cells are primed for releasing p53 from MDM2 to trigger cell death. RG7112 was particularly effective against MLL-7, the only infant *MLL*-rearranged xenograft in the panel and the only xenograft that achieved MCR. With the aim of testing if there was a subtype inherent susceptibility in *MLL*-rearranged leukemias, two additional models (MV4;11 and RS4;11, grown as solid subcutaneous tumors) were tested *in*

vivo. We observed one MCR (MV4;11) while the other xenograft (RS4;11) only had growth delay. Further investigations into the effect of RG7112 on *MLL*-rearranged xenograft models and the basis for the differences in efficacy observed are warranted.

RG7112 *in vivo* activity was correlated with p53 gene expression levels. This correlation is driven in part by the osteosarcoma xenografts, which showed very low basal p53 levels and no response to RG7112. However, even though basal p53 or MDM2 are correlated with EFS activity, the insignificant occurrence of differential expression within this cohort leads us to suspect that basal levels of these genes are not sufficiently specific molecular markers for RG7112 response to consider for clinical utilization as predictive biomarkers. *MDM2* expression levels were higher for the ALL xenografts compared to the solid tumor xenografts with wild-type p53, which is consistent with prior results showing that *MDM2* overexpression is common in ALL cell lines with wild-type p53 [52]. One interesting observation is that among the ALL xenografts ALL-19 had the lowest p53 and highest *MDM2* expression and was the ALL line where RG7112 treatment resulted in low activity (based on EFS) and that did not score a CR or MCR (Figure 4).

The PPTP previously tested serdemetan [53], which was initially developed as an inhibitor of MDM2 [54,55], and a comparison of results from both the *in vitro* and *in vivo* activity evaluations supports prior findings that serdemetan and RG7112 likely have different mechanisms/targets. *In vivo*, although some of the xenografts (i.e., KT-10 and ALL-17) obtained consistent results between the two agents, there were many discrepancies between *in vivo* activity of the two drugs (e.g., OS-31, ALL-4, ALL-7 and ALL-8). Furthermore, *in vitro* sensitivity to serdemetan did not segregate with p53 status, and *in vivo* responses to serdemetan were noted in xenografts with both mutant and wild-type p53 [53].

In conclusion, RG7112 induced tumor regressions in p53 wild-type solid tumors from different tumor panels, and exhibited consistent high-level activity against ALL xenografts. In particular, the very high efficacy against the infant *MLL*-rearranged xenograft is intriguing. The high level of activity reported here for RG7112 against *in vivo* models of pediatric cancer supports prioritization of RG7112 for further evaluation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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A

B

Figure 1.

RG7112 *in vitro* activity. A) The Relative in/out values (as percentage) for RG7112 are shown for the *in vitro* cell panel. Values of -100%, 0%, and 100% are consistent with complete cell kill, cytostasis, and no treatment effect, respectively. Solid black bars indicate cell lines with wild-type p53 and hatched bars indicate cell lines with mutant p53. B) Ratios of rIC_{50} for RG7112i versus rIC_{50} for RG7112 for the *in vitro* cell line panel. Solid black bars indicate wild-type p53 cell lines and hatched bars indicate mutant p53 cell lines.

Figure 2. RG7112 activity against individual solid tumor xenografts, Kaplan-Meier curves for EFS and graphs of median and tumor volumes (controls, gray and treated, black lines), are shown for selected lines: BT-50 (medulloblastoma), Rh65 (alveolar rhabdomyosarcoma), KT-12 (kidney rhabdoid) and CHLA258 (Ewing sarcoma).

Figure 3. RG7112 activity against individual ALL xenografts, Kaplan-Meier curves for EFS and graphs of median and individual %hCD45 (controls, gray and treated, black lines), are shown for selected lines: ALL-4, ALL-7, ALL-8 and MLL-7.

Figure 4.

RG7112 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.

Table 1

In vitro activity of RG7112 and the inactive enantiomer RG7112i

Cell Line	Diagnosis	p53 Status	RG7112 IC ₅₀ (μM)	RG7112 Observed Ymin	RG7112 Relative In/Out%	RG7112i IC ₅₀ (μM)	RG7112i Observed Ymin	RG7112i Relative In/Out%	Ratio IC ₅₀
RD	Rhabdomyosarcoma	Mutant	>10	50.5	48%	8.93	45.9	43%	0.8
Rh41	Rhabdomyosarcoma	Mutant	>10	48.3	34%	3.70	19.6	-12%	0.3
Rh18	Rhabdomyosarcoma	WT	0.47	0.7	-98%	2.25	23.1	-48%	4.8
Rh30	Rhabdomyosarcoma	Mutant	>10	51.2	42%	6.54	35.8	23%	0.6
BT-12	Rhabdoid (CNS)	WT	0.99	26.4	20%	9.66	48.7	44%	9.8
CHLA-266	Rhabdoid (CNS)	WT	0.05	0.0	-100%	2.20	0.1	-100%	45.6
TC-71	Ewing sarcoma	Mutant	2.75	20.8	18%	4.18	6.9	3%	1.5
CHLA-9	Ewing sarcoma	WT	0.9	3.04	-15%	11.0	79.1	78%	12.2
CHLA-10	Ewing sarcoma	Mutant	>10	44.2	40%	3.2	14.01	8%	0.29
CHLA-258	Ewing sarcoma	WT	0.36	1.7	0%	>10	49.8	49%	62.5
SJ-GBM2	Glioblastoma	Mutant	4.92	8.4	-15%	3.56	9.2	-7%	0.7
NB-1643	Neuroblastoma	WT	0.65	0.3	-99%	>10	70.4	63%	16.9
NB-EBc1	Neuroblastoma	WT	0.11	0.0	-100%	0.94	0.0	-100%	8.2
CHLA-90	Neuroblastoma	Mutant	>10	80.0	72%	5.27	34.3	9%	0.5
CHLA-136	Neuroblastoma	WT	0.71	1.0	-96%	6.94	38.4	14%	9.8
NALM-6	Pre-B cell ALL	WT	0.14	0.0	-100%	2.00	0.0	-100%	14.2
COG-LL-317	T-cell ALL	WT	0.41	0.1	-97%	7.50	38.6	36%	18.2
RS4;11	Pre-B cell ALL	WT	0.53	0.4	-98%	4.48	19.7	5%	8.5
MOLT-4	T-cell ALL	WT	0.08	0.0	-100%	1.58	0.0	-100%	20.2
CCRF-CEM	T-cell ALL	Mutant	8.27	45.4	42%	>10	50.7	47%	1.3
Kasumi-1	AML	Mutant	>10	63.3	49%	9.57	48.5	28%	0.9
Karpas-299	ALCL	Mutant	>10	83.0	82%	>10	61.5	58%	1.0
Ramos-RA1	Burkitt lymphoma	Mutant	0.99	17.1	16%	3.04	3.6	3%	3.1
Median			0.99	19.0	17%	5.01	31.1	11%	2.3
Minimum			0.05	0.0	-100%	0.46	0.0	-100%	0.0
Maximum			>10	83.0	82%	>10	70.4	63%	62.5

Table II

In vivo activity of RG7112

Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	T/C Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	> EP	<0.001	> 2.0	1.0	0.31	Int	Int	Int
KT-14	Rhabdoid	.	0.003	>2.5	4.0	0.19	Int	Int	Int
KT-12	Rhabdoid	31.0	<0.001	3.8	>4	0.26	Int	Int	High
KT-10	Wilms	34.0	<0.001	2.5	>4	0.08	High	Int	High
EW5 /	Ewing	7.7	0.622	0.7	>4	1.08	Low	Low	Low
CHLA258	Ewing	9.3	<0.001	>2.5	3.8	0.09	High	Int	High
EW4	Ewing	21.5	0.009	2.4	>4	0.77	Int	Int	Int
ES-6	Ewing	30.9	0.001	1.4		0.67	Low	Low	Low
Rh10 /	ALV RMS	31.1	0.485	.	>4	0.41	Int	NE	Low
Rh28	ALV RMS	38.4	<0.001	2.6	>4	0.13	High	Int	Int
Rh30	ALV RMS	32.4	0.103	1.3	>4	0.28	Int	Low	Low
Rh30R /	ALV RMS	18.1	0.059	0.9	>4	1.59	Low	Low	Low
Rh65	ALV RMS	.	<0.001	3.4	2.5	0.03	High	Int	High
Rh18	EMB RMS	16.8	0.001	1.5	>4	0.57	Low	Low	Low
Rh36	EMB RMS	27.8	<0.001	2.2	>4	0.20	Int	Int	Int
BT-50	Medulloblastoma	.	.	.	0.0	0.66	Low	NE	High
D 456	Glioblastoma	10.3	0.865	1.2	>4	0.96	Low	Low	Low
BT-56	Glioblastoma	25.4	<0.001	2.3	>4	0.40	Int	Int	Int
NB-1691	Neuroblastoma	8.7	0.359	1.2	>4	0.90	Low	Low	Low
NB-EBcl	Neuroblastoma	5.4	0.003	1.4	>4	0.54	Low	Low	Low
CHLA-79	Neuroblastoma	13.7	0.017	1.6	>4	0.71	Low	Low	Int
NB-1643	Neuroblastoma	13.2	0.164	1.3	>4	0.84	Low	Low	Low
NB-1382	Neuroblastoma	24.6	0.008	1.9	>4	0.65	Low	Low	Int
OS-1	Osteosarcoma	26.8	0.234	1.0	>4	0.89	Low	Low	Low
OS-2	Osteosarcoma	19.5	0.166	1.0	>4	0.93	Low	Low	Low

Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	T/C Activity	EFS Activity	Response Activity
OS-17	Osteosarcoma	16.6	0.373	1.1	>4	0.92	Low	Low	Low
OS-9	Osteosarcoma	26.4	0.278	1.1	>4	0.97	Low	Low	Low
OS-31	Osteosarcoma	24.4	0.129	1.1	>4	0.91	Low	Low	Low
OS-29	Osteosarcoma	29.2	0.859	1.0	>4	0.88	Low	Low	Low
ALL-4	ALL B-precursor	31.6	<0.001	3.5	>25	.		Int	High
ALL-7	ALL B-precursor	31.1	<0.001	3.9	>25	.		Int	High
ALL-8	ALL T-cell	41.7	<0.001	5.1	>25	.		Int	High
ALL-17	ALL B-precursor	33.4	0.002	3.8	>25	.		Int	High
ALL-19	ALL B-precursor	22.3	0.170	2.3	>25	.		Low	High
ALL-31	ALL T-cell	33.0	<0.001	7.0	>25	.		Int	High
MLL-7	ALL B-precursor	> EP	<0.001	> 5.6	0.7	.		High	High
RS4;11	Adult ALL	22.8	<0.001	1.9	>4	0.24	High	Low	Int
MV4;11	B-myelomnocyctic	>EP	<0.001	>2.9	0.0	0.01	High	High	High

/ mutant p53