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## Initial Testing (Stage 1) of the Curaxin CBL0137 by the Pediatric Preclinical Testing Program

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### Abstract

**Background**—CBL0137 is a novel drug that modulates FACT (Facilitates Chromatin Transcription), resulting in simultaneous Nuclear factor- $\kappa$ B suppression, Heat shock factor 1 suppression and p53 activation. CBL0137 has demonstrated antitumor effects in animal models of several adult cancers and neuroblastoma.

**Procedures**—CBL0137 was tested against the Pediatric Preclinical Testing Program (PPTP) in vitro cell line panel at concentrations from 1.0 nM to 10.0  $\mu$ M and against the PPTP in vivo solid tumor xenograft and acute lymphocytic leukemia (ALL) panels at 50 mg/kg administered intravenously weekly for 4 weeks.

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**Conflict of interest statement:** Andrei Gudkov was previously Cleveland BioLabs' Chief Scientific Officer and continues to receive compensation from Cleveland Biolabs. Andrei Purmal is an employee of Incuron LLC, which is a joint subsidiary of the Bioprocess Capital Ventures closed-end investment fund and Cleveland BioLabs. The remaining authors consider that there are no actual or perceived conflicts of interest.

**Results**—The median relative IC<sub>50</sub> (rIC<sub>50</sub>) value for the PPTP cell lines was 0.28 μM (range: 0.13 μM to 0.80 μM). There were no significant differences in rIC<sub>50</sub> values by histotype. CBL0137 induced significant differences in event-free survival (EFS) distribution compared to control in 10 of 31 (32%) of the evaluable solid tumor xenografts and in 8 of 8 (100%) evaluable ALL xenografts. Significant differences in EFS distribution were observed in 4 of 6 osteosarcoma lines, 3 of 3 rhabdoid tumor lines, and 2 of 6 rhabdomyosarcoma lines. No objective responses were observed among the solid tumor xenografts. For the ALL panel, 1 xenograft achieved CR and 4 achieved PR.

**Conclusions**—The most consistent in vivo activity for CBL0137 was observed against ALL xenografts, with some solid tumor xenograft lines showing tumor growth delay. It will be important to relate the drug levels in mice at 50 mg/kg to those in humans at the recommended phase 2 dose.

### Keywords

Preclinical Testing; Developmental Therapeutics; curaxin CBL0137

## INTRODUCTION

CBL0137 is a member of a new class of anticancer drugs, named curaxins, that modulate important pathways involved in pathogenesis of cancer. CBL0137 is reported to activate TP53 and inhibit cellular stress pathways mediated by nuclear factor- $\kappa$ B (NF- $\kappa$ B) and Heat Shock Factor-1 (HSF-1)<sup>1–3</sup>. It is considered that apoptosis induced by CBL0137 is mediated through inhibition of FACT (FAcilitates Chromatin Transcription), a chromatin remodeling complex composed of SSRP1 and SUPT16H subunits<sup>1</sup>. This complex is involved in the transcription of genes with highly ordered chromatin structure that are essential for replication, and mitosis<sup>4–6</sup>. Curaxins bind DNA without inducing damage or activating DNA damage sensitive pathways, but alter DNA topology sequestering FACT on DNA making it unable to bind histones that regulate normal chromatin remodeling<sup>1</sup>.

FACT is overexpressed in several tumor types (e.g., pancreatic ductal carcinoma and lung carcinoma) relative to paired normal tissue, and is associated with a more aggressive phenotype with overall poor survival<sup>7</sup>. Less is known regarding FACT expression in pediatric cancer. Overexpression of the SSRP1 subunit was associated with an 11q12 amplicon in an anaplastic medulloblastoma<sup>8</sup>, and Carter et al.<sup>9</sup> observed down regulation of MYCN when either SSRP1 or SUPT16H subunits of FACT were suppressed, suggesting that FACT may regulate expression of MYCN in neuroblastoma cell lines. FACT is expressed in early embryogenesis and in undifferentiated progenitors and stem cells of adult tissues. In contrast FACT subunit proteins are poorly detected in differentiated tissues<sup>10</sup>.

The role of NF $\kappa$ B in cancer is well established<sup>11</sup>. This transcription factor is constitutively activated in acute lymphoblastic leukemia (ALL)<sup>12</sup>, and activation of the classical pathway is considered as one factor in suppression of differentiation in rhabdomyosarcoma<sup>13</sup> and in suppression of apoptosis in murine B lymphocytes, Hodgkins lymphoma cells and some breast cancer cell lines<sup>14–16</sup>. NF $\kappa$ B is also associated with drug resistance in neuroblastoma

cells<sup>17</sup>, possibly through upregulation of MDR1<sup>18</sup> that encodes the ABC drug transporter ABCB1.

CBL0137 has been shown to reduce colony formation in several pancreatic tumor cell lines at sub-micromolar concentrations, suppress growth of PANC-1 tumors and to have additive activity when combined with gemcitabine in this orthotopic xenograft model. CBL0137 also retarded growth of several patient derived pancreatic ductal carcinoma xenografts, the sensitivity of which correlated with level of FACT by immunohistochemistry<sup>19</sup>. Oral CBL0137, significantly extended survival (median survival time from start of treatment = 31.0±9.9 days versus controls = 3.0±0.2 days; P<0.0001), and was as effective as cisplatin or cyclophosphamide single-agent treatment when evaluated in the TH-MYCN transgenic model of neuroblastoma, and the combination of CBL0137 cyclophosphamide/topotecan resulted in more than doubling of lifespan against the BE(2)-C neuroblastoma xenograft model<sup>9</sup>. CBL0137 was selected for testing against the in vitro and in vivo PTP panels because of its novel mechanism of action.

## MATERIALS AND METHODS

### *In vitro* testing

*In vitro* testing was performed using DIMSCAN [20], as previously described in a characterized panel of 24 cell lines<sup>20</sup>. Cells were incubated in the presence of CBL0137 for 96 hours at concentrations from 1.0 nM to 10 µM and analyzed as previously described<sup>21</sup>.

### *In vivo* tumor growth inhibition studies

CB17SC *scid*<sup>-/-</sup> 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<sup>22</sup>. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*<sup>-/-</sup> mice as described previously<sup>23</sup>. 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. Eight to ten mice were used in each control or treatment group. Tumor volumes (cm<sup>3</sup>) [solid tumor xenografts] or percentages of human CD45-positive [%hCD45+] cells [ALL xenografts] were determined and responses were determined using three activity measures as previously described<sup>22</sup>. 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.

## Drugs and Formulation

CBL0137 was provided to the Pediatric Preclinical Testing Program by Cleveland Biolabs Inc., through the Cancer Therapy Evaluation Program (NCI). CBL0137 was formulated in 5% dextrose with a working solution of 10 mg/ml and with dosing at 0.05 ml per 10 gm body weight. CBL0137 was administered by intravenous injection, weekly for 4 consecutive weeks. The total planned treatment and observation period was 6 weeks. Both dose and schedule were proposed by the drug supplier, based upon mouse toxicology studies.

## RESULTS

### In vitro testing

CBL0137 was tested against the PPTP's in vitro cell line panel at concentrations ranging from 1.0 nM to 10.0  $\mu$ M using the PPTP's standard 96 hour exposure period. The median relative IC<sub>50</sub> (rIC<sub>50</sub>) value for the PPTP cell lines was 0.28  $\mu$ M, with a range from 0.13  $\mu$ M to 0.80  $\mu$ M (Table I). The most sensitive cell line, MOLT-4, is an ALL cell line. A metric used to compare the relative responsiveness of the PPTP cell lines to CBL0137 is the ratio of the median rIC<sub>50</sub> of the entire panel to that of each cell line. Higher ratios are indicative of greater sensitivity to CBL0137 and are shown in Figure 1 by bars to the right of the midpoint line.

### In vivo testing

CBL0137 was tested against the PPTP solid tumor xenografts at 50 mg/kg administered by the intravenous route weekly for 4 weeks. Toxicity testing was performed prior to efficacy testing using doses of 90 mg/kg, 68 mg/kg and 50 mg/kg. At 90 mg/kg, weight loss > 10% was observed, as was mortality in 2 of 5 treated animals. The dose selected for efficacy testing was 50 mg/kg, based upon excessive tail necrosis at 68 mg/kg. CBL0137 at 50 mg/kg was generally well tolerated, with a 3.0% mortality rate in the treated groups (12/403), compared to a 0.7% rate for control animals (3/401).

Forty of 42 tested xenograft models were considered evaluable for efficacy. Results from two glioblastoma lines, propagated in athymic nude mice, were excluded from efficacy analysis as toxicity rates exceeded 25%. Complete details of testing are 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.

CBL0137 induced significant differences in EFS distribution compared to control in 10 of 31 (32%) of the solid tumor xenografts evaluable for this measure and in 8 of 8 (100%) of the evaluable ALL xenografts (Table II). BT-41 was not evaluable for differences in EFS distribution because most of the treated and control animals did not experience an event (relative tumor volume > 4) during the treatment/observation period. 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.

CBL0137 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity in 0 of 30 (0%) evaluable solid tumor xenografts. For the ALL panel, 7 of 8 (88%) xenografts met criteria for intermediate activity. Partial responses were observed in ALL B-precursor (ALL-2, ALL-17), T-cell ALL (ALL-8, ALL-31) and a CR was observed in and infant MLL (MLL-7).

No objective responses were observed among the solid tumor xenografts. Six of 31 showed PD2 responses, indicating progressive disease with tumor growth delay greater than 1.5-fold in time to event. Of these six models 5 were wild type for *TP53*. However, 12 other solid tumor models wild type for *TP53* were unresponsive (i.e., PD1 responses) to CBL0137. Figure 2 shows examples of tumor growth curves and Kaplan-Meier EFS curves for solid tumor xenografts with PD2 responses. For the ALL panel, 1 xenograft achieved CR and 4 achieved PR, with Figure 3 showing examples of these responses. For these responding xenograft lines, the human CD45% in peripheral blood was kept at low levels during the treatment period (day 1 to day 22), but increased rapidly after treatment was stopped. Each of the ALL xenograft lines was wildtype for *TP53*. The objective response results for both solid tumor and leukemia models are represented in Figure 4 using a 'COMPARE' format, based on the objective response scoring criteria centered around the midpoint score of 0 that represents stable disease. In the figure, xenografts with PD2 are indicated by a score of -3, and xenografts with regression (PR or CR) are indicated by bars to the right of the midpoint line. Red bars indicate xenografts with significant differences in EFS distribution between the treated and control groups. Figure 4 shows also the objective response activity in a "heat map" format. The relative expression of FACT subunits SSRP1 and SUPT16H for the PPTP in vitro and in vivo panels are shown in Supplemental Figure 1.

## DISCUSSION

Curaxins represent novel anticancer agents that simultaneously activate TP53 and suppress survival pathways such as NF- $\kappa$ B. CBL0137 has been reported to have significant activity against various preclinical models of adult carcinoma, and in the TH-MYC transgenic mouse model of neuroblastoma<sup>9</sup>.

The median relative IC<sub>50</sub> value for the PPTP cell lines was 0.28  $\mu$ M, with a fairly narrow range from 0.13  $\mu$ M to 0.80  $\mu$ M (Table 1). The most sensitive cell line, MOLT-4, is an ALL cell line. There were no significant differences in rIC<sub>50</sub> values by histotype. Of note, the median rIC<sub>50</sub> for the neuroblastoma cell lines exceeded that of the non-neuroblastoma cell lines (0.51  $\mu$ M vs 0.26  $\mu$ M, respectively, p=0.16), while the median rIC<sub>50</sub> for the ALL cell lines was less than that of the non-ALL cell lines (0.20  $\mu$ M vs 0.31  $\mu$ M, respectively, p=0.06). At the highest concentrations used (10  $\mu$ M) CBL0137 was clearly cytotoxic with Ymin values at or approaching -100% for all cell lines.

At present no human pharmacokinetic data are available for CBL0137. However, following a single dose of 30 or 60 mg/kg to CD-1 mice, C<sub>max</sub> levels in plasma reached 6 and 13  $\mu$ M, respectively. At these doses the plasma half-life was 5.62 and 6.47 Hr, respectively (Cleveland Biolabs data, unpublished). Thus, the rIC<sub>50</sub> concentrations determined in vitro are below the C<sub>max</sub> and AUC achieved in mice.

In vivo, CBL0137 was well tolerated at the dose and schedule used. Against solid tumor models the activity of CBL0137 was modest with some delay in tumor growth (PD2) in 6 models, but overall exerted low activity. These data are consistent with results using pancreatic carcinoma xenograft models where tumor growth inhibition varied from approximately 20–60%<sup>19</sup>. Of note, none of five neuroblastoma xenografts responded significantly to CBL0137 treatment in contrast to the TH-MYCN transgenic and BE(2)-C xenograft models previously reported<sup>9</sup>. In the latter report, a higher dose of CBL0137 (60 mg/kg) with more frequent dosing (every 4 days) was employed compared to that used by the PPTP (50 mg/kg every 7 days). CBL0137 demonstrated greater activity against leukemia models with partial responses (PR) measured in 3 B-precursor ALL, 1 T-cell and a complete response (CR) in an infant MLL model. Of note, the responding ALL models had relatively low level expression of both *SSRP1* and *SUPT16H* (that encodes SPT16), considered as markers for sensitivity when expressed at high levels<sup>19</sup>. In contrast, KT-13 (Wilms tumor) expressed the highest level of SUPT16 and had high-level expression of SSRP1, but had a poor response (PD1, T/C EFS = 1.6). Thus, the major activity against these models appears to be in the leukemia panel, although the duration of response was short suggesting that the depth of remission was not deep.

Of importance in considering next steps is how the drug levels in mice at the dose used for testing relate to the systemic exposures to CBL0137 observed in patients. Until human pharmacokinetic data are available for comparison, it is not possible to determine whether the results presented here are likely an under- or over-estimate of the likely efficacy of CBL0137 against pediatric malignancies. Carter et al. reported that CBL0137 enhanced the activity of cytotoxic agents (cyclophosphamide, topotecan) that are used in treatment of neuroblastoma and other solid tumors of childhood<sup>9</sup>. If systemic exposure in mice and humans is reasonably comparable, then further combination studies with standard cytotoxic agents in solid tumor and leukemia models may be valuable in informing pediatric development of CBL0137.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Glossary

<b>PPTP</b>	Pediatric Preclinical Testing Program
<b>EFS T/C</b>	Ratio of Event Free Survival Treated/Control
<b>C<sub>max</sub></b>	Maximum plasma concentration



<b>(NOD)/scid<sup>-/-</sup></b>	Non-Obese diabetic/severe combined immune deficient
<b>FACT</b>	Facilitates Chromatin Transcription
<b>rIC<sub>50</sub></b>	median relative IC <sub>50</sub>
<b>%hCD45+</b>	percentages of human CD45-positive

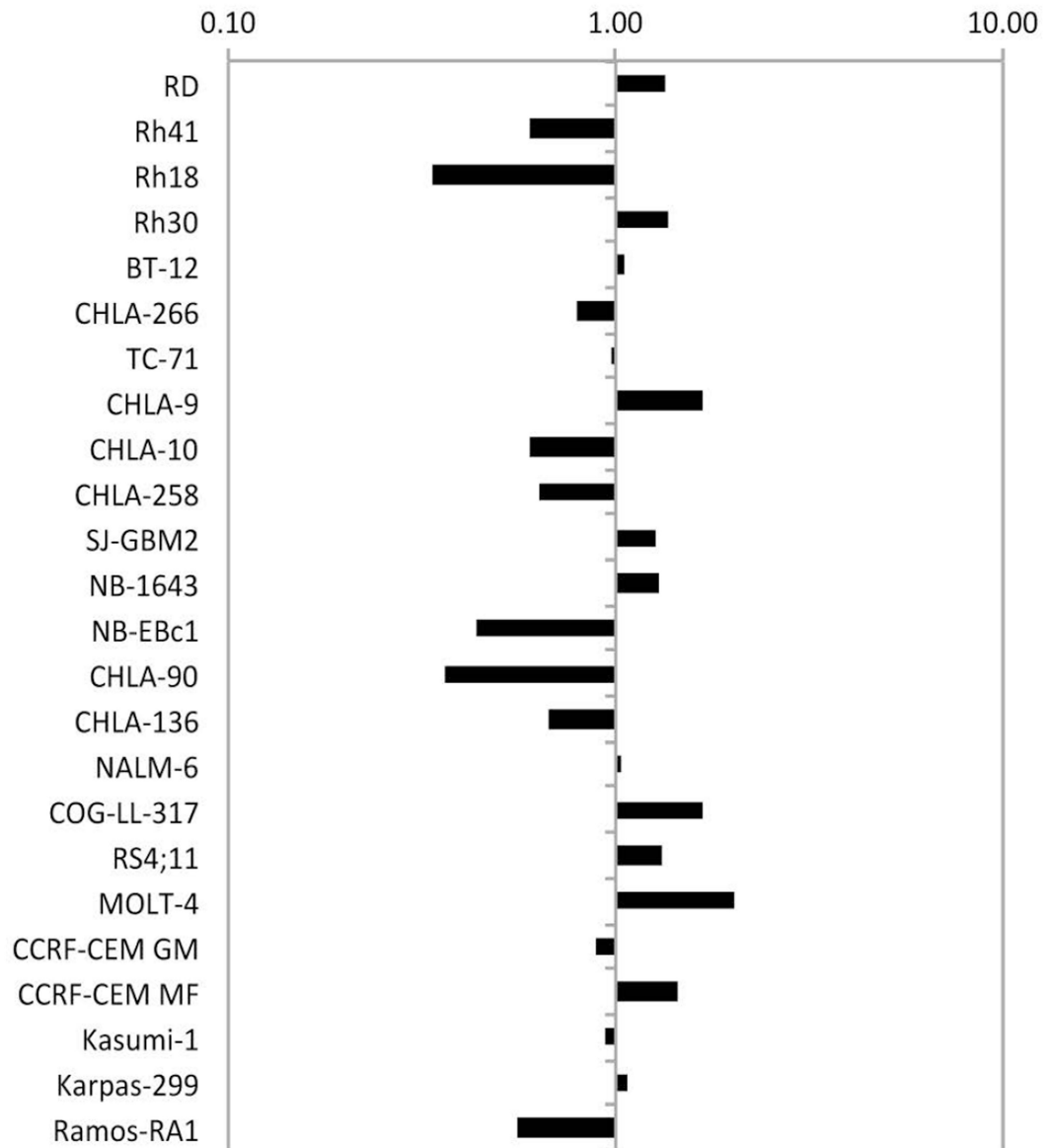
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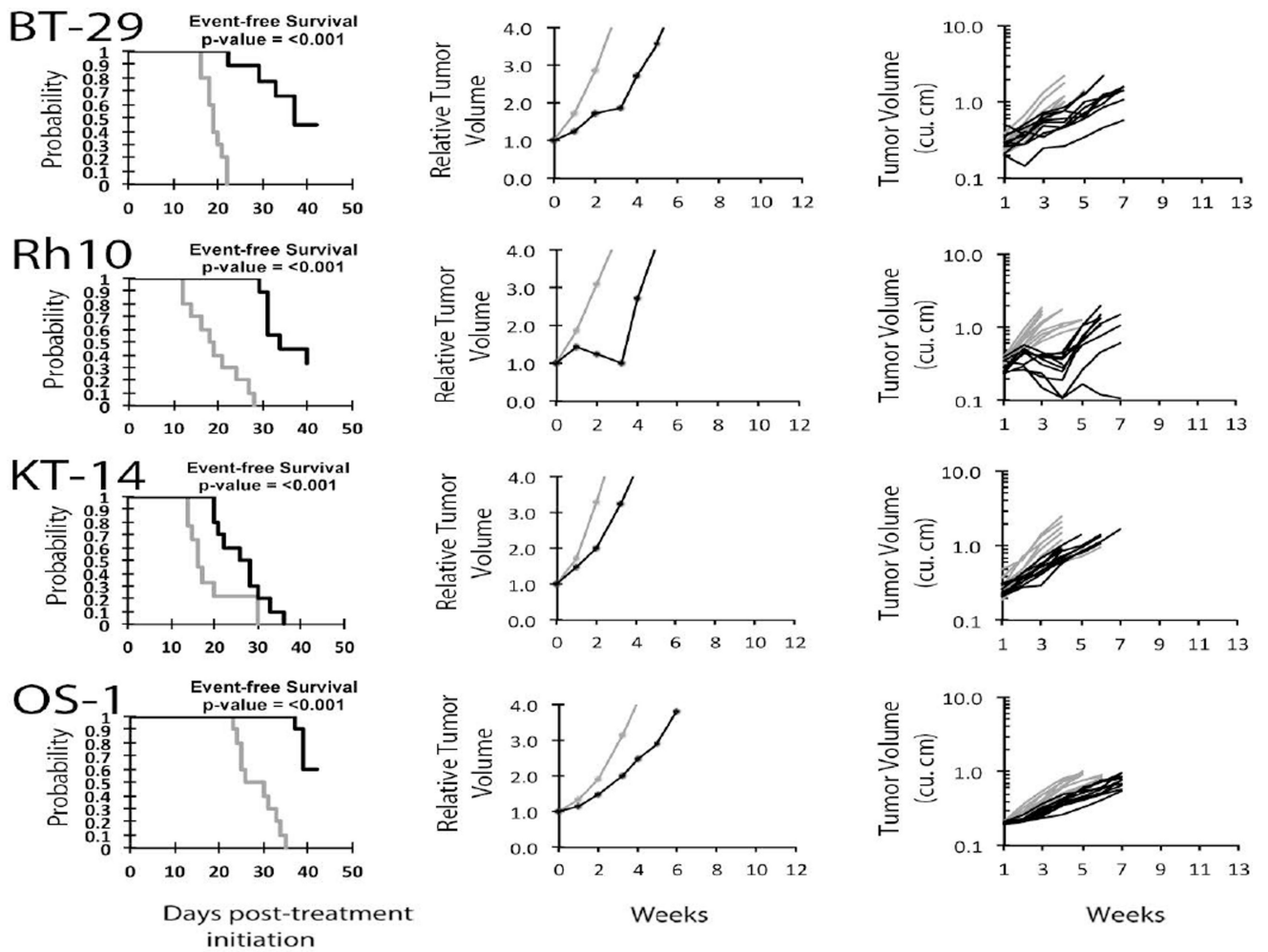
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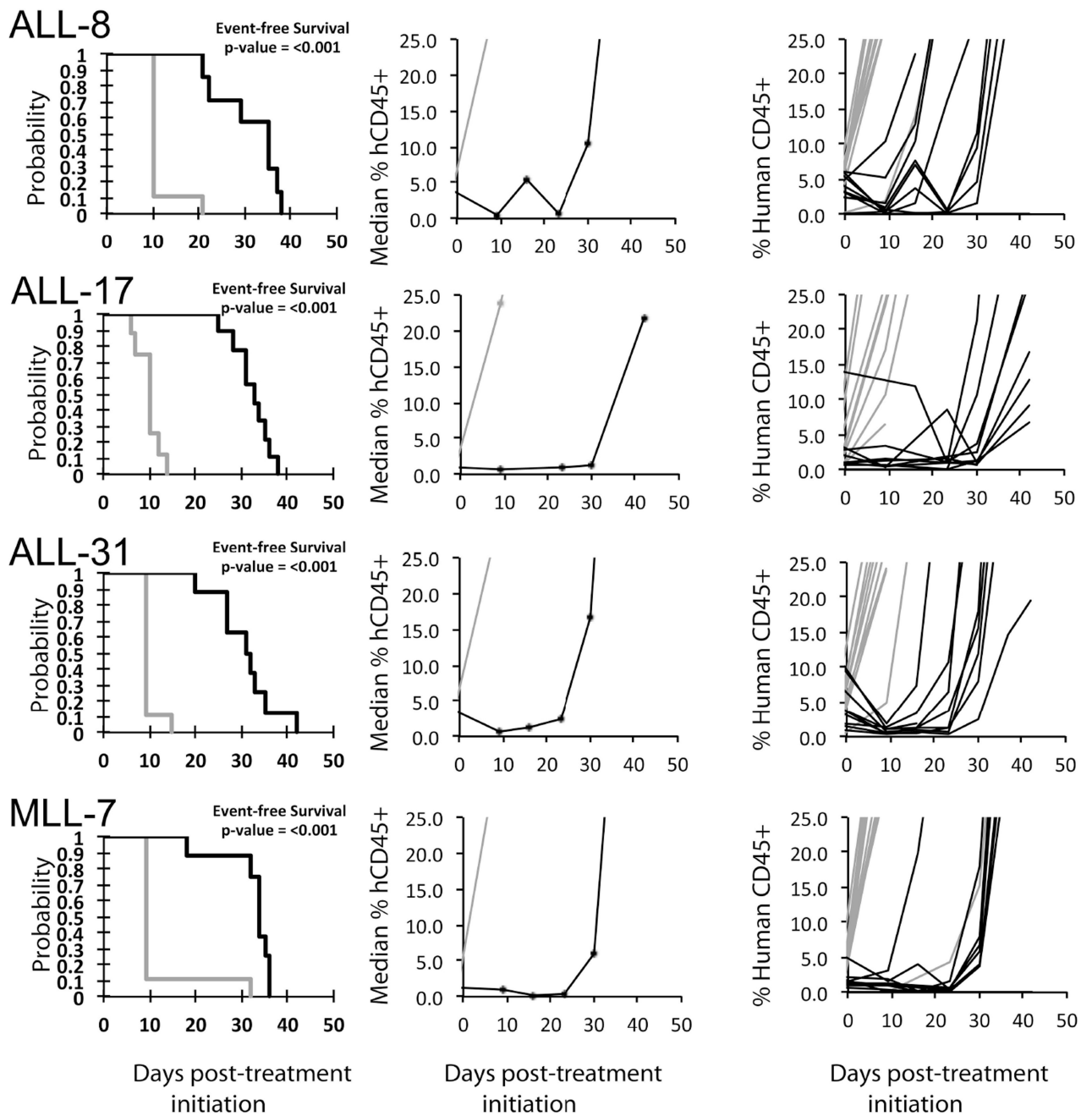
## Panel R\_IC50/ Line R\_IC50



**Figure 1.** CBL0137 *in vitro* activity: The median rIC<sub>50</sub> ratio graph shows the relative rIC<sub>50</sub> values for the cell lines of the PPTP panel. Each bar represents the ratio of the panel rIC<sub>50</sub> to the rIC<sub>50</sub> 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.

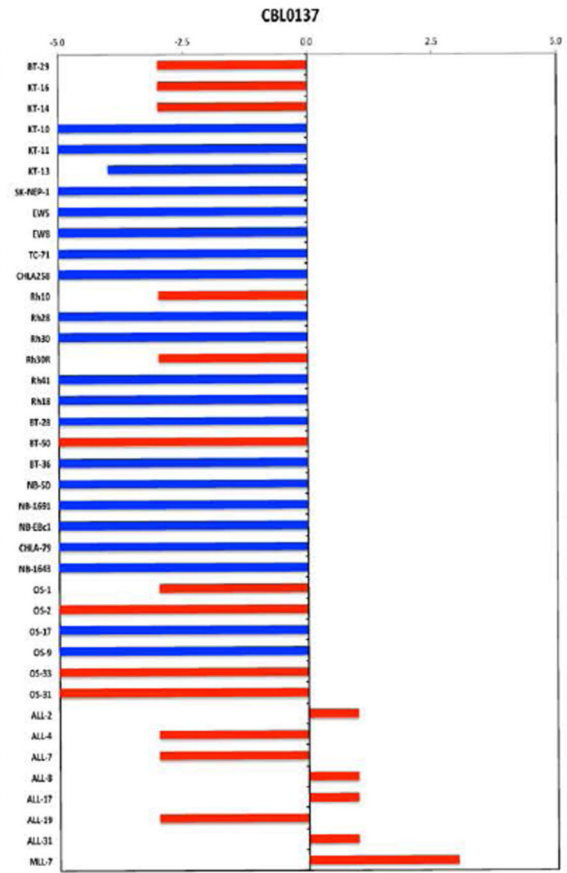


**Figure 2.** CBL0137 *in vivo* objective response activity for more sensitive solid tumors (rhabdoid, BT-28, KT-14), rhabdomyosarcoma (Rh10) and Osteosarcoma (OS-1): Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown for selected lines. Controls (gray lines); Treated (black lines), statistical significance (p values) of the difference between treated and control groups are included.



**Figure 3.** CBL017 *in vivo* objective response activity for leukemia models: Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown for selected lines. Controls (gray lines); Treated (black lines), statistical significance (p values) of the difference between treated and control groups are included.

Xenograft Line	Histology	Log-rank p-value	Median Score	Midpoint Difference	Overall Group Response	Heat Map
BT-29	Rhabdoid	<0.001	2	-3	PD2	
KT-16	Rhabdoid	<0.001	2	-3	PD2	
KT-14	Rhabdoid	0.04	2	-3	PD2	
KT-10	Wilms	0.164	0	-5	PD1	
KT-11	Wilms	0.056	0	-5	PD1	
KT-13	Wilms	0.169	1	-4	PD1	
SK-NEP-1	Ewing	0.13	0	-5	PD1	
EW5	Ewing	0.803	0	-5	PD1	
EW8	Ewing	0.568	0	-5	PD1	
TC-71	Ewing	0.762	0	-5	PD1	
CHLA258	Ewing	0.376	0	-5	PD1	
Rh10	Alveolar RMS	<0.001	2	-3	PD2	
Rh28	Alveolar RMS	0.954	0	-5	PD1	
Rh30	Alveolar RMS	0.101	0	-5	PD1	
Rh30R	Alveolar RMS	<0.001	2	-3	PD2	
Rh41	Alveolar RMS	0.236	0	-5	PD1	
Rh18	Embryonal RMS	0.148	0	-5	PD1	
BT-28	Medulloblastoma	0.093	0	-5	PD1	
BT-50	Medulloblastoma	0.046	0	-5	PD1	
BT-36	Ependymoma	0.01	0	-5	PD1	
NB-SD	Neuroblastoma	0.95	0	-5	PD1	
NB-1891	Neuroblastoma	0.29	0	-5	PD1	
NB-EBc1	Neuroblastoma	0.071	0	-5	PD1	
CHLA-79	Neuroblastoma	0.345	0	-5	PD1	
NB-1843	Neuroblastoma	0.465	0	-5	PD1	
OS-1	Osteosarcoma	<0.001	2	-3	PD2	
OS-2	Osteosarcoma	<0.001	0	-5	PD1	
OS-17	Osteosarcoma	0.196	0	-5	PD1	
OS-9	Osteosarcoma	0.058	0	-5	PD1	
OS-33	Osteosarcoma	0.003	0	-5	PD1	
OS-31	Osteosarcoma	0.045	0	-5	PD1	
ALL-2	ALL B-precursor	<0.001	6	1	PR	
ALL-4	ALL B-precursor	0.002	2	-3	PD2	
ALL-7	ALL B-precursor	<0.001	2	-3	PD2	
ALL-8	ALL T-cell	<0.001	6	1	PR	
ALL-17	ALL B-precursor	<0.001	6	1	PR	
ALL-19	ALL B-precursor	<0.001	2	-3	PD2	
ALL-31	Tertiary T-cell ALL	<0.001	6	1	PR	
MLL-7	ALL MLL (infant)	<0.001	8	3	CR	
Median			0.0			
PD1			PR			
PD2			CR			
SD			MCR			



**Figure 4.**

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. BT-41 is not included because it is not evaluable for the response measure.

**Table 1**

In Vitro Activity of IVT CBL0137 against PPTP Cell Lines

Cell Line	Histotype	rIC <sub>50</sub> (μM)	Panel rIC <sub>50</sub> /Line rIC <sub>50</sub>	T/C Ymin % (Observed)	Relative In/Out (Observed) / Ymin <sup>1</sup>
RD	Rhabdomyosarcoma	0.20	1.33	0.00	-100.0%
Rh41	Rhabdomyosarcoma	0.45	0.60	0.00	-100.0%
Rh18	Rhabdomyosarcoma	0.80	0.34	0.19	-99.6%
Rh30	Rhabdomyosarcoma	0.20	1.35	0.00	-100.0%
BT-12	Rhabdoid	0.26	1.06	0.00	-100.0%
CHLA-266	Rhabdoid	0.34	0.80	0.77	-97.1%
TC-71	Ewing sarcoma	0.28	0.98	0.00	-100.0%
CHLA-9	Ewing sarcoma	0.16	1.69	0.00	-100.0%
CHLA-10	Ewing sarcoma	0.45	0.60	0.00	-100.0%
CHLA-258	Ewing sarcoma	0.43	0.63	0.00	-100.0%
SI-GBM2	Glioblastoma	0.21	1.27	0.01	-99.9%
NB-1643	Neuroblastoma	0.21	1.28	0.01	-100.0%
NB-EBc1	Neuroblastoma	0.61	0.44	0.00	-100.0%
CHLA-90	Neuroblastoma	0.74	0.36	0.02	-99.9%
CHLA-136	Neuroblastoma	0.40	0.67	0.00	-100.0%
NALM-6	ALL	0.26	1.02	0.00	-100.0%
COG-LL-317	ALL	0.16	1.67	0.00	-100.0%
RS4;11	ALL	0.20	1.33	0.17	-98.9%
MOLT-4	ALL	0.13	2.04	0.00	-100.0%
CCRF-CEM (1)	ALL	0.31	0.88	0.00	-100.0%
Kasumi-1	AML	0.29	0.94	0.00	-100.0%
Karpas-299	ALCL	0.25	1.07	0.00	-100.0%
Ramos-RA1	NHL	0.49	0.56	0.00	-100.0%
Median		0.28	1.00	0.00	-100.0%
Minimum		0.13	0.34	0.00	-100.0%

Cell Line	Histotype	rIC <sub>50</sub> ( $\mu$ M)	Panel rIC <sub>50</sub> /Line rIC <sub>50</sub>	T/C Y min % (Observed)	Relative In/Out (Observed Y min)/ Y min
Maximum		0.80	2.04	0.77	-97.1%

The Relative In/Out (I/O)% values compare the relative difference in final cell number compared with the starting cell number for treated cells and for control cells calculated as follows: (Observed Y<sub>min</sub>-Y<sub>0</sub>)/(100-Y<sub>0</sub>) if Observed Y<sub>min</sub>>Y<sub>0</sub>; and (Observed Y<sub>min</sub>-Y<sub>0</sub>)/(Y<sub>0</sub>) if Observed Y<sub>min</sub><Y<sub>0</sub>. Y<sub>0</sub> is an estimate of the starting cell number derived from determinations of the doubling time for each cell line. 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.

Summary of *in Vivo* Activity of CBL0137

Table 2

Line	Tumor Type	Median Time to Event	P-value	EFS T/C <sup>1</sup>	Median RTV/CD45 at End of Study	Tumor Volume T/C	EFS Activity	Median Group Response <sup>2</sup>
BT-29	Rhabdoid	36.7	<0.001	1.9	>	0.43	Low	PD2
KT-14	Rhabdoid	26.9	0.040	1.7	>	0.67	Low	PD2
KT-16	Rhabdoid	13.8	<0.001	1.8	>	0.51	Low	PD2
KT-10	Wilms	16.4	0.164	1.4	>	0.74	Low	PD1
KT-11	Wilms	18.4	0.056	1.2	>	0.59	Low	PD1
KT-13	Wilms	17.3	0.169	1.6	>	0.56	Low	PD1
SK-NEP-1	Ewing	14.6	0.130	1.3	>	0.83	Low	PD1
EW5	Ewing	11.0	0.803	1.1	>	1.10	Low	PD1
EW8	Ewing	12.8	0.568	1.2	>	0.77	Low	PD1
TC-71	Ewing	14.2	0.762	1.1	>	0.82	Low	PD1
CHLA258	Ewing	7.1	0.376	0.7	>	1.00	Low	PD1
Rh10	Alveolar RMS	34.1	<0.001	1.8	>	0.34	Low	PD2
Rh28	Alveolar RMS	25.2	0.954	0.9	>	1.23	Low	PD1
Rh30	Alveolar RMS	17.7	0.101	1.3	>	0.76	Low	PD1
Rh30R	Alveolar RMS	20.7	<0.001	1.6	>	0.48	Low	PD2
Rh41	Alveolar RMS	12.9	0.236	1.1	>	0.83	Low	PD1
Rh18	Embryonal RMS	22.7	0.148	1.1	>	0.90	Low	PD1
BT-28	Medulloblastoma	18.5	0.093	1.4	>	0.67	Low	PD1
BT-50	Medulloblastoma	36.9	0.046	1.2	>	0.86	Low	PD1
BT-36	Ependymoma	25.1	0.010	0.7	>	1.37	Low	PD1
BT-41	Ependymoma	> EP	NE	.	2.5	0.86	NE	PD2
NB-SD	Neuroblastoma	8.2	0.950	0.9	>	1.03	Low	PD1
NB-1691	Neuroblastoma	5.6	0.290	0.9	>	1.13	Low	PD1
NB-EBC1	Neuroblastoma	5.9	0.071	1.2	>	0.74	Low	PD1
CHLA-79	Neuroblastoma	9.7	0.345	1.1	>	0.86	Low	PD1



Line	Tumor Type	Median Time to Event	P-value	EFS T/C <sup>1</sup>	Median RTV/CD45 at End of Study	Tumor Volume T/C	EFS Activity	Median Group Response <sup>2</sup>
NB-1643	Neuroblastoma	6.4	0.465	1.0	>4	0.96	Low	PD1
OS-1	Osteosarcoma	> EP	<0.001	> 1.5	3.8	0.73	NE	PD2
OS-2	Osteosarcoma	32.1	<0.001	1.4	>4	0.72	Low	PD1
OS-17	Osteosarcoma	16.9	0.196	1.2	>4	0.86	Low	PD1
OS-9	Osteosarcoma	28.4	0.058	1.3	>4	0.77	Low	PD1
OS-33	Osteosarcoma	31.7	0.003	1.4	>4	0.75	Low	PD1
OS-31	Osteosarcoma	21.3	0.045	1.2	>4	0.84	Low	PD1
ALL-2	ALL B-precursor	39.9	<0.001	2.4	>25	.	Int	PR
ALL-4	ALL B-precursor	15.3	0.002	3.3	>25	.	Int	PD2
ALL-7	ALL B-precursor	13.2	<0.001	1.7	>25	.	Low	PD2
ALL-8	ALL T-cell	34.6	<0.001	3.5	>25	.	Int	PR
ALL-17	ALL B-precursor	32.9	<0.001	3.2	>25	.	Int	PR
ALL-19	ALL B-precursor	15.1	<0.001	2.4	>25	.	Int	PD2
ALL-31	ALL T-cell	31.9	<0.001	3.5	>25	.	Int	PR
MLL-7 <sup>3</sup>	ALL Infant MLL	34.2	<0.001	3.8	>25	.	Int	CR

<sup>1</sup> EFS T/C value is 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. High activity requires: a) an EFS T/C > 2; b) a significant difference in EFS distributions, 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. Intermediate activity demonstrates criteria a) and b) above, but not having a net reduction in median tumor volume for treated animals at the end of the study. Low activity demonstrates EFS T/C < 2.

<sup>2</sup> Objective response measures are described in detail in the Supplemental Response Definitions. PD1 = progressive disease with EFS T/C 1.5, and PD2 = progressive disease with EFS T/C > 1.5.

<sup>3</sup> *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 T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. High activity = T/C 0.15; Intermediate activity = T/C 0.45 but > 0.15; and Low activity = T/C > 0.45.

<sup>3</sup> KMT2A (MLL) rearranged B-ALL.