Abstract C103

Pediatric Preclinical Testing Program (PPTP) Stage 1 evaluation of the p53-MDM2 antagonist RG7112: Early evidence for high activity against MLL-rearranged leukemias

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RG7112

- Tumor suppressor p53 is a pro-apoptotic molecule frequently inactivated in cancer by gene mutations or defective signaling.
- Mutated p53 is uncommon in many childhood cancers and hence agents able to free p53 from inhibitory mechanisms may have therapeutic benefit in the pediatric setting.
- 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 is a member of the Nutlin family of MDM2 antagonists that has improved potency and pharmacological properties. It is currently in clinical evaluation in adults with leukemias and selected solid tumors.

Methods

In vitro: ***In vitro*** testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005). RG7112 and its inactive enantiomer RG7112i were evaluated against the PPTP *in vitro* panel using 96 hour exposure at concentrations from 1 nM to 10 μM. Relative IC_{50} (rIC_{50}) values were used as a measure of the potency of RG7112 and RG7112i against the PPTP cell lines.

In vivo: Standard PPTP methods for *in vivo* testing were employed (see http://pptp.nchresearch.org/documents.html). RG7112 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 observation.

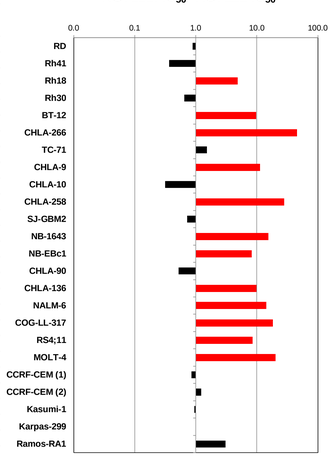
Solid tumor testing: For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm^3. Two perpendicular tumor diameters were measured at either once or twice weekly intervals with digital vernier calipers.

Acute lymphoblastic leukemia (ALL) testing: For each xenograft line, 8 mice were inoculated with 3-5 x 10^6 mononuclear cells purified from the spleens of secondary recipient mice. Engraftment was monitored weekly by flow cytometry, and treatment was initiated when the proportion of human CD45+ cells in the peripheral blood reached 1%. The proportion of human CD45+ cells in the peripheral blood was monitored weekly throughout the course of treatment.

Gene expression: Expression data for p53 and MDM2 are from Affymetrix U133 Plus 2.0 arrays. Molecular characterization of the PPTP models is described in Neale, et al. (Clin Cancer Res 2008;14:4572-83)

RG7112 *In Vitro* Activity

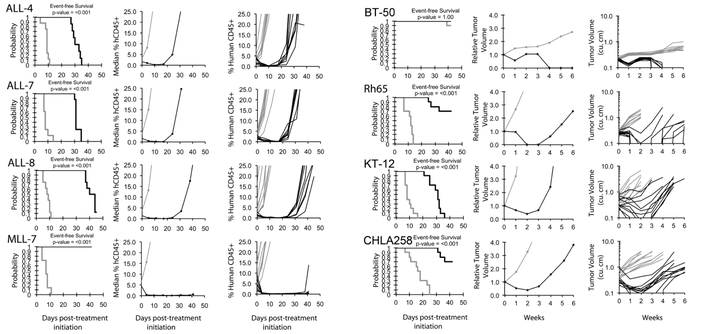
Cell Line	p53 Status	RG7112 IC_{50} (μM)	RG7112i IC_{50} (μM)	Ratio IC_{50}
RD	Mutant	>10	8.93	0.9
Rh41	Mutant	>10	3.7	0.4
Rh18	**WT**	**0.47**	**2.25**	**4.8**
Rh30	Mutant	>10	6.54	0.7
BT-12	**WT**	**0.99**	**9.66**	**9.8**
CHLA-266	**WT**	**0.05**	**2.2**	**45.6**
TC-71	Mutant	2.75	4.18	1.5
CHLA-9	**WT**	**0.90**	**>10**	**11.3**
CHLA-10	Mutant	>10	3.2	0.3
CHLA-258	**WT**	**0.36**	**>10**	**28.0**
SJ-GBM2	Mutant	4.92	3.56	0.7
NB-1643	**WT**	**0.65**	**>10**	**15.5**
NB-EBc1	**WT**	**0.11**	**0.94**	**8.2**
CHLA-90	Mutant	>10	5.27	0.5
CHLA-136	**WT**	**0.71**	**6.94**	**9.8**
NALM-6	**WT**	**0.14**	**2**	**14.2**
COG-LL-317	**WT**	**0.41**	**7.5**	**18.2**
RS4;11	**WT**	**0.53**	**4.48**	**8.5**
MOLT-4	**WT**	**0.08**	**1.58**	**20.2**
CCRF-CEM	Mutant	>10	8.6	0.9
CCRF-CEM	Mutant	8.27	>10	1.2
Kasumi-1	Mutant	>10	9.57	0.9
Karpas-299	Mutant	>10	>10	1.0
Ramos-RA1	Mutant	0.99	3.04	3.1
Median		0.99	5.92	3.9
Minimum		0.05	0.94	0.3
Maximum		>10	>10	45.6



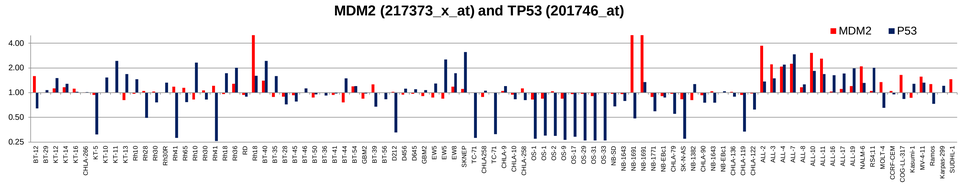
- The median rIC_{50} for RG7112 was ~0.4 μM for p53 wild-type (WT) cell lines versus >10 μM for p53 mutant cell lines.
- p53 WT cell lines (red bars in figure) were approximately 13-fold more sensitive to RG7112 compared to its inactive enantiomer RG7112i.
- p53 mutant cell lines (black bars) showed similar sensitivity to RG7112 and RG7112i.

RG7112 *in Vivo* Activity

Line	Tumor Type	Median Time to Event	P-value	EFS T/C	RTV/CD45 at End of Study	Tumor Volume T/C	Response
BT-29	Rhabdoid	> EP	**<0.001**	**> 2.0**	1.0	0.31	SD
KT-14	Rhabdoid	> EP	**0.003**	**> 2.5**	4.0	0.19	PD2
KT-12	Rhabdoid	31.0	**<0.001**	**3.8**	>4	0.26	PR
KT-10	Wilms	34.0	**<0.001**	**2.5**	>4	0.08	PR
EW5	Ewing	7.7	0.622	0.7	>4	1.08	PD1
CHLA258	Ewing	> EP	**<0.001**	**> 2.5**	3.8	0.09	PR
ES-4	Ewing	19.3	**0.001**	**2.1**	>4	0.34	PD2
ES-6	Ewing	30.9	**<0.001**	1.4	>4	0.67	PD1
Rh10	ALV RMS	31.1	0.485	.	>4	0.41	PD1
Rh28	ALV RMS	38.4	**<0.001**	**2.6**	>4	0.13	PD2
Rh30	ALV RMS	32.4	0.103	1.3	>4	0.28	PD1
Rh30R	ALV RMS	18.1	0.059	0.9	>4	1.59	PD1
Rh65	ALV RMS	> EP	**<0.001**	**> 3.4**	2.5	0.03	CR
Rh18	EMB RMS	16.8	**0.001**	1.5	>4	0.57	PD1
Rh36	EMB RMS	27.8	**<0.001**	**2.2**	>4	0.2	PD2
BT-50	Medulloblastoma	> EP	1.000	.	0.0	0.66	MCR
D456	Glioblastoma	10.3	0.865	1.2	>4	0.96	PD1
BT-56	Glioblastoma	25.4	**<0.001**	**2.3**	>4	0.4	PD2
NB-1691	Neuroblastoma	8.7	0.359	1.2	>4	0.9	PD1
NB-EBc1	Neuroblastoma	5.4	**0.003**	1.4	>4	0.54	PD1
CHLA-79	Neuroblastoma	13.7	**0.017**	1.6	>4	0.71	PD2
NB-1643	Neuroblastoma	13.2	0.164	1.3	>4	0.84	PD1
NB-1382	Neuroblastoma	24.6	**0.008**	1.9	>4	0.65	PD2
OS-1	Osteosarcoma	26.8	0.234	1.0	>4	0.89	PD1
OS-2	Osteosarcoma	19.5	0.166	1.0	>4	0.93	PD1
OS-17	Osteosarcoma	16.6	0.373	1.1	>4	0.92	PD1
OS-9	Osteosarcoma	26.4	0.278	1.1	>4	0.97	PD1
OS-31	Osteosarcoma	24.4	0.129	1.1	>4	0.91	PD1
OS-29	Osteosarcoma	29.2	0.859	1.0	>4	0.88	PD1
ALL-2	ALL B-precursor	23.7	0.188	1.7	>25	.	SD
ALL-4	ALL B-precursor	30.1	**<0.001**	3.3	>25	.	CR
ALL-7	ALL B-precursor	31.1	**<0.001**	3.9	>25	.	CR
ALL-8	ALL T-cell	41.7	**<0.001**	5.1	>25	.	CR
ALL-17	ALL B-precursor	33.4	**0.002**	3.8	>25	.	CR
ALL-19	ALL B-precursor	22.3	0.170	2.3	>25	.	PR
ALL-31	ALL T-cell	33.0	**<0.001**	7.0	>25	.	CR
MLL-7	ALL (MLL, Infant)	> EP	**<0.001**	> 5.6	0.7	.	MCR
MLL-2	ALL (MLL, Infant)	> EP	**<0.001**	> 4.3	24.5	.	CR
MLL-3	ALL (MLL, Infant)	32.2	**<0.001**	3.1	>25	.	CR
MLL-5	ALL (MLL, Infant)	23.3	**<0.001**	2.9	>25	.	CR
MLL-6	ALL (MLL, Infant)	34.8	**<0.001**	7.4	>25	.	CR
MLL-8	ALL (MLL, Infant)	33.4	**0.001**	4.8	>25	.	CR
MLL-14	ALL (MLL, Infant)	33.6	**<0.001**	4.8	>25	.	CR
RS4;11	ALL (MLL, adult)	22.8	**<0.001**	1.9	>4	0.24	PD2
MV4;11	Biphenotypic (MLL)	> EP	**<0.001**	> 2.9	0.0	0.01	MCR



MDM2 and p53 Expression for PPTP Models



- MDM2 and p53 expression (relative to the panel median for each) are shown in the figure.
- The osteosarcoma xenografts were p53 WT, but had very low p53 expression and low MDM2 expression. They did not respond to RG7112. *in vivo*.
- The ALL xenografts expressed the highest levels of p53 and MDM2 among the PPTP panels and showed the most consistent *in vivo* responses to RG7112.
- Two PPTP xenografts have MDM2 amplification, Rh18 and NB-1691, and both showed high MDM2 expression. Neither responded to RG7112.

In Vivo Results Summary &Conclusions

- RG7112 was well tolerated *in vivo* at a dose of 100 mg/kg daily for 14 days.
- RG7112 induced significant differences in EFS (event-free survival) distribution compared to control in 15 of 27 (56%) evaluable p53 WT solid tumor xenografts.
- RG7112 induced a two-fold or greater delay in time to event (EFS T/C > 2) in 10 of 25 (40%) p53 WT solid tumor xenografts, including: 2/2 rhabdoid tumor, 2/2 Wilms tumor, 2/3 Ewing, and 3/6 rhabdomyosarcoma models.
- No neuroblastoma (n=5) or osteosarcoma (n=6) models showed EFS T/C > 2, and the two solid tumor xenografts with mutant p53 (Rh30R and EW5) showed no response to RG7112 as expected.
- 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 (PR) for a Wilms tumor, rhabdoid tumor, and Ewing tumor xenograft.
- For the ALL panel, among 13 xenografts there were 11 CR, 1 MCR and 1 PR.
- Each of the 7 ALL xenografts with MLL rearrangement was highly responsive to RG7112 with 6 CR and 1 MCR.
- Two additional MLL-rearranged xenografts (MV4;11 and RS4;11) grown subcutaneously were also tested, with the former showing MCR and the latter showing tumor growth delay (PD2).
- The consistent high level activity of RG7112 against ALL models, particularly those with MLL-rearrangement, supports prioritization of RG7112 for evaluation in the acute leukemia setting. Preclinical evaluations of RG7112 with standard agents are planned for both solid tumor and ALL models.

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