Abstract #3575

Pediatric Preclinical Testing Program (PPTP) efficacy and pharmacodynamic evaluation of the Hsp90 inhibitor 17-DMAG

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Abstract

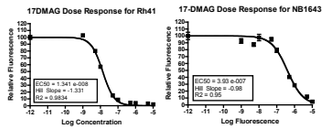
Background: 17-DMAG is a potent small-molecule inhibitor of the protein chaperone HSP90 that is being developed as an anticancer agent because of the multiple HSP90 client proteins that are involved in cancer cell growth and survival.
Methods: The PPTP includes an *in vitro* panel (n=27) as well as panels of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. 17-DMAG was tested against the *in vitro* panel at concentrations from 1 nM to 10 microM and was tested against the *in vivo* tumor panels by i.p. administration using a 50 mg/kg BID twice weekly x 6 weeks dose and schedule. The PPTP's 3 measures of antitumor activity were applied (Houghton et al. Ped Blood Cancer 2006): 1) an objective response measure; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (EFS T/C) measure. HSP70 induction was used as a pharmacodynamic measure of HSP90 inhibition and was determined in tumor and liver tissue at 8 and 24 hours following the second of two doses of 17-DMAG (50 mg/kg i.p.) administered at 12 hour intervals.
Results: 17-DMAG had an EC50 of 62 nM against the PPTP's *in vitro* panel, with a trend for lower EC50 values for the rhabdomyosarcoma panel (median EC50 31 nM) compared to the remaining PPTP *in vitro* cell lines (p=0.06) and for higher EC50 values for the neuroblastoma lines (median EC50 396 nM, p=0.01). 17-DMAG induced significant differences in EFS distribution in 15 of 30 of the solid tumor xenografts, and in 4 of 6 of the evaluable ALL xenografts. Using the time to event activity measure, 17-DMAG had intermediate or high activity against 4 of 28 evaluable solid tumor xenografts (1 of 2 rhabdoid tumor and 3 of 4 alveolar rhabdomyosarcoma). The only objective response observed (PR) was for an alveolar rhabdomyosarcoma xenograft. HSP70 induction was observed in both liver and tumor tissue, with robust induction (up to 450% increase versus control) occurring in both responding and non-responding xenografts.
Conclusions: 17-DMAG produced its greatest antitumor activity against alveolar rhabdomyosarcoma xenografts. Robust HSP70 induction was observed in both responding and non-responding xenografts, suggesting that tumor-specific downstream effects of HSP90 inhibition are primary determinants of response.

PPTP *In Vitro* Testing Methods

Methods: *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 (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005). Testing was for 96 hours at concentrations from 1.0 nM to 10.0 μM with replicates of 6 per data point. Data were analyzed using Prism (GraphPad), fitting a non-linear regression, sigmoidal dose-response model to the response, relative fluorescence values *vs.* the concentration. The PPTP in vitro panel contains cell lines for neuroblastoma (4), Ewing sarcoma (4), rhabdomyosarcoma (4), ALL (5), NHL (2), and others.

17-DMAG *In Vitro* Activity

- 17-DMAG was uniformly active against the cell lines of the PPTP *in vitro* panel, with a median EC_{50} of 60 nM.
- EC_{50} values ranged from 11 and 13 nM for two rhabdomyosarcoma cell lines (Rh41 and RD) to 372 and 439 nM for two neuroblastoma cell lines (NB-1643 and NB-EBc1), as illustrated below.



- The median EC_{50} values for the 4 rhabdomyosarcoma cell lines were lower than the median EC_{50} values for the remaining lines in the panel (29 nM versus 93 nM, p=.055), whereas the median EC_{50} values for the 4 neuroblastoma cell lines were higher than the remaining lines in the panel (367 nM versus 45 nM, p=.01).
- The maximal inhibition values for 17-DMAG exceeded 90% for all of the PPTP's cell lines, with a median maximal inhibition of 97%.
- 17-DMAG activity data for the entire *in vitro* panel are shown below (yellow shaded rows indicate cell lines with EC_{50} values < the panel median EC_{50}):

Line	Histology	EC_{50} (nM)	Maximal Inhibition (%)	Panel EC_{50}/ Line EC_{50}
RD	Rhabdomyosarcoma	13	94	4.60
Rh41	Rhabdomyosarcoma	11	96	5.27
Rh18	Rhabdomyosarcoma	45	97	1.34
Rh30	Rhabdomyosarcoma	46	95	1.33
BT-12	Rhabdoid	33	95	1.86
CHLA-266	Rhabdoid	150	98	0.40
TC-71	Ewing	89	99	0.68
CHLA-9	Ewing	254	97	0.24
CHLA-10	Ewing	140	93	0.43
CHLA-258	Ewing	13	100	4.77
GBM2	GBM	29	92	2.11
NB-1643	Neuroblastoma	372	93	0.16
NB-EBc1	Neuroblastoma	439	96	0.14
CHLA-90	Neuroblastoma	96	93	0.63
CHLA-136	Neuroblastoma	361	100	0.17
NALM-6	ALL	41	100	1.46
RS4;11	ALL	63	95	0.95
MOLT-4	ALL	58	100	1.05
CCRF-CEM	ALL	231	99	0.26
KASUMI-1	AML	18	99	3.42
KARPAS-299	NHL	38	99	1.60
RAMOS-RA1	NHL	156	100	0.39
MEDIAN		60	97	1.00
MINIMUM		11	100	0.14
MAXIMUM		439	92	5.27

Methods for PPTP *In Vivo* Testing

Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD or at a dose selected based on PK/PD studies using adult preclinical models.
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 once weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula $(\pi/6)\times d^3$, where d represents the mean diameter.
Acute lymphoblastic leukemia 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.
Drug: 17-DMAG was provided to the PPTP by Kosan Biosciences through the Cancer Therapy Evaluation Program (NCI). 17-DMAG was dissolved in a sodium citrate/citric acid buffer at pH 3.2 and administered IP using a 50 mg/kg BID dose in the solid tumor models and 25mg/kg BID dose in the ALL tumor models, on a twice weekly X 6 schedule. 17-DMAG was provided to each PPTP testing site in coded vials for blinded testing, according to the PPTP program standard operating procedures .

Solid Tumor Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	>25% ↑ in tumor volume, TGD value ≤1.5	0
PD2 (Progressive Disease 2)	>25% ↑ in tumor volume, TGD value >1.5	2
SD (Stable Disease)	<25% ↑ in tumor volume, <50% regression	4
PR (Partial Response)	≥50% regression, but no CR	6
CR (Complete Response)	<0.1 cm^3 tumor volume	8
MCR (Maintained CR)	<0.1 cm^3 tumor volume at the end of study	10

Leukemia Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	No PR & TGD value of ≤1.5 & events at EOS	0
PD2 (Progressive Disease 2)	No PR & TGD value >1.5 & events at EOS	2
SD (Stable Disease)	No PR and no events at EOS	4
PR (Partial Response)	CD45% <1% for only 1 week	6
CR (Complete Response)	CD45% <1% for 2 consecutive weeks	8
MCR (Maintained CR)	CD45% <1% for last 3 weeks of study	10

Median Group Response: Each individual mouse in the treatment group was assigned a response score (see Tables above) and a Median Score (MS) for the treatment group was calculated. Then each treatment group was assigned an overall response as follows: PD1, MS ≤ 1; PD2, 1 < MS ≤ 3; SD, 3 < MS ≤ 5; PR, 5 < MS ≤ 7; CR, 7 < MS ≤ 9, and MCR, 9 < MS.

Time to Event (EFS T/C Activity Measure): *High activity* required all 3 of the criteria below to be met, and *Intermediate activity* required meeting the first two criteria.
a) EFS T/C > 2; and
b) p≤0.050 for EFS distributions; and
c) final median RTV < 1 for treated animals

Statistical Methods: Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant.

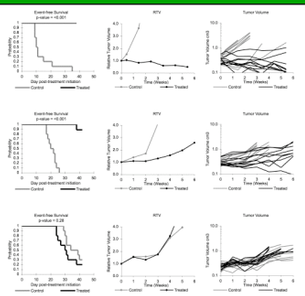
17-DMAG *In Vivo* Activity

- 17-DMAG induced significant differences in EFS distribution (treated compared to controls) in 15/30 (50%) of the solid tumor models and 5/6 (83%) of the ALL models .
- Nine of 34 evaluable xenografts met criteria for intermediate or high activity for the EFS T/C time to event activity measure: 3 of 4 alveolar rhabdomyosarcoma xenografts, 1 of 2 rhabdoid tumor xenografts, and 5 of 6 ALL xenografts.
- The single objective response among the solid tumor xenografts was a partial response for the alveolar rhabdomyosarcoma xenograft Rh30.
- No objective responses were observed in the ALL panel.

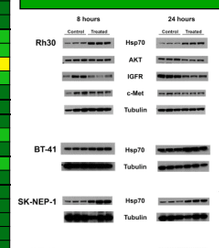
Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	Objective Response
BT-29	Rhabdoid	<0.001	> 2.2	3.4	0.47	PD2
KT-14	Rhabdoid	0.345	0.8	>4	1.1	PD1
KT-10	Wilms	0.601	0.9	>4	1.22	PD1
KT-13	Wilms	0.016	1.6	>4	0.61	PD1
SK-NEP-1	Ewing	0.065	1.4	>4	0.59	PD1
EW5	Ewing	0.021	1.3	>4	0.64	PD1
EW8	Ewing	0.706	1.1	>4	0.71	PD1
Rh10	ALV RMS	<0.001	> 2.0	2.6	0.33	PD2
Rh28	ALV RMS	0.005	2	>4	0.47	PD2
Rh30	ALV RMS	<0.001	> 3.6	0.5	0.31	PR
Rh41	ALV RMS	<0.001	3.4	>4	0.38	PD2
Rh18	EMB RMS	0.445	0.8	>4	1.21	PD1
BT-45	Medulloblastoma	0.015	1.1	>4	0.86	PD1
BT-36	Ependymoma	0.28	0.8	>4	0.98	PD1
BT-41	Ependymoma	0.361	> 1.4	2.3	1.06	PD2
BT-44	Ependymoma	0.303	0.9	>4	1.24	PD1
GBM2	Glioblastoma	<0.001	1.7	>4	0.58	PD2
BT-39	Glioblastoma	0.172	1	>4	0.71	PD1
NB-1771	Neuroblastoma	0.044	1.4	>4	0.72	PD1
NB-1691	Neuroblastoma	0.782	1	>4	0.91	PD1
NB-EBc1	Neuroblastoma	0.018	1.1	>4	0.82	PD1
CHLA-79	Neuroblastoma	0.296	1	>4	0.96	PD1
NB-1643	Neuroblastoma	0.001	1.4	>4	0.57	PD1
SK-N-AS	Neuroblastoma	<0.001	1.6	>4	0.62	PD2
OS-1	Osteosarcoma	<0.001	1.5	>4	0.48	PD2
OS-2	Osteosarcoma	0.788	1.2	>4	0.74	PD1
OS-17	Osteosarcoma	0.197	1.1	>4	0.81	PD1
OS-9	Osteosarcoma	0.002	> 1.4	3.5	0.8	PD2
OS-33	Osteosarcoma	0.054	0.8	>4	1.22	PD1
OS-31	Osteosarcoma	0.166	0.7	>4	0.93	PD1
ALL-2	ALL B-precursor	0.026	2.9	>25	.	SD
ALL-3	ALL B-precursor	0.004	2.7	>25	.	PD2
ALL-4	ALL B-precursor	0.069	4.3	>25	.	PD2
ALL-8	ALL T-cell	<0.001	> 4.8	17.9	.	SD
ALL-17	ALL B-precursor	0.028	3.8	>25	.	PD2
ALL-19	ALL B-precursor	0.005	2.4	>25	.	PD2

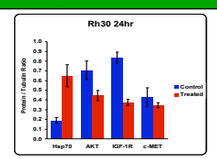
Red shading in the p-value column indicates a significant difference in EFS distribution between treated and control groups.
Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (gray) activity.

17-DMAG *In Vivo* Activity Examples



17-DMAG Pharmacodynamic Effects





- Protein levels were determined by western blot analysis of tumor tissue at 8 and 24 hours following the second of two doses of 17-DMAG (50 mg/kg i.p.) administered at 12 hour intervals.
- Hsp70 induction was 3.4-fold for Rh30, versus 1.5-, 3.1-, & 3.2-fold for 3 non-responsive xenografts.
- 17-DMAG treatment reduced levels of AKT and IGF-1R in the responsive xenograft Rh30.

CONCLUSIONS

- 17-DMAG potently inhibited growth of the cell lines of the PPTP's *in vitro* panel, with the rhabdomyosarcoma cell lines having lower EC_{50} values compared to the remaining cell lines of the panel.
- 17-DMAG treatment produced a partial response in an alveolar rhabdomyosarcoma xenograft, and it produced 2-fold differences in time to event (EFS T/C > 2) in an additional 8 xenografts.
- 17-DMAG induced increased levels of Hsp70 in both the responsive xenograft Rh30 and in non-responding xenografts.
- Despite demonstrating pharmacodynamic effects indicative of Hsp90 inhibition, 17-DMAG showed limited antitumor activity against the PPTP's *in vivo* childhood cancer preclinical models.

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