#4350 Evaluation of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG, KOS-1022) against Childhood Cancer Models by the Pediatric Preclinical Testing Program (PPTP)

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Abstract

17-DMAG is a water-soluble analog of 17-allylamino-17-demethoxygeldanamycin (17-AAG), an agent currently in pediatric phase 1 trials. The geldanamycins inhibit Hsp90, a chaperone that is involved in the folding, conformational activation, and assembly of proteins. In the presence of Hsp90 inhibitors, Hsp90 client proteins [e.g., transmembrane tyrosine kinases (e.g., HER2, EGFR, IGF-IR), intermediary signaling kinases (e.g., AKT and Raf), and chimeric signaling proteins (NPM-ALK and Bcr-Abl)] are unable to undergo conformational maturation and instead are targeted for proteasomal degradation.

The PPTP was established by NCI to identify novel agents that have significant activity against preclinical models of childhood cancers. The PPTP is based upon prior experience showing that preclinical models can identify drugs known to be active against their respective clinical diseases and can prospectively identify agents that are subsequently shown to have significant activity in phase 1 and 2 clinical trials in children with cancer. The PPTP includes an *in vitro* panel as well as panels of xenograft models representing kidney tumors/rhabdoid tumors (8), sarcoma (10), non-glioblastoma brain tumors (8), glioblastoma (GBM) (6), neuroblastoma (8), osteosarcoma (8) and acute lymphocytic leukemia (ALL) (10). Tumors were selected based upon their growth characteristics and upon gene expression profiles similar to their respective clinical counterparts. Models have also been selected to represent tumors and histologies that have poor prognosis (e.g., rhabdoid tumors, GBM). To 'calibrate' the system, standard agents such as vincristine and cyclophosphamide have been evaluated, with the result that the activity patterns observed for the PPTP panels have generally matched the clinical spectrum of activity of these agents.

17-DMAG was tested against the PPTP *in vivo* tumor panels at 50 mg/kg IP, twice daily 2x per week for 6 weeks. Responses by tumor panel were as follows: Wilms – 2 Progressive Disease (PD); Rhabdoid tumor – 2PD; Rhabdomyosarcoma – 1 partial response (PR) & 5PD; Ewing sarcoma – 3PD; Medulloblastoma – 1PD; Glioblastoma - 4PD; Neuroblastoma - 6PD; Osteosarcoma 2PD; and ALL - 8PD. Evidence for antitumor activity was observed in two slow-growing ependymoma lines, with tumor regressions meeting criteria for CR observed in each.

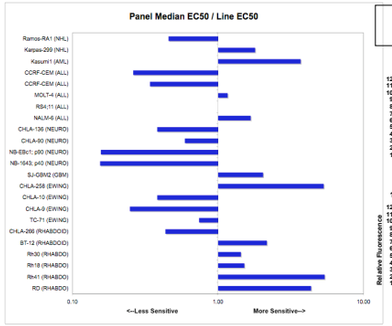
Conclusions: Based on tumor regressions observed for 17-DMAG against two ependymoma lines, further evaluation is warranted for this diagnosis (e.g., confirmation of activity in an expanded ependymoma panel and evaluation of activity against orthotopic models). 17-DMAG demonstrated limited evidence of activity in the other PPTP *in vivo* tumor panels. Antitumor activity for 17-DMAG against molecular subtypes not represented in the PPTP *in vivo* panels (e.g., MLL leukemias) can not be ruled out by these results.

In Vitro Test Results for 17-DMAG

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 μM with replicates of 6-12 per data point. Data were analyzed using GraphPad Prism, fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values *vs.* the concentration.**

Results:

- **EC50 values ranged ~30-fold from 13 nM to 0.4 μM, with a median of 76 nM.**
- **The 4 rhabdomyosarcoma lines tested were more sensitive than the median, while the neuroblastoma lines were less sensitive than the median.**



Examples of Dose Response Curves

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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 either once or twice weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula $(\pi/6)\times d3$, 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.**
- **17-DMAG was provided by Kosan Pharmaceuticals through the Cancer Therapy Evaluation Program (NCI). 17-DMAG was dissolved in a Sodium Citrate/Citric Acid buffer pH 3.2 and administered IP twice weekly for 6 weeks at a dose of 50mg/kg BID.**
- **Vincristine and cyclophosphamide were provided by the Developmental Therapeutics Program, NCI. Vincristine was dissolved in saline and administered IP weekly x 6 weeks at a dose of 1 mg/kg. Cyclophosphamide was dissolved in saline and administered IP weekly x 6 weeks at a dose of 1 mg/kg.**

- **Solid Tumor Response Criteria:**

Response		Definition	Score
PD2	Progressive Disease 2	>25% increase in tumor volume, TGD value of ≤1.5	0
PD1	Progressive Disease 1	>25% increase in tumor volume, TGD value of >1.5	2
SD	Stable Disease	≤25% increase, <50% regression	4
PR	Partial Response	≥50% regression	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:**

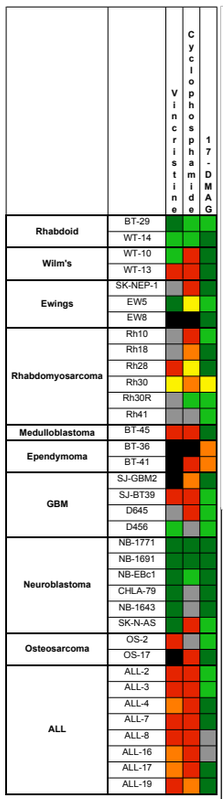
Response		Definition	Score
PD2	Progressive Disease 2	CD45% never drops below 1%, events before end of study, TGD value of ≤1.5	0
PD1	Progressive Disease 1	CD45% never drops below 1%, events before end of study, TGD value of >1.5	2
SD	Stable Disease	CD45% never drops below 1%, no events before end of study	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 2 consecutive weeks and at end of study CD45% <1%	10

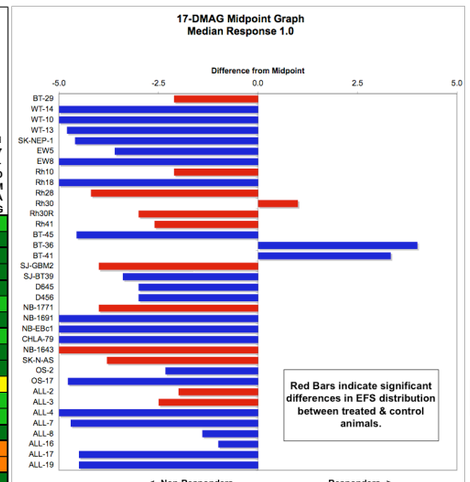
- **Average Group Response: Each individual mouse in the treatment group was assigned a response score (see Tables above) and an average score for the treatment group was calculated and then each treatment group was assigned an overall response according to the table below.**

If Average Score (AS) from (1):	Overall Group Response
0 ≤ AS ≤1	PD2
1 < AS ≤3	PD1
3 < AS ≤5	SD
5 < AS ≤7	PR
7 < AS ≤9	CR
9 < AS	MCR

- **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 Activity: PPTP *In Vivo* Lines





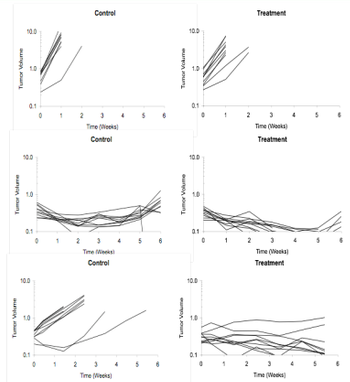
***17-DMAG antitumor activity*:**

- **Progressive disease (with or without tumor growth delay) was the most commonly observed response.**
- **Significant differences (p<0.05) in EFS distributions were observed in 1 of 2 rhabdoid lines, 5 of 6 rhabdomyosarcoma lines, 1 of 4 glioblastoma lines, 3 of 6 neuroblastoma lines, and 2 of 8 ALL lines.**
- **In lines where EFS distributions were significantly different, overall treatment group responses were still PD1 or PD2, with the exception of the rhabdomyosarcoma line, Rh30, which was a PR.**
- **Objective responses (CR) were observed in the two ependymoma lines tested. However, these lines were slow growing and the EFS distributions were not significantly different between treated and control lines. Further testing is required to confirm activity against ependymoma.**

***Vincristine and Cyclophosphamide antitumor activity*:**

- **Testing of these agents serves as a positive control for the PPTP preclinical models.**
- **The responses observed in multiple solid tumor lines and in the ALL panel lines were generally consistent with the expected antitumor activity for vincristine and cyclophosphamide.**

Examples of 17-DMAG *In Vivo* Activity



DISCUSSION & CONCLUSIONS

- **17-DMAG, an Hsp90 inhibitor, is of potential pediatric relevance because of the multiple Hsp90 client proteins [e.g., transmembrane tyrosine kinases, intermediary signaling kinases (e.g., AKT and Raf), and chimeric signaling proteins (NPM-ALK and Bcr-Abl)] affected by Hsp90 inhibition.**
- **The 2 primary measures used by the PPTP for assessing *in vivo* antitumor activity are objective response rate and comparison of EFS distributions between treated and control animals for statistical significance. The two ependymoma lines in which CRs were observed were slow growing, and there was not a statistically significant difference in EFS distributions between treated & control animals. Further evaluation of these and other ependymoma lines is needed before claiming significant antitumor activity for 17-DMAG against preclinical models of ependymoma.**
- **Most rhabdomyosarcoma lines showed significant differences in EFS distribution (treated vs control), but only one line demonstrated an objective response (PR). The rhabdomyosarcoma cell lines were among the most sensitive lines to 17-DMAG in the *in vitro* panel.**
- **The paucity of objective responses observed for 17-DMAG in the *in vivo* panel could reflect drug levels inadequate to inhibit Hsp90 in tumor tissues. Experiments to address this possibility are planned and will use increased tumor levels of Hsp70 in animals treated with 17-DMAG as a pharmacodynamic readout for Hsp90 inhibition.**
- **Comparisons of achievable 17-DMAG systemic exposures between humans (from ongoing phase 1 studies) and mice (previously published) will allow determination of whether PK differences between mice & humans need to be considered when interpreting the activity of 17-DMAG against the PPTP's *in vivo* panel.**
- **In summary, Stage 1 testing for 17-DMAG against the PPTP's childhood cancer preclinical models suggests limited single agent activity, but identifies areas of potential activity that warrant further evaluation.**

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