Pediatric Preclinical Testing Program (PPTP) Stage 2 Evaluation of Eribulin, a Novel Anti-Microtubule Agent

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ERIBULIN

- Eribulin is a pharmaceutically and structurally optimized analog of the marine sponge natural product halichondrin B.
- Eribulin differs from both Vinca alkaloids and taxanes in its mode of binding to tubulin polymers and in its effects on microtubule dynamics.
- Eribulin mesylate (Halaven®) is clinically used in the US, the EU, and Japan for the treatment of certain heavily pretreated patients with metastatic breast cancer, who previously received an anthracycline and a taxane.
- Stage 1 PPTP testing of eribulin showed its high activity, with complete responses (CRs) or maintained CRs (MCR) observed for multiple histotypes, including Wilms tumor, Ewing sarcoma, rhabdomyosarcoma, osteosarcoma, and acute lymphoblastic leukemia (ALL).
- To further evaluate the potential of eribulin against selected pediatric cancers, dose-response testing was performed to define the exposure range over which eribulin was effective. Additionally, a large panel of Ewing sarcoma cell lines was tested to assess the range of in vitro responses to eribulin.

ERIBULIN *IN VITRO* ACTIVITY

- Initial testing of eribulin documented its potent cytotoxic activity in vitro:
  - The median relative IC_{50} (rIC_{50}) value for the PPTP cell lines was 0.27 nM, with a range from <0.10 nM to 14.8 nM.
  - The cell line panels differed in whether they showed plateau survival effects at 96 hours, with the 3 of 4 rhabdomyosarcoma and 2 of 4 Ewing sarcoma cell lines showing plateau effects compared to 0 of 5 ALL cell lines.
- A large panel of Ewing sarcoma cell lines was tested. Results are shown in the table at the right.
  - The median rIC_{50} value for the Ewing lines was 0.38 nM, similar to the median for all PPTP cell lines in Stage 1 testing.
  - The Ewing cell lines differed in whether they showed a plateau survival effect at 96 hours. 7 lines showed Ymin% values near 0%, indicative of complete cytotoxicity, while 13 lines had statistically significant Ymin% plateau values.

PPTP IN VITRO & IN VIVO TESTING METHODS

In vitro: 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 (Kang MH, et al. Pediatr Blood Cancer 56:239-249, 2011). Testing was for 96 hours at concentrations from 0.01 nM to 100 nM with replicates of 6-12 per data point. Data were analyzed by fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values vs. the concentration.

In vivo: Standard PPTP methods for in vivo testing were employed (http://pptp.nchresearch.org/documents/detailedAnalysisMethods.pdf).

Eribulin was tested *in vivo* using intraperitoneal (IP) administration on a Q4D x 3 (repeated at day 21) schedule. Testing was also performed using a Q4D x 2 (repeated at day 21) schedule. Eribulin doses were 1 mg/kg, 0.5 mg/kg and 0.25 mg/kg.

For comparison, vincristine was tested at 1 mg/kg weekly x 6.

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 (π/6)×d3, where d represents the mean diameter.

The primary activity measures were the objective response measure (see legend to figure at right) and the EFS T/C measure. The 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.

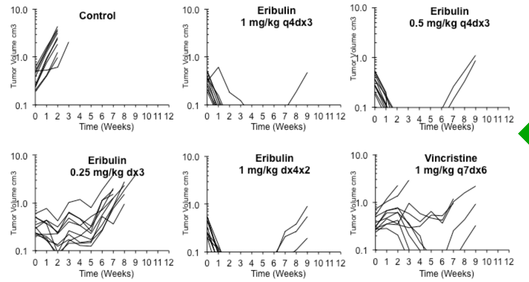
Cell Line	rIC_{50} (nM)	Panel rIC_{50}/ Line rIC_{50}	Observed Ymin%	Estimated Ymin%
6647	0.35	1.08	2.4	3.6
A-673	0.24	1.57	0.1	0.0
CB-AGPN	0.12	3.14	0.1	0.0
SK-N-MC	0.15	2.48	0.1	0.0
TC-32	0.41	0.93	0.4	0.0
TC-71	0.22	1.75	0.0	0.0
TC-106	0.15	2.46	0.3	0.0
TC-138	0.17	2.29	13.5	15.2
TC-205	0.31	1.24	3.9	5.7
TC-248	0.59	0.65	0.1	0.0
CHLA-9	0.47	0.82	1.4	3.8
CHLA-10	0.42	0.90	2.8	3.4
CHLA-25	0.52	0.73	7.1	8.8
CHLA-32	0.13	2.90	4.0	3.8
CHLA-56	0.15	2.46	9.7	9.8
CHLA-57	0.65	0.59	14.5	23.3
CHLA-99	0.48	0.80	4.4	5.7
CHLA-218	0.51	0.74	24.7	30.5
CHLA-258	0.65	0.59	7.5	6.2
COG-E-352	0.56	0.67	5.8	7.1
Median	**0.38**	**1.01**	**3.4**	**3.8**
Minimum	**0.12**	**0.59**	**0.0**	**0.0**
Maximum	**0.65**	**3.14**	**24.7**	**30.5**

ERIBULIN IN VIVO ACTIVITY

Line	Tumor Type	Group	Median Time to Event	P-value	EFS T/C	Median RTV at End of Study	Median Group Response
EW8	Ewing	1 mg/kg x 3 x 2	>98	<0.001	> 4.3	0.0	MCR
EW8	Ewing	0.5 mg/kg x 3 x 2	>98	<0.001	> 4.3	0.0	MCR
EW8	Ewing	0.25 mg/kg x 3 x 2	>98	<0.001	> 4.3	0.0	MCR
EW8	Ewing	1 mg/kg x 2 x 2	>98	<0.001	> 4.3	0.0	MCR
CHLA258	Ewing	1 mg/kg x 3 x 2	>91	<0.001	>10.0	0.0	MCR
CHLA258	Ewing	0.5 mg/kg x 3 x 2	>91	<0.001	>10.0	0.0	MCR
CHLA258	Ewing	0.25 mg/kg x 3 x 2	48.1	<0.001	5.3	>4	SD
CHLA258	Ewing	1 mg/kg x 2 x 2	>91	<0.001	>10.0	0.0	MCR
Rh30R	Rhabdomyosarcoma	1 mg/kg x 3 x 2	88.5	<0.001	9.7	>4	CR
Rh30R	Rhabdomyosarcoma	0.5 mg/kg x 3 x 2	87.2	<0.001	9.5	>4	CR
Rh30R	Rhabdomyosarcoma	0.25 mg/kg x 3 x 2	72.3	<0.001	7.9	>4	CR
Rh30R	Rhabdomyosarcoma	1 mg/kg x 2 x 2	83.6	<0.001	9.1	>4	CR
Rh41	Rhabdomyosarcoma	1 mg/kg x 3 x 2	49.7	<0.001	7.1	>4	SD
Rh41	Rhabdomyosarcoma	0.5 mg/kg x 3 x 2	24.2	<0.001	3.5	>4	PD2
Rh41	Rhabdomyosarcoma	0.25 mg/kg x 3 x 2	16.1	<0.001	2.3	>4	PD2
Rh41	Rhabdomyosarcoma	1 mg/kg x 2 x 2	25.8	<0.001	3.7	>4	PD2
EW8	Ewing	VCR 1 mg/kg x 6	>98	<0.001	>10.8	0.0	MCR
CHLA258	Ewing	VCR 1 mg/kg x 6	63.7	<0.001	7.0	>4	SD
Rh30R	Rhabdomyosarcoma	VCR 1 mg/kg x 6	88.5	<0.001	3.8	>4	CR
Rh41	Rhabdomyosarcoma	VCR 1 mg/kg x 6	21.5	<0.001	1.8	>4	PD2

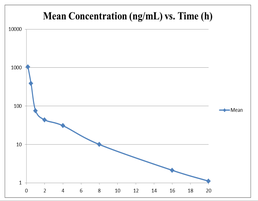
- **PD2 (Progressive Disease 2):** >25% ↑ in tumor volume, TGD value >1.5;
- **SD (Stable Disease):** <25% ↑ in tumor volume, <50% regression
- **CR (Complete response):** disappearance of measurable tumor mass (< 0.10 cm^3)
- **MCR (Maintained CR):** tumor volume < 0.10 cm^3 at the end of the study period

- Eribulin was administered IP at 1, 0.5 and 0.25 mg/kg using a Q4D x 3 schedule or at 1 mg/kg Q4D X2, with cycles repeated at day 21.
- Vincristine was administered at 1 mg/kg using a Q7D x 6 IP schedule with a period of observation for all treatment groups up to 12 weeks.
- Both Ewing sarcoma xenografts were highly sensitive to eribulin, with maintained CR (MCR) observed using doses below 1 mg/kg and using only 2 doses per course.
- Eribulin was also highly active against the rhabdomyosarcoma xenograft, Rh30, inducing CRs at all doses. Only Rh41 failed to show objective responses to eribulin.
- Compared to vincristine at 1 mg/kg, eribulin at 1 mg/kg was as (EW8 and Rh30R) or more effective (CHLA-258 and Rh41) than vincristine.



Dose response to eribulin, and response to vincristine at its MTD in CHLA258 Ewing sarcoma xenografts.

- Graphs show individual tumor growth for control (no treatment), or eribulin administered at 1, 0.5 and 0.25 mg/kg on the q4dx3 schedule, or at 1 mg/kg on the q4dx2 schedule, all repeated at day 21 (10 animals per treatment group).
- Eribulin (1 mg/kg) induced complete regressions (CR) of all tumors on both schedules, as did eribulin at 0.5 mg/kg.
- Vincristine (1 mg/kg q7dx6) induced some regressions with regrowth directly after stopping therapy at week 6.



Dose (mg/kg)	Route	Tissue	Cmax (ng/mL)	Cmax/D (ng/mL/D)	tmax (h)	t1/2 (h)	AUC0-t (ng·h/mL)	AUC0-inf (ng·h/mL)	AUC0-inf/D (ng·h/mL/D)
1	i.p.	plasma	1032.354	1032.354	0.167	0.18	651.627	657.629	657.629

- The preclinical AUC for eribulin at the 1 mg/kg dose is 658 ng*hr/ml (see figure and table above).
- The 1.0 mg/kg/dose used by the PPTP provides systemic exposures for eribulin that are reasonably comparable to the ~ 790 ng*hr/ml exposures achieved in humans treated at the recommended phase 2 dose of 1.4 mg/m^2.

IN VIVO RESULTS AND CONCLUSIONS

- Eribulin demonstrated high level antitumor activity against 3 of 4 sarcoma xenografts over a 2- to 4-fold dose range as well as when the drug administration schedule was reduced to more closely mimic the 2-dose per course clinical schedule of administration.
- Eribulin demonstrated equivalent or superior activity to vincristine in all models tested.
- High-level activity for eribulin was observed using doses and schedules that produce systemic exposures below those that are achievable in humans, supporting a potential therapeutic window for eribulin against selected childhood sarcomas.
- Future preclinical studies with eribulin will examine the agent in combination with other cytotoxic agents, and clinical trials are currently being planned.

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This poster will be available at: http://pptp.nchresearch.org/presentations.html