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Initial Testing (Stage 1) of AT13387, an HSP90 Inhibitor, by the Pediatric Preclinical Testing Program

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Abstract

AT13387, a non-geldanamycin inhibitor of heat-shock protein 90 (HSP90), was tested against the PPTP *in vitro* panel (1.0 nM to 10 μ M) and against the PPTP *in vivo* panels (40 mg/kg or 60 mg/kg) administered orally twice weekly. *In vitro* AT13387 showed a median EC₅₀ value of 41 nM and exhibited activity consistent with a cytotoxic effect. *In vivo* AT13387 induced significant differences in EFS distribution compared to controls in 17% evaluable solid tumor xenografts, but in none of the ALL xenografts. No objective tumor responses were observed. *In vivo* AT13387 demonstrated only modest single agent activity.

Keywords

Preclinical Testing; Developmental Therapeutics; HSP90 inhibitors

INTRODUCTION

The heat-shock protein 90 (HSP90), an evolutionarily conserved molecular chaperone is involved in regulating *de novo* protein folding during protein synthesis, translocation of proteins across membranes, and proteolytic turnover of important mediators of cell growth, cell differentiation, and cell survival [1]. HSP90 interacts with a number of proteins, referred to as 'client proteins', for the post-translational regulation, stabilization, activation, and

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assembly/disassembly of protein complexes [2]. HSP90 is considered to play a central role in many biological processes, including stabilization of several oncogenic proteins required to maintain the malignant phenotype [3]. HSP90 is considered to be a promising target for anti-cancer drug development because HSP90 inhibition interrupts signal transduction pathways that are essential for cell growth and survival [4].

Despite the systemic toxicities related to the first formulation, 17-allylamino-17-demethoxygeldanamycin (17-AAG, tranespimycin), the first-in-class HSP90 inhibitor, is entering into phase III clinical trials with an improved formulation. Since the discovery of 17-AAG, many HSP90 inhibitors have been identified, and 13 of them are in clinical development as single agents or in combination. Two phase I clinical trials of 17-AAG are completed in pediatric solid tumor patients [5,6]. Although drug exposures consistent with those required for anticancer activity in preclinical models were achieved, and systemic modulation of HSP90 was seen, no objective responses were reported in both studies.

Preclinical activity of 17-DMAG (alvespimycin) in cell culture and xenograft models of pediatric cancer was previously evaluated by the PPTP, in which 17-DMAG showed minimal activity against xenograft models of the majority of the pediatric cancers except alveolar rhabdomyosarcoma [7]. AT13387, structurally unrelated to geldanamycin, has high affinity for binding HSP90 ($K_d = 0.5\text{nM}$), and optimized pharmaceutical properties [8]. Further, AT13387 demonstrated long tumor-specific drug retention, which may allow less frequent dosing. Although having a similar target (HSP90) to geldanamycins, AT13387 is distinct from 17-DMAG, and the PPTP performed preclinical testing of AT13387 against pediatric cancers to evaluate the differences in preclinical activity of 17-DMAG.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN, as previously described [9]. Cells were incubated in the presence of AT13387 for 96 hours at concentrations from 1 nM to 10 μM and analyzed as previously described [10].

In vivo tumor growth inhibition studies

CB17SC *scid*^{-/-} 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 [11,12]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [13]. 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^3) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [11]. Responses were determined using three activity measures as previously described [11]. 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 SASR, 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

AT13387 was provided to the Pediatric Preclinical Testing Program by Astex Therapeutics, through the Cancer Therapy Evaluation Program (NCI). Powder was stored at 4°C, protected from light. Drug was formulated in 17.5% hydroxy-propyl- β -cyclodextrin, in sterile water for injection, and made fresh prior to administration. AT13387 was administered intraperitoneally using a twice-weekly schedule for 6 weeks at a dose of 40 mg/kg, or 60 mg/kg for 3 weeks. AT13387 was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In vitro testing

The median AT13387 IC₅₀ value (concentration inhibiting growth 50% relative to controls) for the PPTP cell lines was 41 nM, (range 14 nM - 201 nM). The ratio of the median EC₅₀ of the entire panel to that of each cell line was calculated Table I. The median EC₅₀ (concentration causing 50% maximum effect) for the Ewing sarcoma panel was significantly lower ($p=0.015$) than that of the remaining PPTP cell lines. AT13387 demonstrated an activity pattern consistent with cytotoxic activity for many of the PPTP cell lines with minimum (Ymin) T/C% values approaching 0%.

In vivo testing

AT13387 was tested *in vivo* using a 40 mg/kg dose administered IP twice-weekly (Mon-Thurs) repeated weekly for 6 weeks. All 43 xenograft models studied were considered evaluable for efficacy. A complete summary of results is provided in Supplemental Table I.

AT13387 induced significant differences in EFS distribution compared to control in 6 of 35 (17%) evaluable solid tumor xenografts, Table II. AT13387 did not induce high or intermediate (EFS T/C>2) activity in any solid tumor xenografts evaluable. For the ALL panel, no xenografts showed a significant difference in EFS distribution between treated and control animals. AT13387 did not induce objective responses (PR or CR) in the PPTP solid tumor panels. The best response in the solid tumor panel was PD2 (progressive disease with growth delay), which was observed in 4 of 35 xenografts (11%).

Because of the relative lack of toxicity in the initial studies at 40 mg/kg, secondary screening against seven models was undertaken using a dose of 60 mg/kg administered twice weekly for 3 weeks. AT13387 was well tolerated with a mortality of 4.3% (3 of 70 mice). At this higher dose AT13387 produced only intermediate activity against Rh18 using response criteria (> 2-fold increase in EFST/C), but activity was low using all other criteria for the 7 tumor models evaluated, Table II, and Supplemental Table II.

DISCUSSION

AT13387 is a non-geldanamycin small molecule inhibitor of HSP90 discovered using a high-throughput x-ray crystallography fragment-based drug discovery platform, and has been shown to result in client protein degradation, suppression of signaling, and induce cell cycle arrest and apoptosis [14]. Xenograft studies of AT13387 demonstrated long tumor-specific drug retention, which may allow less frequent dosing [15]. AT13387 exhibited potent cytotoxic activity *in vitro*. The median EC₅₀ values varied by histotype, from 24 nM for the Ewing sarcoma panel to 80 nM for the neuroblastoma panel. However, some cell

lines, particularly those in the neuroblastoma panel, had nadir values that plateaued well above 0%, suggesting a cytostatic effect. AT13387 did not induce objective responses (PR or CR) in the PPTP solid tumor panels. The best response in the solid tumor panel was PD2 (progressive disease with growth delay), which was observed in 4 of 35 xenografts (11%), being most commonly observed in the rhabdoid tumor panel (3 of 3). No objective responses were observed in the ALL panel. However, at 40 mg/kg there was only minor toxicity (1.5% deaths in the treatment groups), hence further testing at 60 mg/kg was undertaken against selected tumors demonstrating the greatest sensitivity to AT13387 at the lower dose. At the higher dose AT13387 induced some drug-related toxicity (4.3%), but did not induce biologically meaningful responses against seven tumor models tested.

While phase I studies reported minimal single agent activity with a HSP90 inhibitor in pediatric cancer patients [5,6], moderate activity was seen in adults treated with HSP90 inhibitor(s) as single agents or in combination with other anticancer drugs. Further, HER2-positive metastatic breast cancer patients who progressed on trastuzumab may benefit from a HSP90 inhibitor [16], based on the idea of disrupting HSP90/HER-2 interaction that subsequently results in impaired downstream signaling [17]. Another example of the relevant biology supports the use of HSP90 inhibitors in combination with a tyrosine kinase inhibitor (TKI) in non-small cell lung cancer that progressed on TKI therapy [18,19]. An HSP90 inhibitor may block oncogenic switching to signaling *via* other receptor tyrosine kinases, shown to be a mechanism for acquired resistance to TKIs [18]. These alternative tyrosine kinases are often HSP90 client proteins that are modulated by HSP90 inhibition. Further, 17-AAG resulted in rapid degradation of EML4-ALK *in vitro* and transient tumor regression in a murine EML4-ALK-driven lung cancer tumor model *in vivo* [20]. However, AT13387 did not exert significant activity against tumor models in the PPTP panel that overexpress wildtype or mutant ALK. As single agents HSP90 inhibitors have not shown impressive activity in the PPTP models, or significant activity in pediatric clinical trials. Demonstration of significant activity of HSP90 inhibitors with targeted agents or cytotoxic agents in relevant preclinical models of pediatric cancers together with a greater understanding of molecular mechanisms of disease and resistance to anticancer therapy may enable more efficacious utilization of this class of agent.

In summary, the non-geldanamycin HSP90 inhibitor AT13387 demonstrated low activity as a single agent against solid tumor and leukemia models of the PPTP. Thus, antitumor activity of AT13387 against this panel of models is similar to alvespimycin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1Summary of AT13387 Activity *in Vitro*

Cell Line	Histotype	Relative IC ₅₀ (nM)	Absolute IC ₅₀ (nM)	Observed Ymin	Median Relative IC ₅₀ Ratio	Relative I/O
RD	Rhabdomyosarcoma	55	59	1.0	0.74	-82%
Rb41	Rhabdomyosarcoma	39	41	1.4	1.03	-94%
Rh18	Rhabdomyosarcoma	90	88	2.2	0.45	-95%
Rh30	Rhabdomyosarcoma	41	43	3.4	1.00	-79%
BT-12	Rhabdoid	153	173	3.0	0.27	-64%
CHLA-266	Rhabdoid	127	145	5.9	0.32	-78%
TC-71	Ewing sarcoma	24	24	0.2	1.71	-87%
CHLA-9	Ewing sarcoma	23	23	1.2	1.80	-67%
CHLA-10	Ewing sarcoma	24	25	3.7	1.68	-41%
CHLA-258	Ewing sarcoma	16	16	0.0	2.62	-100%
GBM2	Glioblastoma	66	71	6.0	0.62	-40%
NB-1643	Neuroblastoma	41	50	7.9	0.99	-63%
NB-EBc1	Neuroblastoma	36	37	4.3	1.14	-81%
CHLA-90	Neuroblastoma	153	201	9.7	0.27	-65%
CHLA-136	Neuroblastoma	118	155	11.1	0.35	-61%
NALM-6	Pre-B cell ALL	34	34	0.8	1.21	-72%
COG-LL-317	T-cell ALL	14	14	0.0	2.89	-99%
RS4;11	Pre-B cell ALL	50	55	7.2	0.82	-52%
MOLT-4	T-cell ALL	36	38	0.1	1.12	-99%
CCRF-CEM	T-cell ALL	32	32	1.0	1.26	-84%
Kasumi-1	AML	16	17	0.2	2.54	-99%
Karpas-299	ALCL	48	48	0.5	0.86	-93%
Ramos-RA1	Burkitt Lymphoma	41	41	0.0	0.99	-100%
Median		41	41	1.4	1.00	-81%
Minimum		14	14	0.0	0.27	-100%
Maximum		153	201	11.1	2.89	-40%

ALCL, anaplastic large cell lymphoma.

Table II

Summary of AT13387 Activity *In Vivo*

Line Description	Tumor Type	Median Time to Event	P- value	EFS T/C	Median RTV/ hCD45 at end of study	Tumor Volume T/C	T/C Volume Activity	EFS Activity	Response Activity
40 mg/kg twice weekly x 6									
BT-29	Rhabdoid	17.3	0.009	1.6	>4	0.58	Low	Low	Int
KT-14	Rhabdoid	> EP	0.206	.	2.1	0.58	Low	NE	Int
KT-12	Rhabdoid	15.8	0.071	1.8	>4	0.84	Low	Low	Int
KT-10	Wilms	10.0	0.295	0.8	>4	1.22	Low	Low	Low
KT-11	Wilms	11.3	0.134	0.9	>4	1.21	Low	Low	Low
KT-13	Wilms	13.1	0.550	1.0	>4	1.11	Low	Low	Low
SK-NEP-1	Ewing	9.7	0.564	0.9	>4	1.09	Low	Low	Low
EW5	Ewing	12.8	0.771	0.6	>4	0.80	Low	Low	Low
EW8	Ewing	9.2	0.040	0.7	>4	1.65	Low	Low	Low
TC-71	Ewing	6.4	0.822	1.0	>4	1.17	Low	Low	Low
CHLA258	Ewing	14.8	0.053	1.2	>4	0.82	Low	Low	Low
Rh28	ALV RMS	17.2	0.993	1.2	>4	0.82	Low	Low	Low
Rh30	ALV RMS	16.8	0.066	1.3	>4	0.76	Low	Low	Low
Rh30R	ALV RMS	12.7	0.200	1.1	>4	0.79	Low	Low	Low
Rh41	ALV RMS	9.7	0.262	1.1	>4	0.85	Low	Low	Low
Rh18	EMB RMS	9.9	0.258	1.2	>4	0.85	Low	Low	Low
BT-28	Medulloblastoma	7.2	0.271	1.4	>4	0.81	Low	Low	Low
BT-45	Medulloblastoma	22.5	0.016	1.4	>4	0.47	Low	Low	Low
BT-50	Medulloblastoma	35.8	0.423	1.2	>4	0.65	Low	Low	Low
BT-44	Ependymoma	12.2	0.007	1.4	>4	0.64	Low	Low	Low
GBM2	Glioblastoma	12.7	0.935	1.0	>4	1.06	Low	Low	Low
BT-39	Glioblastoma	11.8	0.786	1.0	>4	0.92	Low	Low	Low
D645	Glioblastoma	13.7	0.267	1.4	>4	0.67	Low	Low	Low
D456	Glioblastoma	13.8	<0.001	1.7	>4	0.57	Low	Low	Int
NB-SD	Neuroblastoma	8.2	0.659	1.1	>4	0.86	Low	Low	Low

Line Description	Tumor Type	Median Time to Event	P- value	EFS T/C	Median RTV/ hCD45 at end of study	Tumor Volume T/C	T/C Volume Activity	EFS Activity	Response Activity
NB-1771	Neuroblastoma	25.1	0.235	1.1	>4	0.79	Low	Low	Low
NB-1691	Neuroblastoma	12.3	0.610	1.1	>4	0.72	Low	Low	Low
NB-EBc1	Neuroblastoma	10.7	0.082	1.5	>4	0.78	Low	Low	Low
NB-1643	Neuroblastoma	6.1	0.076	1.2	>4	0.76	Low	Low	Low
OS-1	Osteosarcoma	29.7	0.514	1.5	>4	0.73	Low	Low	Low
OS-2	Osteosarcoma	24.0	0.001	1.3	>4	0.72	Low	Low	Low
OS-17	Osteosarcoma	16.8	0.582	1.0	>4	0.97	Low	Low	Low
OS-9	Osteosarcoma	16.6	0.578	1.1	>4	0.94	Low	Low	Low
OS-33	Osteosarcoma	23.9	<0.001	1.3	>4	0.68	Low	Low	Low
OS-31	Osteosarcoma	22.5	0.056	1.2	>4	0.83	Low	Low	Low
ALL-2	ALL B-precursor	13.1	0.089	1.6	>25	.		Low	Int
ALL-3	ALL B-precursor	6.0	0.526	0.6	>25	.		Low	Low
ALL-4	ALL B-precursor	9.2	0.471	1.1	>25	.		Low	Low
ALL-7	ALL B-precursor	2.0	0.044	0.6	>25	.		Low	Low
ALL-8	ALL T-cell	5.9	0.069	1.3	>25	.		Low	Low
ALL-16	ALL T-cell	14.4	0.472	1.1	>25	.		Low	Low
ALL-17	ALL B-precursor	6.7	0.412	1.1	>25	.		Low	Low
ALL-19	ALL B-precursor	11.6	0.083	2.2	>25	.		Low	Int
60 mg/kg twice weekly x 6									
BT-29	Rhabdoid	16.9	0.021	1.3	4	0.63	Low	Low	Low
Rh28	ALV RMS	22.8	0.524	1.4	>4	0.64	Low	Low	Low
Rh30	ALV RMS	21.8	0.879	1.3	>4	0.94	Low	Low	Low
Rh18	EMB RMS	16.4	0.084	2.5	>4	0.52	Low	Low	Int
OS-2	Osteosarcoma	2405	0.044	1.1	>4	0.82	Low	Low	Low
OS-9	Osteosarcoma	18.5	0.062	1.3	>4	0.81	Low	Low	Low
OS-31	Osteosarcoma	21.6	0.019	1.1	>4	0.81	Low	Low	Low