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Initial Testing (Stage 1) of Ganetespib, an Hsp90 Inhibitor, by the Pediatric Preclinical Testing Program

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Abstract

Ganetespib, an Hsp90 inhibitor, was tested against the PPTP *in vitro* cell line panel and selected xenografts *in vivo*, including JAK2- and BRAF-mutated models. Ganetespib demonstrated potent *in vitro* cytotoxic activity (median rIC_{50} 8.8 nM, range 4.4–27.1 nM). *In vivo*, ganetespib induced significant differences in EFS distribution for 4 of 11 xenografts. Intermediate activity (EFS T/C > 2) was noted only for the MV4;11 xenograft, and there were no objective responses. Administered as single agents, Hsp90 inhibitors examined by the PPTP have shown limited evidence for a therapeutic window against both solid tumor and leukemia pediatric preclinical models.

Keywords

Developmental therapeutics; Hsp90 inhibitors; preclinical testing

INTRODUCTION

Heat shock protein 90 (Hsp90) is an essential molecular chaperone that functions as part of a multi-protein complex in the post-translational stabilization of its protein substrates (client proteins) [1]. Hsp90 has emerged as an attractive target for the development of novel anti-

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cancer therapeutics, since many of its client proteins are implicated in the etiology of human cancer. Hsp90 substrates include protein kinases (e.g., BRAF, JAK) transcription factors (e.g., HIF-1α) and chimeric signaling proteins (e.g., EML4-ALK) [1–5]. Thus, inhibition of Hsp90 may result in simultaneous blockade of many oncogenic signaling pathways, leading to tumor cell death or enhanced sensitivity to chemotherapeutic drugs.

Ganetespib (formerly known as STA-9090) is a resorcinolic triazolone Hsp90 inhibitor currently in clinical trials for several adult human cancers [6]. Ganetespib, in contrast to some other Hsp90 inhibitors that have entered clinical evaluation, appears to lack ocular toxicities, an effect that is likely related to its more favorable retinal distribution and elimination [7]. Ganetespib induced objective responses in non-small cell lung cancer (NSCLC) patients with ALK translocations [8]. It is under evaluation as monotherapy for NSCLC patients with ALK gene rearrangement (NCT01562015), and it is being studied in a phase 2B/3 clinical trial in combination with docetaxel in patients with advanced NSCLC (NCT01348126).

The Pediatric Preclinical Testing Program (PPTP) utilizes well-characterized panels of *in vitro* cell lines and *in vivo* xenografts derived from a broad spectrum of pediatric malignancies to evaluate novel drugs for potential inclusion in pediatric cancer clinical trials [9]. Therefore, it was of interest to test ganetespib against the PPTP cell lines and a focused panel of xenografts with biological characteristics suggestive of susceptibility to Hsp90 inhibition.

MATERIALS AND METHODS

In Vitro Testing

In vitro testing was performed using DIMSCAN, as previously described [10]. Cells were incubated in the presence of ganetespib for 96 hours at concentrations from 0.1 nM to 1 μM and analyzed as previously described [11].

In Vivo Tumor Growth Inhibition Studies

CB17SC *scid*$^{-/-}$ mice (Taconic Farms, Germantown, NY), were used to propagate subcutaneously implanted neuroblastoma, astrocytoma, and MV4;11 tumors, as previously described [9]. Human leukemia cells were propagated by intravenous inoculation in non-obese diabetic (NOD)/*scid*$^{-/-}$ mice as described previously [12]. Responses were determined using three activity measures as previously described [9]. 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 SAS®, was used to compare event-free survival (EFS) 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

Ganetespib was provided to the PPTP by Synta Pharmaceuticals Corp., through the Cancer Therapy Evaluation Program (NCI). Powder was stored at 4°C, protected from light. Drug for *in vivo* treatments was formulated in DMSO/cremophor RH40/5% dextrose in sterile water (at 10:18:72 parts), and administered immediately. Ganetespib was administered I.V. at 100 mg/kg (ALL xenografts in NOD-SCID mice) and 125 mg/kg (solid tumor xenografts and MV4;11 in SCID mice) using a weekly 3× schedule followed by 3 weeks observation. Ganetespib was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In Vitro Testing

Ganetespib was tested against the PPTP's *in vitro* cell line panel at concentrations ranging from 0.1 nM to 1.0 μM using a 96-hour exposure period. Ganetespib demonstrated potent cytotoxic activity, with T/C% values approaching 0% for most of the cell lines at the highest concentration tested and with median Relative In/Out value of −87% (Table I). The median relative IC_{50} (rIC_{50}) value for the PPTP cell lines was 8.8 nM (range 4.4–27.1 nM; Table I).

In Vivo Testing

The *in vivo* testing panel for ganetespib included selected translocation-positive sarcomas as well as the following models that were tested as subcutaneous xenografts: NB-1643, a neuroblastoma xenograft with an activating ALK mutation (R1275Q); BT-40, a juvenile pilocytic astrocytoma model with a BRAF V600E mutation; and MV4;11, an MLL-rearranged AML cell line with FLT3-ITD, reported to be responsive to ganetespib [6]. Additionally, four JAK-mutated ALL xenografts were tested using a systemic disease protocol. Ganetespib was tolerated at the doses employed, with a 4.9% toxicity rate for treated animals.

Ganetespib induced a significant difference in EFS distribution compared to control for MV4;11 TC-71, Rh41 and NB-1643, but not for the other seven tested xenografts (Table II, Supplemental Table I). Ganetespib induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity (EFS T/C > 2) for only MV4;11, which had an EFS T/C value of 2.3. Objective responses were not noted for the 11 tested xenografts.

DISCUSSION

The median rIC_{50} value of 8.8 nM observed for the PPTP cell lines is somewhat lower than a previous report using cell lines from adult tumors [6]. Ewing sarcoma cell lines were more sensitive to ganetespib than other PPTP cell lines, a finding that was also noted for the Hsp90 inhibitor AT13387 [13]. However, the difference in rIC_{50} for the Ewing cell lines and the non-Ewing cell lines is only twofold (5.1 nM vs. 10.5 nM, respectively).

MV4;11 was included for *in vivo* testing as this cell line was noted in a prior report to be especially responsive to ganetespib when grown as a subcutaneous xenograft [6]. In our study, although ganetespib showed a significant treatment effect against MV4;11 with EFS T/C > 2, tumor regression was not observed.

Ganetespib did not show activity against the BRAF-mutated astrocytoma xenograft, BT-40, a tumor responsive to the MEK inhibitor AZD6244 [14]. Mutated BRAF has been reported to form an Hsp90–cdc37 complex that is required for its stability and function, and treatment of BRAF-mutant melanoma cells with an Hsp90 inhibitor resulted in degradation of mutant BRAF and induction of apoptosis [2]. However, the *in vivo* preclinical activity of 17-AAG against BRAF-mutant melanoma xenografts was modest [2], as was its clinical activity [15].

Point mutations activating the ALK tyrosine kinase domain are reported to occur in most cases of familial neuroblastoma and 10% of sporadic cases [16,17]. One hotspot mutation (F1174L) has been shown to confer *in vitro* resistance to the ALK inhibitor crizotinib, but not to 17-AAG [18]. Therefore, it was of interest to test the *in vivo* efficacy of ganetespib against the NB-1643 neuroblastoma xenograft, which harbors another ALK hotspot mutation, R1275Q. Although ganetespib significantly delayed time to event for NB-1643, the treatment effect was not large (EFS T/C = 1.6). Modest activity was also observed for ganetespib against translocation positive Ewing sarcoma and rhabdomyosarcoma xenografts.

Ganetespib was evaluated against a panel of JAK-mutated ALL xenografts. JAK mutations are noted in 5–10% of B-precursor high-risk ALL and almost always occur with concurrent over-expression of CRLF2 resulting from genomic alterations fusing CRLF2 with either the IGH locus or with P2RY8 [19–21]. Ganetespib showed no evidence of activity against the JAK-mutated xenografts, a result similar to that obtained by the PPTP for the JAK inhibitor AZD1480 [22]. Weigert et al. [5] have described results that were interpreted as representing promising activity for an Hsp90 inhibitor (AUY922) against a JAK2-mutated ALL xenograft. However, the methods employed in that report do not allow an assessment of remission status, and the extension in time to event for treated versus control animals (EFS T/C using PPTP terminology) was less than twofold. This level of activity would not be considered promising using the stringent activity assessments mployed by the PPTP.

The results presented here as well as prior reports by the PPTP provide limited evidence for an *in vivo* therapeutic window for Hsp90 inhibitors against solid tumor and ALL pediatric preclinical models [13,23]. These results contrast with the clinical activity observed for ganetespib for non-small cell lung cancer (NSCLC) patients with EML4-ALK translocations [8], and point to the possibility that future research may identify a pediatric cancer(s) with biological features that result in comparable responsiveness to Hsp90 inhibition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE I

In Vitro Activity for Ganetespib

Cell line	Histotype	rIC_{50} (nM)	Panel rIC_{50}/line rIC_{50}	Ymin (observed)	Relative in/out (observed Ymin) (%)
RD	Rhabdomyosarcoma	8.0	1.10	3.9	−28
Rh41	Rhabdomyosarcoma	10.4	0.85	2.2	−90
Rh18	Rhabdomyosarcoma	6.2	1.41	6.0	−87
Rh30	Rhabdomyosarcoma	5.6	1.58	3.2	−81
BT-12	Rhabdoid	14.3	0.61	2.2	−74
CHLA-266	Rhabdoid	27.1	0.32	2.5	−90
TC-71	Ewing sarcoma	4.5	1.97	0.9	−30
CHLA-9	Ewing sarcoma	4.6	1.91	0.2	−94
CHLA-10	Ewing sarcoma	5.7	1.55	6.1	−3
CHLA-258	Ewing sarcoma	6.4	1.38	0.0	−100
SJ-GBM2	Glioblastoma	12.9	0.68	2.0	−80
NB-1643	Neuroblastoma	7.4	1.19	7.5	−64
NB-EBc1	Neuroblastoma	16.8	0.52	4.8	−79
CHLA-90	Neuroblastoma	22.3	0.39	6.7	−76
CHLA-136	Neuroblastoma	23.2	0.38	3.0	−90
NALM-6	ALL	11.7	0.75	0.2	−94
COG-LL-317	ALL	4.4	2.02	0.0	−99
RS4;11	ALL	13.5	0.65	6.4	−58
MOLT-4	ALL	10.6	0.83	0.1	−99
CCRF-CEM (1)	ALL	12.5	0.71	1.2	−81
CCRF-CEM (2)	ALL	7.2	1.22	0.8	−87
Kasumi-1	AML	5.8	1.52	0.5	−98
Karpas-299	ALCL	9.6	0.92	0.3	−96
Ramos-RA1	NHL	7.4	1.20	0.0	−100
Median		8.8	1.01	2.1	−87
Minimum		4.4	0.32	0.0	−100
Maximum		27.1	2.02	7.5	−3

TABLE II

Summary of *In Vivo* Activity of Ganetespib

Xenograft Line	Histology	Median time to event (days)	*P*-value	EFS T/C	Median final RTV	T/C[a]	T/C activity	EFS activity[b]	Response activity[c]
BT-40	BRAF mutated astrocytoma	14.9	0.791	0.9	>4	0.96	Low	Low	Low
NB-1643	Neuroblastoma	12.8	0.006	1.6	>4	0.5	Low	Low	Int
TC-71	Ewing sarcoma	16.4	<0.001	1.7	>4	0.41	Int	Low	Low
CHLA-258	Ewing sarcoma	14.7	0.445	1.0	>4	0.88	Low	Low	Low
Rh10	ALV rhabdomyosarcoma	20.2	0.125	1.2	4	0.74	Low	Low	Low
Rh41	ALV rhabdomyosarcoma	17.6	0.048	1.1	>4	0.73	Low	Low	Low
MV4;11	MLL-rearranged AML	26.7	<0.001	2.3	>4	0.24	Int	Int	Int
ALL-10	ALL JAK1 V658	5	0.200	1.0	>25			Low	Low
TGT_020	ALL JAK2 R867Q	8.8	0.892	1.1	>25			Low	Low
TGT_047	ALL JAK2 R683G	5.1	0.433	1.2	>25			Low	Low
TGT_174	ALL JAK2 P933R	8.4	0.055	1.4	>25			Low	Low

[a]Tumor volume T/C value: Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if <21 days). The T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. High activity = T/C ≤ 0.15; Intermediate activity = T/C ≤ 0.45 but >0.15; and low activity = T/C > 0.45;

[b]EFS T/C values: The ratio of the median time to event of the treatment group and the median time to event of the respective control group. High activity requires: (a) an EFS T/C > 2; (b) a significant difference in EFS distributions; and (c) a net reduction in median tumor volume for animals in the treated group at the end of treatment as compared to at treatment initiation. Intermediate activity = criteria (a) and (b) above, but not having a net reduction in median tumor volume for treated animals at the end of the study. Low activity = EFS T/C < 2;

[c]Objective response measures are described in detail in the Supplemental Response Definitions. PD1 = progressive disease with EFS T/C ≤ 1.5, and PD2 = progressive disease with EFS T/C > 1.5.