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Evaluation of Arsenic Trioxide by the Pediatric Preclinical Testing Program with a Focus on Ewing Sarcoma

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Abstract

Arsenic trioxide was tested against the PPTP *in vitro* panel (1.0 nM to 10 μM) and against the PPTP Ewing sarcoma *in vivo* panel administered intraperitoneally at a dose of 2.5 mg/kg daily × 5 per week for a planned treatment duration of 3 weeks. Arsenic trioxide showed a median relative IC_{50} value of 0.9 μM, with Ewing sarcoma cell lines having IC_{50} values similar to those of the remaining PPTP cell lines. Arsenic trioxide did not induce significant differences in EFS distribution compared to control in any of the Ewing sarcoma xenografts studied, and no objective responses were observed.

Keywords

Preclinical Testing; Developmental Therapeutics; Arsenic trioxide; Ewing sarcoma

INTRODUCTION

Arsenic trioxide induces remission as a single agent for most patients with acute promyelocytic leukemia (APL) [1], and disease-free survival rates of 90% or greater are reported for regimens incorporating arsenic trioxide [2]. The more common form of APL results from fusion of PML with RARα, with the resulting fusion protein (PML-RARα) being central to the pathogenesis and maintenance of the disease. The anti-leukemia activity of arsenic results from its binding to cysteine residues in zinc fingers located within a specific domain of PML-RARα and PML, inducing oligomerization and leading to enhanced SUMOylation, ubiquitination, and proteasome-mediated degradation of PML-RARα and PML [3]. The degradation of PML-RARα in APL cells is followed by partial differentiation and/or apoptosis [4]. Analogous to its mechanism of efficacy for APL, the toxicity of arsenic trioxide results from the high affinity of trivalent arsenic for sulfhydryl groups of biomolecules such as glutathione and for cysteinyl residues of proteins [5]. While chronic environmental exposure is carcinogenic as a result of these effects, this same

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reactivity is the basis for the remarkable high level activity of arsenic trioxide for acute promyelocytic leukemia APL.

Of relevance in the pediatric cancer setting is the observation that arsenic trioxide is an inhibitor of the Hedgehog (Hh) signaling pathway, an effect that is mediated at least in part through direct binding to the GLI1 protein resulting in inhibition of its transcriptional activities [6]. Arsenic trioxide demonstrated *in vitro* cytotoxicity against medulloblastoma cell lines with activation of Hh pathway through SMO mutations, and showed modest tumor growth delay against a Ptch-mutant mouse model when treatment was initiated after tumors were palpable [7]. Arsenic trioxide was cytotoxic against Ewing sarcoma cell lines with upregulated GLI1 expression, and slowed the growth of a Ewing sarcoma xenograft *in vivo* [6]. Based on these results for arsenic trioxide, the PPTP evaluated arsenic trioxide against its *in vitro* panel, which includes four Ewing sarcoma cell lines, and against five Ewing sarcoma xenografts.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN, as previously described [8].

In vivo tumor growth inhibition studies

CB17SC *scid*$^{-/-}$ female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted tumors as previously described. Ten mice were used in each control or treatment group. Responses were determined using three activity measures as previously described, and statistical methods were as previously described [9]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Drugs and Formulation

Clinical grade arsenic trioxide (Trisenox®) was used for both in vitro and in vivo experiments. Arsenic trioxide was administered intraperitoneally using a daily × 5 schedule (3 weeks of treatment followed by 3 weeks of observation) at a dose of 2.5 mg/kg.

RESULTS

In vitro testing

Table I shows the relative IC_{50} (rIC_{50}) values and the Ymin (%) values for each cell line evaluated. The median arsenic trioxide rIC_{50} for the PPTP cell lines was 0.9 μM, (range 0.2 μM – 4.7 μM). Arsenic trioxide demonstrated cytotoxic activity with Ymin values approaching 0% for each of the cell lines evaluated. The ratio of the median rIC_{50} for the panel to the rIC_{50} for an individual cell line provides a measure of the comparative sensitivity of each cell line to arsenic trioxide, with values > 1 indicating greater sensitivity and values < 1 representing relative resistance. Other than the neuroblastoma cell lines having a median rIC_{50} significantly greater than the remaining PPTP cell lines (2.8 versus 0.8 μM, p=0.003), there were no significant differences in the median rIC_{50} values for the different cell line panels. Specifically, the median rIC_{50} for the Ewing panel (0.8 μM) was similar to that of the rhabdomyosarcoma (0.9 μM) and ALL (0.9 μM) panels.

In vivo testing

Arsenic trioxide was tested *in vivo* using a 2.5 mg/kg dose administered IP daily × 5 with a planned treatment duration of 3 weeks. No toxicity was observed using this dose and

schedule, and each of the five Ewing sarcoma xenograft models studied was considered evaluable for efficacy. A complete summary of results is provided in Supplemental Table I. Arsenic trioxide did not induce significant differences in EFS distribution compared to control in any of the Ewing sarcoma xenografts studied (Table II), and no objective responses were observed. Kaplan-Meier event free survival data are presented as Supplemental Figure 1.

DISCUSSION

Arsenic trioxide has previously been tested for *in vivo* activity against a number of preclinical cancer models, with a daily (or daily × 5 repeated weekly) administration schedule generally being used [6,7,10–13]. The 2.5 mg/kg dose employed by the PPTP corresponds to a human dose of 0.2 mg/kg using standard allometric scaling conversion methods [14], and hence relates closely to the approved clinical dose of arsenic trioxide for APL (0.15 mg/kg administered daily). This arsenic trioxide dose induces remissions in mouse genetic models of APL preclinical models [13], further supporting its clinical relevance. Regression has not been observed for preclinical *in vivo* testing outside of the APL setting, with best response limited to tumor growth inhibition that generally has been of limited extent [6,7].

The clinical pharmacology of arsenic trioxide provides relevant information to address what accounts for the discrepancy between the preclinical *in vivo* activity and the clinical activity for arsenic trioxide against APL compared to other malignancies. Plasma levels of trivalent arsenic are maximal during the 2 hour infusion period, with C_{max} values ranging between 0.1 and 0.5 μM [15,16]. Plasma levels quickly drop at the end of the infusion into the 0.1 μM range and then diminish further. These concentrations contrast with those typically studied to document *in vitro* effects of arsenic trioxide against proteins such as GLI1, Bcr-Abl, and AML1/MDS1/EVI1, which range from 0.5 to 10 μM. By contrast, arsenic trioxide induces partial differentiation of APL cells at concentrations of 0.1 to 0.5 μM, and it causes degradation of PML-RARα at concentrations as low as 0.1 μM [4].

The PPTP *in vivo* results show no significant activity for arsenic trioxide against Ewing sarcoma xenografts. The *in vitro* testing results show little histotype-specificity for arsenic trioxide, as illustrated by the very similar IC_{50} values for the ALL, rhabdomyosarcoma, and Ewing sarcoma cell lines. Activation of the Hedgehog pathway has been reported for several cancers including Ewing sarcoma, and recently GLI1 has been proposed as a direct target for EWS-FLI1 [17] Reference to the PPTP Affymetrix expression profiling database showed there was also no obvious relationship between cell line sensitivity to arsenic trioxide and expression of genes regulated through the SHH signaling pathway (Supplemental Figure 2). Further, although GLI2 and -3 are expressed in most Ewing sarcoma xenografts, GLI1 was detected only in EW-5 tumors.

The PPTP results are consistent with a broad *in vitro* cytotoxic effect for arsenic trioxide against pediatric cancer cell lines with IC_{50} values in the 1.0 μM range, and the lack of a therapeutic window in the *in vivo* setting suggests that this cytotoxic effect extends to normal tissues as well. The negative phase 2 clinical experience of arsenic trioxide in adults for non-APL cancer indications is consistent with this conclusion [18–20]. Our results do not provide evidence to support clinical investigations of arsenic trioxide outside of the APL setting in children with cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table I

Summary of Arsenic Trioxide *in Vitro* Activity

Cell Line	Histology	Relative IC_{50} (μM)	R^2	Panel rIC_{50}/ Line rIC_{50}	Ymin (%)
RD	Rhabdomyosarcoma	0.9	0.95	0.95	0.1
Rh41	Rhabdomyosarcoma	0.5	0.99	1.66	0.0
Rh18	Rhabdomyosarcoma	1.6	0.97	0.56	2.0
Rh30	Rhabdomyosarcoma	0.8	0.97	1.08	0.3
BT-12	Rhabdoid	0.8	0.99	1.09	0.1
CHLA-266	Rhabdoid	0.4	0.91	2.06	0.2
TC-71	Ewing sarcoma	0.6	0.99	1.40	0.0
CHLA-9	Ewing sarcoma	1.2	0.98	0.73	0.1
CHLA-10	Ewing sarcoma	1.0	0.98	0.90	0.1
CHLA-258	Ewing sarcoma	0.5	0.88	1.77	0.0
SJ-GBM2	Glioblastoma	0.9	0.95	0.96	1.6
NB-1643	Neuroblastoma	1.9	0.95	0.47	1.1
NB-EBc1	Neuroblastoma	1.5	0.99	0.57	1.1
CHLA-90	Neuroblastoma	3.7	0.97	0.24	3.5
CHLA-136	Neuroblastoma	4.7	0.92	0.18	3.3
NALM-6	ALL	0.9	0.98	1.00	0.0
COG-LL-317	ALL	0.2	0.96	4.68	0.0
RS4-11	ALL	0.9	0.99	1.00	0.1
MOLT-4	ALL	1.3	0.91	0.69	2.1
CCRF-CEM (1)	ALL	0.7	0.99	1.25	0.0
CCRF-CEM (2)	ALL	0.6	0.96	1.48	0.0
Kasumi-1	AML	0.8	0.99	1.05	0.4
Karpas-299	ALCL	0.4	0.98	1.95	0.0
Ramos-RA1	NHL	0.9	0.95	0.95	0.0
Median Summary Data		0.9	0.97	1.00	0.10
Minimum		0.2	0.88	0.18	0.00
Maximum		4.7	0.99	4.68	3.49

Table II

Summary of Arsenic Trioxide *in Vivo* Activity Against Ewing Sarcoma Xenografts

Xenograft Line	Median Time to Event	P-value	EFS T/C	Median RTV at End of Study	Tumor Volume T/C	Median Group Response	T/C Volume Activity	EFS Activity
SK-NEP-1	8.2	0.400	1.3	>4	0.76	PD1	Low	Low
EW5	10.7	0.926	1.5	>4	0.88	PD2	Low	Low
EW8	10.3	0.119	0.9	>4	1.19	PD1	Low	Low
TC-71	6.2	0.952	1.0	>4	0.97	PD1	Low	Low
CHLA258	17.2	0.452	1.0	>4	0.88	PD1	Low	Low