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Initial Testing (Stage 1) of the Topoisomerase II inhibitor Pixantrone, by the Pediatric Preclinical Testing Program

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Abstract

Pixantrone, a novel aza-anthracenedione with cytotoxic activity, was tested against the PPTP *in vitro* panel (3.0 nM to 30.0 μ M) and against a limited panel of PPTP Wilms tumors and sarcomas (7.5 mg/kg) administered intravenously using an every 4 day x 3 schedule. *In vitro* pixantrone showed a median relative IC₅₀ value of 54 nM, (range <3 nM to 1.03 μ M). *In vivo* pixantrone induced significant differences in EFS distribution compared to controls in 2 of 8 solid tumor xenografts at dose levels relevant to human drug exposure. A complete response was observed for one Wilms tumor xenograft.

Keywords

Preclinical Testing; Developmental Therapeutics; Topoisomerase 2 inhibitor

INTRODUCTION

Pixantrone is an aza-anthracenedione that lacks the quinone-hydroquinone site responsible for iron binding that is present in doxorubicin and mitoxantrone [1]. Preclinical testing supports reduced cardiotoxicity for pixantrone, as it induced minimal or no significant cardiotoxicity in treatment-naïve mice, in contrast to the marked degenerative cardiomyopathy observed for mice treated similarly with doxorubicin or mitoxantrone [2]. The failure of pixantrone to induce hydrogen peroxide and superoxide anion in *ex vivo* human cardiac strips, in contrast to the effect of doxorubicin, further supports the reduced cardiac toxicity of pixantrone [3].

Multiple mechanisms may be responsible for the cytotoxicity of pixantrone. Pixantrone shows intercalative interaction with DNA, and it stimulates topoisomerase-II mediated DNA cleavage and DNA-protein cross-links [4–6]. Pixantrone can also form a covalent adduct with DNA following activation by formaldehyde [7,8]. The Preclinical Pediatric Testing

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Program (PPTP) performed testing of pixantrone to assess its activity against the PPTP *in vitro* panel and a subset of the PPTP solid tumor models.

MATERIALS AND METHODS

In vitro testing

Testing was performed using DIMSCAN, as previously described in a characterized panel of 24 cell lines [9]. Cells were incubated in the presence of pixantrone for 96 hours at concentrations from 3.0 nM to 30 μ M 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 Wilms tumors, Ewing sarcomas and rhabdomyosarcomas. 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. Ten mice were used in each control or treatment group. Tumor volumes (cm³) were determined and 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.

Pharmacokinetic Study

Pixantrone was formulated in sterile 0.9% NaCl and administered at 30mg/kg intravenously (0.1ml/10g of body weight). Three mice were used per timepoint: 0, 10, 30min, 1, 2, 6, and 24hr. Blood specimens (0.8–1ml) were obtained at sacrifice in sodium heparin, rapidly centrifuged (4°C) and stored at –20°C. See the Supplemental Methods for details of the pixantrone assay methodology.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, 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

Pixantrone was provided to the Pediatric Preclinical Testing Program by Cell Therapeutics, Inc., through the Cancer Therapy Evaluation Program (NCI). Pixantrone was formulated in sterile saline and stored for up to 7 days at 4°C, protected from light. Pixantrone was administered intravenously at 7.5 mg/kg to mice using a q4days x 3 schedule. Pixantrone was provided in coded vials for blinded testing.

RESULTS

In vitro testing

Pixantrone was tested against the PPTP's *in vitro* cell line panel at concentrations ranging from 3.0 nM to 30 μ M using the PPTP's standard 96 hour exposure period. The median relative IC₅₀ (rIC₅₀) value for the PPTP cell lines was 54 nM, with a range from <3 nM (CHLA-9) to 1033 nM (Rh18) (Table I). Observed Y_{min} values approached 0% for all cell lines at the highest concentrations tested. The median rIC₅₀ values were lowest for the Ewing sarcoma panel (14 nM) and highest for the rhabdomyosarcoma panel (412 nM).

In vivo testing

Pixantrone was tested against eight PPTP solid tumor xenografts using a dose of 7.5 mg/kg administered intravenously q4d x 3. This dose was based on toxicity testing in non-tumored SCID mice. The planned treatment and observation period was 6 weeks. Toxicity was not observed in either treated or control groups at the 7.5 mg/kg dose. Eight of 8 tested xenograft models were considered evaluable for efficacy. Complete details of testing are provided in Supplemental Table I including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

Pixantrone induced significant differences in event free survival (EFS) distribution compared to control in 25% (2 of 8) of the evaluable solid tumor xenografts, Table II. Pixantrone induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity in 12.5% (1 of 8) evaluable solid tumor xenografts. An objective response (KT-10, Wilms tumor) was observed in 1 of 8 solid tumor xenografts.

Pharmacokinetic analysis

Pixantrone pharmacokinetics was determined following a single dose of pixantrone (30 mg/kg) administered intravenously. The bioanalytical method for pharmacokinetic analysis for pixantrone was validated in the range 50 to 2500 ng/mL. The concentrations at 24 hours, though below the quantitation limit of the method, were well quantifiable and were characterized by low inter-animal variability (%CV = 14%), and hence were included in the pharmacokinetic analysis. (Supplemental Figure 1). Plasma pharmacokinetic parameters are provided in Supplemental Table II and include C_{max} of 5690 nM, $AUC_{0-t(last)}$ of 13600 nM*h and $AUC_{0-\infty}$ of 14000 nM*h.

DISCUSSION

The aza-anthracenedione pixantrone was synthesized with the intent to reduce the cardiotoxicity of anthracycline derivatives such as doxorubicin and mitoxantrone [12]. It was shown preclinically to have little or no cardiotoxicity in mice compared to those treated with doxorubicin and mitoxantrone [2]. Multiple mechanisms including intercalative interaction with DNA, induction of DNA strand breaks, and interaction with topoisomerase II may be responsible for the cytotoxicity of pixantrone [4–8]

Pixantrone exhibited potent cytotoxic activity *in vitro* against the PPTP cell lines, with a median relative IC_{50} (rIC_{50}) value for the PPTP cell lines of 54 nM. The continuous exposure for 96 hours likely explains the lower rIC_{50} values for the PPTP cell lines compared to prior reports of the *in vitro* activity of pixantrone that employed shorter exposure periods.

Pixantrone administered at 7.5 mg/kg q4d x 3 did not induce toxicity in treated mice, and its *in vivo* activity was modest. A complete response was observed in the Wilms tumor model KT-10, and a statistically significant delay in time to event was measured in Rh36 (embryonal rhabdomyosarcoma). Pixantrone did not significantly inhibit tumor growth for any of the three Ewing sarcoma xenografts.

The dose-normalized systemic AUC for pixantrone obtained for the CB17-SCID mice used by the PPTP to study pixantrone was compared to that observed in humans (Supplemental Table III). Pixantrone systemic exposure in humans varies according to dose administered, ranging from 3680 nM*h for 84 mg/m² to 8741 nM*h for 180 mg/m² [13–15]. Using these published data and normalizing to the dose and schedule used in the phase 3 trial of

pixantrone in NHL [16], the estimated AUC per course is 11171 – 17866 nM·h, similar to the systemic exposure per course observed for SCID mice by the PPTP (10500 nM·h).

In summary, pixantrone showed tumor regressing activity against a Wilms tumor xenograft, but not against 6 pediatric sarcoma models. Because doxorubicin is poorly tolerated by SCID mice [17,18], it is not possible to compare the activity of pixantrone to that of doxorubicin against the PPTP pediatric solid tumor models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table I*In vitro* activity of pixantrone against PPTP cell lines.

Cell Line	Histotype	rIC ₅₀ (nM)	Panel rIC ₅₀ /Line rIC ₅₀	Ymin (Observed)
RD	Rhabdomyosarcoma	145	0.4	0.00
Rh41	Rhabdomyosarcoma	679	0.1	0.00
Rh18	Rhabdomyosarcoma	1033	0.1	0.00
Rh30	Rhabdomyosarcoma	140	0.4	0.00
BT-12	Rhabdoid	67	0.8	0.00
CHLA-266	Rhabdoid	31	1.7	0.08
TC-71	Ewing sarcoma	56	1.0	0.00
CHLA-9	Ewing sarcoma	<3	18.6	0.00
CHLA-10	Ewing sarcoma	12	4.4	0.00
CHLA-258	Ewing sarcoma	15	3.6	0.00
SJ-GBM2	Glioblastoma	384	0.1	0.00
NB-1643	Neuroblastoma	64	0.8	0.14
NB-EBc1	Neuroblastoma	60	0.9	0.01
CHLA-90	Neuroblastoma	382	0.1	0.01
CHLA-136	Neuroblastoma	51	1.0	0.00
NALM-6	ALL	28	1.9	0.00
COG-LL-317	ALL	25	2.2	0.00
RS4;11	ALL	29	1.9	0.00
MOLT-4	ALL	21	2.6	0.00
CCRF-CEM (1)	ALL	49	1.1	0.00
CCRF-CEM (2)	ALL	29	1.9	0.00
Kasumi-1	AML	146	0.4	0.00
Karpas-299	ALCL	166	0.3	0.00
Ramos-RA1	NHL	47	1.1	0.00
Median		54	1.0	0.00
Minimum		<3	0.1	0.00
Maximum		1033	18.6	0.14

Summary of *in vivo* activity of pixantrone

Table II

Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	T/C Activity	EFS Activity	Response Activity
KT-10	Wilms Tumor	> EP	<0.001	> 4.2	0.9	0.05	High	High	CR
KT-13	Wilms Tumor	12.9	0.889	1.0	>4	0.94	Low	Low	PD1
SK-NEP-1	Ewing Sarcoma	11.9	0.125	1.1	>4	0.98	Low	Low	PD1
EW8	Ewing Sarcoma	11.7	0.598	1.0	>4	0.90	Low	Low	PD1
CHLA258	Ewing Sarcoma	10.5	0.430	1.0	>4	0.85	Low	Low	PD1
Rh28	Rhabdomyosarcoma	16.9	0.201	1.3	>4	0.72	Low	Low	PD1
Rh30	Rhabdomyosarcoma	18.8	0.842	0.9	>4	1.11	Low	Low	PD1
Rh36	Rhabdomyosarcoma	13.3	0.003	1.9	>4	0.63	Low	Low	PD2

¹ *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 less than 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 > 0.15 but > 0.15; and Low activity = T/C > 0.45. ² 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. ³ 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.