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Initial Testing (Stage 1) of BAL101553, a Novel Tubulin Binding Agent, by the Pediatric Preclinical Testing Program

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Abstract

BAL101553 is a highly water soluble prodrug of BAL27862 that arrests tumor cell proliferation and induces cell death in cancer cells through disruption of the microtubule network. In vitro BAL27862 demonstrated potent activity, with the median relative IC₅₀ (rIC₅₀) of 13.8 nM (range 5.4 to 25.2 nM). The *in vitro* activity of BAL27862 against the PPTP cell lines is distinctive from that previously described for vincristine. BAL101553 induced significant differences in EFS distribution compared to control in 16 of 30 (53%) solid tumor xenografts and in 2 of 4 (67%) of the evaluable ALL xenografts. No objective responses were observed.

Keywords

Preclinical Testing; Developmental Therapeutics; vascular-disrupting agents; microtubule-binding agents

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Introduction

Targeting microtubules is a widely used strategy to treat many malignancies in children and adults. Constitutive and acquired resistance to microtubule-targeting agents may be mediated through p-glycoprotein expression, alteration in tubulin binding sites and modification of apoptotic pathways through down regulation of BCL-2 [1]. BAL101553 is a highly water soluble prodrug of BAL27862, a novel microtubule destabilizing agent with activity in taxane and vinca alkaloid resistant tumor models [2-4] and vascular disrupting properties [5]. Phase I trials in adult cancer patients have been initiated with an aqueous I.V. BAL101553 formulation. BAL101553 is also orally bioavailable and efficiently penetrates the brain.

Vincristine, a microtubule-targeted vinca alkaloid with broad activity in pediatric malignancies, has been tested against the Pediatric Preclinical Testing Program (PPTP) tumor panels. The wide range of activity across different histologies among the PPTP xenograft lines was predicted by the wide use of vincristine in pediatric patients with leukemia, sarcomas, neuroblastoma, Wilms tumor, and brain tumors [6]. As the only microtubule-targeted agent commonly utilized in children, constitutive and acquired resistance to vincristine is a problem. Alternate microtubule-targeted therapies are needed. Given the novel mechanism of action and the capacity to potentially circumvent resistance to conventional microtubule inhibitors, the PPTP evaluated BAL101553 to better define its potential in pediatric malignancies.

Materials And Methods

In vitro testing

In vitro testing was performed using DIMSCAN, as previously described [7,8]. Cells were incubated with the active moiety BAL27862 for 96 hours at concentrations from 0.1 nM to 1 μ M.

In vivo tumor growth inhibition studies

CB17SC *scid*^{-/-} mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted solid tumors and athymic nude mice were used to propagate glioblastoma models, as previously described [9]. Human leukemia cells were propagated by intravenous inoculation in non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [10]. Responses were determined using three activity measures [9]. An in-depth description of the analysis methods is included in the Supplemental Appendix I.

Drugs and Formulation

The water soluble prodrug, BAL101553, was provided to the PPTP by Basilea Pharmaceutica International Ltd, Basel, Switzerland through the Cancer Therapy Evaluation Program (NCI). Drug was formulated in 0.9% NaCl, adjusted to pH 5.0 using acetate buffer, and was stable at 4°C protected from light. Following MTD evaluation, BAL101553 was administered I.V. at 14 mg/kg using a weekly $\times$ 3 schedule followed by 3 weeks

observation. BAL101553 was provided to each consortium investigator in coded vials for blinded testing.

Results

In vitro testing

BAL27862 was evaluated against 23 cell lines of the PPTP in vitro panel using 96 hour exposure at concentrations from 0.1 nM to 1.0 μ M. BAL27862 demonstrated potent activity, with the median relative IC₅₀ (rIC₅₀) value for the PPTP cell lines being 13.8 nM [range 5.4 to 25.2 nM], Table I. Observed Ymin% values ranged from 0% to 53.0%, with 10 of 23 models having non-zero model-based Ymin% values. The in vitro pattern of response to BAL27862 was unrelated to that for vincristine, previously reported by the PPTP ($R^2 < 0.1$ for rIC₅₀ comparison), consistent with reports that BAL27862 has a different anticancer profile [3,4].

In vivo testing

BAL101553 was tested against PPTP solid tumor xenografts using a dose of 14 mg/kg administered I.V. weekly for 3 weeks. 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, numbers of responses and T/C values.

Thirty-four of 42 tested xenograft models were evaluable for efficacy, with 8 studies excluded for excessive toxicity. BAL101553 induced significant differences in EFS distribution compared to control in 18 of 30 (53%) of the evaluable solid tumor xenografts and in 2 of 4 of the evaluable ALL xenografts (Table II). BAL101553 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity (EFS T/C > 2) in 3 of 27 (11.1%) evaluable solid tumor xenografts, and in 1 of 4 of evaluable ALL xenografts. Intermediate activity for the EFS T/C metric was observed for two of four rhabdomyosarcoma xenografts and for a neuroblastoma xenograft. Objective responses were not observed in either solid tumor or ALL xenografts. The BAL101553 dose used was based on an initial MTD evaluation in non-tumor-bearing mice; however, animal deaths in treated groups (14.6%) were higher than in controls (0.25%). More than 50% of deaths occurred in 5 models, raising the possibility of a tumor-related influence on drug tolerability.

Discussion

BAL27862 and its prodrug BAL101553 have a broad anticancer activity in vitro and in mouse models bearing adult solid tumors, including models refractory to taxanes and vinca alkaloids [2-4]. Consistent with this, BAL27862 demonstrated potent in vitro growth inhibition in the PPTP in vitro panel, with a median IC₅₀ in the nanomolar range (13.8 nM) and a profile distinct from vincristine. Nonetheless, the prodrug BAL101553 demonstrated limited activity against the PPTP's *in vivo* solid tumor panels. While there were clearly treatment effects as demonstrated by significant differences in EFS distribution between treated and control animals, these effects were modest and in only 11% of the xenograft lines did the time to event for a treated group exceed that for a control group by a factor of

two or greater. The extent of activity observed for BAL101553 was less than that previously noted for standard agents (e.g., vincristine and cyclophosphamide) [6]. The reason for the relative lack of activity for BAL101553 compared to vincristine is unclear. However, tubulin binding agents such as the taxanes (microtubule stabilizers) have only modest activity against childhood cancers [11-13], and ABT-751, an agent that binds the colchicine site in β -tubulin, showed only modest activity against many of the models used in the PPTP screen [14], and demonstrated a low objective response rate and failure to prolong time to progression in patients with refractory neuroblastoma [15]. Further pharmacodynamic studies to determine whether BAL101553 induced mitotic arrest and tumor cell death at the dose and schedule used would be required to elucidate whether the limited activity observed was a consequence of poor drug access to tumor tissue.

Interim results for the adult phase 1 trial of BAL101553 have been reported [16]. Through the 60 mg/m² dose level (administered on days 1, 8, and 15), the most frequent side effects are nausea/vomiting and transient blood pressure elevations. A patient with ampullary adenocarcinoma achieved a partial response (among 16 evaluable patients). Of particular relevance, the pharmacokinetic profiles show dose-proportionality with exposure to the active drug BAL27862 at 60 mg/m² being 1.5- and 3-fold, respectively, above the exposure in rats and dogs at their maximum-tolerated doses.

The PPTP Stage 1 testing results do not point to specific childhood cancers against which BAL101553 shows substantial activity as a single agent. Further adult experience with BAL101553, combined with preclinical drug combination analysis, may provide data that can inform potential pediatric applications of BAL101553.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

In vitro activity for BAL27862

Cell Line	Histotype	rIC ₅₀ (nM)	Panel rIC ₅₀ /Line Rel IC ₅₀	Ymin% (Observed)	Ymin% (Model Based)
RD	Rhabdomyosarcoma	8.9	1.55	3.4	4.9
Rb41	Rhabdomyosarcoma	14.2	0.97	5.1	7.6
Rh18	Rhabdomyosarcoma	5.4	2.58	53.0	58.0
Rh30	Rhabdomyosarcoma	11.8	1.17	18.8	22.9
BT-12	Rhabdoid	17.3	0.80	2.9	3.3
CHLA-266	Rhabdoid	14.0	0.99	3.4	3.9
TC-71	Ewing sarcoma	14.0	0.99	0.0	0.0
CHLA-9	Ewing sarcoma	16.7	0.83	2.5	4.1
CHLA-10	Ewing sarcoma	12.5	1.11	1.6	0.0
CHLA-258	Ewing sarcoma	12.5	1.11	5.5	4.6
SJ-GBM2	Glioblastoma	18.0	0.77	1.1	0.0
NB-1643	Neuroblastoma	9.1	1.52	0.4	0.0
NB-EBc1	Neuroblastoma	5.5	2.52	0.2	0.0
CHLA-90	Neuroblastoma	13.6	1.01	12.2	13.3
CHLA-136	Neuroblastoma	7.2	1.91	0.5	0.0
NALM-6	ALL	15.9	0.87	0.0	0.0
COG-LL-317	ALL	17.3	0.80	0.0	0.0
RS4;11	ALL	13.7	1.01	0.5	0.0
MOLT-4	ALL	19.6	0.70	0.0	0.0
CCRF-CEM (1)	ALL	21.8	0.64	0.0	0.0
CCRF-CEM (2)	ALL	25.2	0.55	0.0	0.0
Kasumi-1	AML	20.4	0.68	0.3	1.9
Karpas-299	ALCL	11.0	1.26	0.1	0.0
Ramos-RA1	NHL	13.0	1.07	0.0	0.0
Median		13.8	1.00	0.5	0.0
Minimum		5.4	0.55	0.0	0.0

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Cell Line	Histotype	rIC ₅₀ (nM)	Panel rIC ₅₀ /Line Rel IC ₅₀	Ymin % (Observed)	Ymin % (Model Based)
Maximum		25.2	2.58	53.0	58.0

Table II

Summary of *in Vivo* Activity of BAL101553

Line	Tumor Type	Median Time to Event	P-value	EFS T/C ¹	Median Final RTV/CD45	Tumor Volume T/C ²	EFS Activity	Response ³
BT-29	Rhabdoid	> EP	0.668	> 1.2	>4	0.78	NE	PD1
KT-14	Rhabdoid	27.1	0.066	1.2	>4	0.76	Low	PD1
KT-12	Rhabdoid	33.6	0.279	1.1	>4	0.65	Low	PD1
KT-10	Wilms	11.6	0.123	1.2	>4	0.7	Low	PD1
KT-11	Wilms	26.2	<0.001	1.7	>4	0.53	Low	PD2
KT-13	Wilms	17.4	0.001	1.7	>4	0.46	Low	PD2
SK-NEP-1	Ewing	8.7	0.751	1.1	>4	0.99	Low	PD1
EW5	Ewing	8.8	0.003	1.4	>4	1	Low	PD1
CHLA258	Ewing	20.6	<0.001	1.6	>4	0.39	Low	PD2
Rh30	Alveolar RMS	24.1	<0.001	2.7	>4	0.43	Int	PD2
Rh30R	Alveolar RMS	20.2	<0.001	2.1	>4	1	Int	PD2
Rh41	Alveolar RMS	16.4	0.106	1.6	>4	0.69	Low	PD2
BT-45	Medulloblastoma	40.6	0.108	1.3	>4	0.61	Low	PD1
BT-50	Medulloblastoma	> EP	0.033	.	2.4	0.4	NE	PD2
BT-36	Ependymoma	> EP	1.000	.	3.3	0.85	NE	PD2
GBM2	Glioblastoma	17.9	0.003	1.9	>4	0.4	Low	PD2
BT-39	Glioblastoma	8.7	0.065	1.2	>4	0.82	Low	PD1
D645	Glioblastoma	7.2	0.329	1.1	>4	0.9	Low	PD1
D456	Glioblastoma	10.8	0.041	1.7	>4	0.66	Low	PD2
NB-SD	Neuroblastoma	16.7	<0.001	1.9	>4	0.76	Low	PD2
NB-1771	Neuroblastoma	19.3	0.040	2.5	>4	0.75	Int	PD2
NB-1691	Neuroblastoma	11.1	0.910	1.5	>4	0.86	Low	PD1
NB-EBc1	Neuroblastoma	4.9	0.102	1.2	>4	0.66	Low	PD1
CHLA-79	Neuroblastoma	12.8	0.001	1.4	>4	0.68	Low	PD1
NB-1643	Neuroblastoma	11.4	0.586	1.1	>4	0.97	Low	PD1
OS-1	Osteosarcoma	28.4	<0.001	1.3	>4	0.72	Low	PD1

Line	Tumor Type	Median Time to Event	P-value	EFS T/C ¹	Median Final RTV/CD45	Tumor Volume T/C ²	EFS Activity	Response ³
OS-2	Osteosarcoma	20.6	0.012	1.1	>4	0.82	Low	PD1
OS-17	Osteosarcoma	26.5	0.002	1.3	>4	0.84	Low	PD1
OS-33	Osteosarcoma	23.5	0.014	1.2	>4	0.73	Low	PD1
OS-31	Osteosarcoma	25.2	0.176	1.0	>4	0.95	Low	PD1
ALL-2	ALL B-precursor	28.1	<0.001	1.9	>25	.	Low	PD2
ALL-4	ALL B-precursor	8.0	<0.001	0.8	>25	.	Low	PD1
ALL-8	ALL T-cell	28.5	<0.001	3.8	>25	.	Int	PD2
ALL-19	ALL B-precursor	10.7	0.179	1.4	>25	.	Low	PD1

¹ 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. NE, not evaluable.

² *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 0.45 but > 0.15; and Low activity = T/C > 0.45.

³ 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.