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Small Molecule Therapeutics

Molecular Cancer Therapeutics

A Phase I Study of the SMAC-Mimetic Birinapant in Adults with Refractory Solid Tumors or Lymphoma

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Abstract

The inhibitor of apoptosis (IAP) family of antiapoptotic proteins has been identified as a target for small molecule inhibitors in cancer. Second mitochondrial-derived activator of caspases (SMAC) efficiently and naturally antagonizes IAPs, and preclinical studies have determined that SMAC mimetics have potent anticancer properties. Here, we report a first-in-human trial designed to determine the maximum tolerated dose (MTD), safety, and pharmacokinetics/pharmacodynamics (PK/PD) of birinapant, a novel SMAC mimetic. Patients with advanced solid tumors or lymphoma were enrolled in a 3+3 dose escalation design with birinapant administered intravenously from 0.18 to 63 mg/m^2 once weekly every 3 of 4 weeks. Fifty patients were enrolled to 12 dose cohorts. Birinapant 47 mg/m^2 was determined to be the MTD. At 63 mg/m^2, dose-limiting toxicities included headache, nausea, and vomiting. Two cases of Bell's palsy (grade 2) also occurred at 63 mg/m^2. Birinapant had a plasma half-life of 30 to 35 hours and accumulated in tumor tissue. Birinapant suppressed cIAP1 and increased apoptosis in peripheral blood mononuclear cells and tumor tissue. Prolonged stable disease was observed in 3 patients: non–small cell lung cancer (5 months), colorectal cancer (5 months), and liposarcoma (9 months). Two patients with colorectal cancer had radiographic evidence of tumor shrinkage. In conclusion, birinapant was well tolerated with an MTD of 47 mg/m^2 and exhibited favorable PK and PD properties. Several patients demonstrated stable disease and evidence of antitumor activity. These results support the ongoing clinical trials of birinapant in patients with cancer. *Mol Cancer Ther; 14(11); 2569–75. ©2015 AACR.*

Introduction

Inhibitors of apoptosis (IAP) proteins, such as X-linked IAP (XIAP), cellular IAPs (cIAP1 and cIAP2), and melanoma IAP (ML-IAP), suppress apoptosis and activate a tumor necrosis factor (TNF)-dependent prosurvival NF-κB pathway that is important in tumor cells and infectious diseases (1–6). IAP genes that encode these proteins are amplified in many tumors and overexpression of cIAP1 and cIAP2 can suppress apoptosis of a variety of tumor cells (1). Also, overexpression of IAP genes has been found to contribute to tumor resistance to conventional cancer chemotherapeutic agents (5, 7, 8). The antiapoptotic function of IAPs and their overexpression in many cancers make them attractive antitumor therapeutic targets.

IAP proteins are antagonized by another protein called second mitochondrial-derived activator of caspase (SMAC). SMAC binds to IAP proteins via its N-terminal AVPI sequence of its processed form, which results in the autoubiquitylation and subsequent degradation of IAP proteins (9–12). Thus, SMAC-induced degradation of IAP proteins suppresses TNF-dependent NF-κB activation and allows apoptosis to occur in tumor cells (13, 14). This has stimulated development of SMAC mimetics that bind strongly to tumor-expressed IAP proteins, disrupt their function, and stimulate tumor cell death (12, 15–17).

Birinapant (TL32711) is a bivalent SMAC mimetic that displays preferential binding to cIAP1 relative to cIAP2 and XIAP (12, 15, 18). In preclinical studies, birinapant demonstrated potent single-agent antitumor activity while sparing normal cells (15, 18, 19). In addition, birinapant showed significant activity in preclinical models of ovarian, colorectal, head and neck squamous cell carcinoma, leukemia, and melanoma (18–21). Finally, birinapant demonstrated synergistic preclinical antitumor activity with multiple chemotherapies, such as irinotecan, gemcitabine, and azacitidine (18).

Here, we report the first-in-human phase I, open-label, nonrandomized, dose-escalation study with birinapant in patients with advanced solid tumors or lymphoma. The aims of this study were to assess the safety and tolerability of birinapant as a single agent in patients with advanced cancer, to investigate the pharmacodynamic (PD) and pharmacokinetic (PK) properties of birinapant, and to assess the preliminary efficacy of single-agent birinapant in these patients.

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Patients and Methods

Patients

Patients were ≥18 years with advanced treatment-refractory malignancies (or for which no effective therapy existed). Included patients had measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1) or Revised Response Criteria for Malignant Lymphoma, were Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2, and had adequate renal [≤1.5× upper limit of normal (ULN)], hepatic (≤3× ULN) and bone marrow function [defined as absolute neutrophil (ANC) ≥1,500/mm^3 (≥1.5 × 10^6/L), platelet count ≥75,000/mm^3 (≥75 × 10^6/L), and hemoglobin ≥10 g/dL (in the absence of transfusion within 24 hours prior to dosing)]. Patients were ineligible if they had a history of autoimmune disease within the previous 5 years, or any immunosuppressive therapy 4 weeks prior to study. The study protocol was approved by Institutional Review Boards and registered at www.clinicaltrials.gov (NCT00993239); all patients gave informed consent.

Treatment administration and patient monitoring

Patients received birinapant by intravenous infusion more than 30 minutes once weekly for 3 weeks of each 4-week dosing cycle. The doses of birinapant administered to patients ranged from 0.18 to 63 mg/m^2.

Dose escalation and dose-limiting toxicities

Patients were enrolled in cohorts of 3 to 6 following a standard "3+3" phase I dose escalation design (22). The starting dose of 0.18 mg/m^2 was chosen as this dose level corresponded to one-sixth the highest nonseverely toxic dose (HNSTD) in dog, the most sensitive species (23). Dose escalation used a modified Fibonacci sequence; intracohort doses were increased by 100% if no dose-limiting toxicity (DLT) were observed in the first cycle, and were increased either 50 or 33% if a drug-related DLT of National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE; version 4) grade 2 AE occurred, depending on the event type and grade. An AE was considered dose limiting if it met one of the following criteria: grade 4 neutropenia (ANC < 500/mm^3) >7 days; grade 3 or 4 neutropenia with fever; grade 4 thrombocytopenia (<25,000/mm^3); grade ≥3 nausea and vomiting despite maximal anti-emetic treatment; grade ≥3 diarrhea despite maximal antidiarrheal treatment; or any other nonhematologic or laboratory grade ≥3 AE, with the exception of transient grade 3 hypotension. Owing to expectations based upon the mechanism of action of birinapant, which may cause an increase in serum cytokines and subsequent transient hypotension, if hypotension could be resolved with administration of ≤3 L of intravenous fluids within the 8-hour period of outpatient observation after drug infusion, then it was not considered a DLT.

Patients who experienced a DLT during the first treatment cycle discontinued study treatment but remained in the study for follow-up. Patients who experienced a clinically significant AE that was not considered dose limiting, and recovered within 28 days with standard supportive care, could be re-treated per the protocol treatment schedule.

Patients who experienced a clinically significant hematologic toxicity or nonhematologic toxicity determined to be study drug-related (definitely, probably, or possibly related) to birinapant were able to continue in the study with either the current dose or with a dose reduction for subsequent cycles, depending on the severity, nature of, and duration of the AE. Birinapant was delayed for a maximum of 14 consecutive days before being restarted.

A dose expansion cohort of 20 patients was planned at or below the maximum tolerated dose (MTD), which was determined upon completion of the first treatment cycle.

Safety and antitumor activity assessment

The tolerability of birinapant was assessed using criteria of NCI CTCAE (version 4). Patients were evaluable for safety if they signed informed consent and received at least one dose of birinapant.

Cardiac safety was assessed by serial electrocardiograms (ECG) and left ventricular ejection fraction (LVEF) measurement. ECGs were obtained during cycle 1 dosing and at study completion. LVEF was assessed by echocardiogram or MUGA scan within 30 days of the initial dose and during week 4 of the second treatment cycle. Vital signs were assessed within 30 days of screening, prior to each dose, and at week 4.

Disease status was assessed within 30 days of the first dose and at the end of every 2 cycles using the RECIST version 1.1 (24) or revised response criteria for malignant lymphoma (25). Patients were evaluable for efficacy if they completed 2 cycles of birinapant treatment and underwent radiographic assessment. The number of patients that met criteria for safety and efficacy assessments is detailed in Supplementary Table S1.

Sample collection and PK analysis

Blood and peripheral blood mononuclear cell (PBMC) samples were collected for PK studies at baseline and during the first birinapant treatment cycle. Optional tumor biopsies were obtained at baseline (pretreatment) and during and upon completion of 2 cycles of birinapant treatment.

Birinapant concentrations in biopsy samples and in plasma were analyzed by liquid chromatography/tandem mass spectrometry (LC/MS-MS) using methods developed by TetraLogic Pharmaceuticals, Inc.

During the first cycle of birinapant treatment, the following PK parameters were calculated using noncompartmental analysis (WinNonlin Version 5.2; Pharsight): maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the concentration–time curve from time zero to the last quantifiable time point (AUC_{0-t}) (day 1 and day 15), AUC extrapolated from time zero to infinity ($AUC_{0-\infty}$) day 1, and elimination half-life ($t_{1/2}$), clearance (CL), and apparent volume of distribution (V_{ss}). Only plasma concentrations with greater than or equal to the limit of quantification (LOQ) of the assay were used for PK determination. Peak (end of infusion) and trough plasma birinapant concentrations were determined on day 8.

Drug accumulation [week 3/week 1 AUC ratio (R)] and dose proportionality were assessed using an appropriate power model (26). In PBMC and plasma sample(s) collected at the time of posttreatment optional biopsy collection, birinapant concentration was assessed.

For evaluation of potential drug metabolites and the amount of drug excreted within a 24-hour period (Ae%), a predose urine sample and a 24-hour urine sample were collected prior to birinapant treatment from patients who enrolled in the expansion cohort.

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PBMC, tumor, and plasma PD markers

Biologic response to birinapant was evaluated using cellular markers such as cIAP1, cIAP2, pro–caspase-8, and poly(ADP-ribose) polymerase (PARP) that were associated with birinapant mechanism of action in preclinical studies (18).

Blood samples for cytokeratin-18 (CK-18), caspase-3/7, and multicytokine analysis were collected at baseline, 4 hours after birinapant treatment on days 1, 8, and 15 of cycle 1, and 24 hours after dosing on C1D1 and C1D15. CK-18 was assayed using M30-Apoptosense Peviva AB (Bromma) and M65 enzyme-linked Peviva AB (Bromma) immunosorbent assays. Caspase-3/7 was measured using Caspase-Glo 3/7 (Promega). Multicytokine analysis was performed by Rules-Based Medicine, Inc.

Varicella-zoster virus (VZV) and herpes simplex virus (HSV) DNA were evaluated as previously described (27, 28).

Statistical analysis

Data collected throughout the study were analyzed using descriptive nonparametric statistics. For categorical data, the number and percentage of patients are presented.

Results

Between December 2009 and August 2012, 50 patients were enrolled at Fox Chase Cancer Center (Philadelphia, (PA), Roswell Park Cancer Institute (Buffalo, NY), and Abramson Cancer Center, University of Pennsylvania (Philadelphia, PA). The demographics of the patients enrolled in the study are presented in Table 1.

The median age of the patients was 59 years (range, 31–85). Fifty-six percent of the patients were male. Tumor types evaluated in the study were colorectal (15), head and neck (7), soft-tissue sarcomas (5), lung (4), pancreatic (4), melanoma (4), and miscellaneous other tumors (11). A majority of patients had received prior systemic cancer treatments (92%) and had an ECOG PS of 1 (64%) (Table 1).

Table 1. Patient demographics

Characteristic	Patients ($n = 50$)
Age, y	
Median (range)	58.5 (31–85)
Gender, *n* (%)	
Female	22 (44)
Race, *n* (%)	
White	45 (90)
Black	4 (8)
American Indian or Alaska Native	1 (2)
ECOG performance status, *n* (%)	
1	32 (64)
Primary tumor site	
Gastrointestinal	19 (38%)
Endocrine	5 (10%)
Genitourinary	1 (2%)
Gynecologic	3 (6%)
Head and Neck	7 (14%)
Lung	4 (8%)
Musculoskeletal	5 (10%)
Skin	4 (8%)
Lymphoma	2 (4%)
Number of prior systemic therapies	
0	1 (2%)
1–3	19 (38%)
4–6	22 (44%)
7–9	5 (10%)
Not specified	3 (6%)

Maximum tolerated dose

Preclinical IND-enabling animal toxicity studies suggested that 0.18 mg/m^2 would be a tolerable initial dose for the first birinapant treatment cycle. Further, birinapant at $\geq$5.6 mg/m^2 was predicted to be potentially therapeutically active. Patients were enrolled to 1 of 12 dosing cohorts with doses that ranged from 0.18 to 63 mg/m^2 (Table 2). No DLTs were observed in any patient after 4 weeks of birinapant treatment enrolled to the first 10 cohorts of 0.18 to 35 mg/m^2. At 47 mg/m^2 (cohort 11), 1 of 6 patients developed grade 3 hypophosphatemia and a macular rash, which were considered DLTs. In the 63-mg/m^2 treatment group, 2 of 3 patients experienced a drug-related DLT. One patient experienced headache, nausea, and vomiting (all grade 3). This patient developed Bell's palsy (grade 2), with an unknown duration (patient lost to follow-up). Another patient developed headache (grade 3) and Bell's palsy (grade 2), which resolved without treatment after 2 days. These events were deemed clinically significant, and birinapant administered at 63 mg/m^2 was determined to have exceeded the MTD. Based on these results, 9 additional patients were enrolled in a 47-mg/m^2 expansion cohort. Of the 9 patients, only 1 exhibited a first-cycle DLT of asymptomatic elevations of amylase and lipase.

Safety

Fifty patients in the study were evaluable for safety. Table 3 shows related AEs that occurred >5% of patients receiving birinapant treatment. Thirty-three patients (66%) experienced at least one grade 1 or grade 2 AE, whereas only 18% experienced at least 1 grade 3 or grade 4 AE. The most common grade 1 or grade 2 AEs were fatigue, lymphopenia, vomiting, hypotension, nausea, headache, decreased appetite, and diarrhea (Table 3). The most common grade 3 or grade 4 AEs were fatigue, headache, lymphopenia, elevated blood alkaline phosphatase, decreased appetite, dyspnea, pain in extremities, and aspiration pneumonia.

Serious adverse events (SAE) related to birinapant were observed only in patients treated with birinapant administered at >35 mg/m^2. A presumptive clinical diagnosis of "cytokine release syndrome" characterized by a rash and fever in the absence of hypotension was reported for 1 patient (grade 1) treated with 35 mg/m^2 birinapant; 3 patients (2 grade 1; 1 grade 2) treated with 47 mg/m^2; and 1 patient treated (grade 1) with 63 mg/m^2. "Cytokine release syndrome" was observed only during the first treatment cycle and did not recur with additional birinapant treatment. Although not considered a DLT, Bell's palsy was observed in 2 of 3 patients treated with 63 mg/m^2 (2 grade 2; 1 SAE). These results suggest that administration of birinapant at 47 mg/m^2 could be tolerated as a once-weekly treatment regimen for 3 weeks with the cycle repeated every 4 weeks.

Pharmacokinetics

The pharmacokinetics of birinapant administration was studied over the 0.18 to 63 mg/m^2 dose range. Birinapant exposure (C_{max} and $AUC_{0-\infty}$) on days 1 and 15 increased in a dose-proportional manner following a 30-minute intravenous infusion (Table 4, Fig. 1). C_{max} values were generally coincident with the end of infusion (approximately 0.5 hours). Birinapant was eliminated from plasma in a triexponential manner with an estimated mean terminal $t_{1/2}$ of 19 to 47 hours on day 1. There was moderate variability in birinapant clearance (CL) with mean day 1 values of approximately 21.0 $\pm$ 7.4 L/h (35 CV%) at a dose of 47 mg/m^2 ($n = 17$; Table 4).

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Table 2. Safety cohort review DLTS and related SAE

Cohort	Birinapant dose	Number of patients	Cycle 1 DLTs ($n = 48$)	Related[a] SAE, any cycle ($n = 50$)
1	0.18 mg/m^2	3	0	None
2	0.36 mg/m^2	3	0	None
3	0.72 mg/m^2	3	0	None
4	1.44 mg/m^2	3	0	None
5	2.88 mg/m^2	3	0	None
6	5.76 mg/m^2	3	0	None
7	11.5 mg/m^2	3	0	None
8	17.2 mg/m^2	3	0	None
9	26.0 mg/m^2	3	0	None
10	35.0 mg/m^2	3	0	None
11	47.0 mg/m^2	6[b]	Hypophosphatemia[c] Macular rash[c]	None
12	63.0 mg/m^2	3	Headache[c] Nausea[c] Vomiting[c] Headache	Cytokine release syndrome Headache[c] VIIth nerve paralysis (Bell's palsy)[c] Nausea[c] Vomiting[c]
Expansion	47.0 mg/m^2	9	Amylase/lipase increase[c]	None

[a]Adverse events judged by the investigator to be definitely related, probably related, or possibly related.
[b]Six patients evaluable for DLT and 8 patients evaluable for SAE.
[c]Occurred in the same patient.

There was no accumulation in plasma with the weekly dosing regimen as reflected in the day 15/day 1 C_{max} and $AUC_{0-\infty}$ accumulation ratios (1.12 ± 0.24 and 1.02 ± 0.22, respectively). The mean volume of distribution was high (approximately 710.4 ± 357.7 L at a dose of 47 mg/m^2) on day 1, indicative of extensive tissue uptake. The urinary elimination of birinapant over 0 to 24 hours was low at 47 mg/m^2 [mean (SD) Ae%; $4.4\% \pm 3.4\%$].

Target inhibition

Birinapant treatment resulted in a greater than 75% reduction in cIAP1 in PBMC samples at doses of 5.6 mg/m^2 and above. This was consistent with analysis of biopsies taken from three different tumors (Fig. 2A–C). Suppression of cIAP1 was observed 1.5 hours after treatment in tumor biopsies and after 4 hours in PBMC samples. Suppression of cIAP1 was sustained in PBMC and in tumor tissue biopsies for at least 7 days after birinapant treatment.

In one biopsy sample that was available for additional study, suppression of cIAP1 was associated with increases in the activated form of caspase-8, and a reduction in PARP levels consistent with PARP cleavage (Fig. 2B). This suggested activation of apoptotic pathways in the tumor following intravenous administration of birinapant.

Examination of PD markers: CK-18 and caspase-3/7

CK-18 is an intermediate filament protein found in the cytoplasm of many epithelial cells. Full-length and caspase-cleaved CK-18 (detected as M65 and M30 antigens) represent circulating indicators of apoptosis. There was a drug effect in terms of increased levels of M30 and M65 following birinapant treatment, although a clear correlation with drug dose was not observed (Supplementary Table S2). The lack of a clear dose relationship was not surprising given the interindividual variation in these

Table 3. Adverse events (related)

	All patients ($n = 50$)					
System organ class, preferred term, *n* (%)	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
Patients with at least one related adverse event, *n*	21 (42)	11 (22)	6 (12)	3 (6)	0	41 (82)
Blood and lymphatic systems disorders						
Anemia	0	3 (6)	0	0	0	3 (6)
Lymphopenia	1 (2)	3 (6)	2 (4)	2 (4)	0	8 (16)
Gastrointestinal disorders						
Diarrhea	5 (10)	0	0	0	0	5 (10)
Nausea	4 (8)	2 (4)	1 (2)	0	0	7 (14)
Vomiting	5 (10)	2 (4)	1 (2)	0	0	8 (16)
General disorders and administration site conditions						
Fatigue	2 (4)	5 (10)	1 (2)	0	0	8 (16)
Pyrexia	4 (8)	0	0	0	0	4 (8)
Immune system disorders						
Cytokine release syndrome	4 (8)	1 (2)	0	0	0	5 (10)
Metabolism and nutrition						
Decreased appetite	4 (8)	1 (2)	0	0	0	5 (10)
Nervous system disorders						
Headache	2 (4)	2 (4)	2 (4)	0	0	6 (12)
Bell's palsy	0	2 (4)	0	0	0	2 (4)
Skin and subcutaneous tissue disorder						
Rash	2 (4)	1 (2)	0	0	0	3 (6)
Vascular disorders						
Hypotension	4 (8)	3 (6)	0	0	0	7 (14)

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Table 4. Plasma birinapant PK parameter estimates [0.18 to 63 mg/m^2; 30-minute i.v. infusion (mean ± SD)]

Dose		C_{max} (ng/mL)		$AUC_{0-\infty}$ (ng×h/mL)		$t_{1/2}$ (h)	
(mg/m^2)	*n*	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
0.18	3	8.9 ± 3.2	11.0 ± 5.1	10.1 ± 1.7	11.2 ± 2.2	1.9 ± 0.4	1.9 ± 0.6
0.36	3	19.3 ± 6.9	25.6 ± 15.4	20.3 ± 1.2	20.8 ± 2.0	2.6 ± 0.1	2.4 ± 0.8
0.72	3	34.8 ± 14.3	47.9 ± 19.3	33.4 ± 6.8	43.8 ± 10.1	2.5 ± 0.3	4.5 ± 3.8
1.44	3	79.3 ± 27.2	92.9 ± 40.6	118.3 ± 31.1	108.2 ± 39.8	18.6 ± 21.1	5.6 ± 0.6
2.88	3	275.3 ± 166.7	241.2 ± 98.9	295.4 ± 95.2	225.4 ± 71.6	46.9 ± 37.5	6.9 ± 1.0
5.76	3	254.6 ± 50.7	330.4 ± 36.9	316.9 ± 100.4	352.8 ± 98.0	5.7 ± 0.3	6.5 ± 0.8
11.5	3	606.5 ± 143.4	888.4 ± 489.0	663.9 ± 70.7	816.0 ± 276.3	5.8 ± 0.4	5.6 ± 0.8
17.2	3	1,579.1 ± 926.7	1,097.7 ± 259.2	1,703.7 ± 575.4	2,417.2 ± 1,312.4	30.7 ± 3.3	9.6 ± 6.3
26	3	1,653.7 ± 352.1	1,320.5 ± 58.0	2,466.8 ± 606.5	1,801.5 ± 1,38.8	28.3 ± 1.5	6.4 ± 0.6
35	3	2,342.5 ± 417.1	2,756.9 ± 1,080.9	4,180.7 ± 3,464.5	3,510.6 ± 2,781.4	22.1 ± 14.6	5.8 ± 2.9
47	17	3,995.6 ± 1,634.4	3,966.6 ± 1,991.8[a]	4,648.4 ± 1,278.4	4,522.3 ± 1,429.7[a]	24.5 ± 9.7	6.3 ± 0.7[a]
63	3	5,498.6 ± 2,494.8	5,837.7[b]	5,626.6 ± 1,629.0	4,599.0[b]	20.9 ± 14.0	7.3[b]

[a]$n = 14$.
[b]$n = 1$.

assays. However, for the three lowest dose cohorts, there was no increase in m30 or m65 with the first two doses of the drug (cycle 1, day 1; C1D1 and cycle 1, day 8; C1D8), implying that the lowest doses had little PD effect, whereas a dose as low as 1.44 mg/m^2 increased these levels.

The magnitude of increase in circulating M30 and M65 was modest following birinapant treatment. At birinapant doses of 47 mg/m^2, the median baseline M30 level was 174 U/L (range, 90–1583), whereas 24 hours after the third dose on day 15 (C1D15), the median M30 level was 270 U/L (86–2004). This reflected a maximum increase of approximately 1.5-fold. Similarly with M65, the median baseline level was 522 U/L (range, 190–7515), whereas on C1D15, 24 hours after dose, it was 719 (range, 308–8591).

There was also a suggestion that levels of both M30 and M65 increased with each cycle of drug. Even for the 3 lowest dose cohorts, which showed no clear increase in these markers with the first 2 doses of drug, there was an increase after the third dose of drug (Supplementary Table S2). This suggested a cumulative PD effect, in the absence of evidence of drug accumulation. This cumulative PD effect was further supported when pretreatment levels are compared. For example, M65 (Supplementary Table S3) levels at baseline were lowest, pretreatment levels were increased prior to the second dose, and further increased prior to the third dose. These conclusions were further supported by the results of the caspase-3/7 assays (Supplementary Table S4). Taken together, these results provided evidence of cell-death pathway activation in response to birinapant.

Serum cytokine assays

Levels of serum cytokines were measured following birinapant treatment from all 50 patients. Results of these analyses indicated that birinapant treatment ranging from 0.18 to 63 mg/m^2 did not significantly increase serum levels of TNF, monocyte chemoattractant protein-1 (MCP-1), or IL1 IL6, or IL8 (unpublished observations). However, 5 patients treated with 47 and 63 mg/m^2 of birinapant exhibited transient elevation of serum MCP-1 4 hours after treatment. MCP-1 returned to normal levels in all of these patients within 24 hours following birinapant administration. Interestingly, elevated cytokine levels were not detected in the serum of 5 patients who presented with presumptive "cytokine release syndrome."

Efficacy

Twenty-six patients treated with birinapant were evaluable for clinical efficacy. No patient exhibited a complete response (CR) or partial response (PR) to birinapant treatment as best response. Stable disease was observed in 7 treated patients (28%), whereas progressive disease was identified in 18 patients (72%).

Of the 7 patients who exhibited stable disease, 3 experienced stable disease for more than 4 months, whereas 2 patients with non–small cell lung cancer (treated with 47 mg/m^2 of birinapant) and 1 patient with colorectal cancer (0.36 mg/m^2 of birinapant) experienced stable disease for 5 months. In addition, 1 patient with liposarcoma received 3 cycles of birinapant treatment (17.2 mg/m^2 for over 9 months). Finally, 2 patients with colorectal

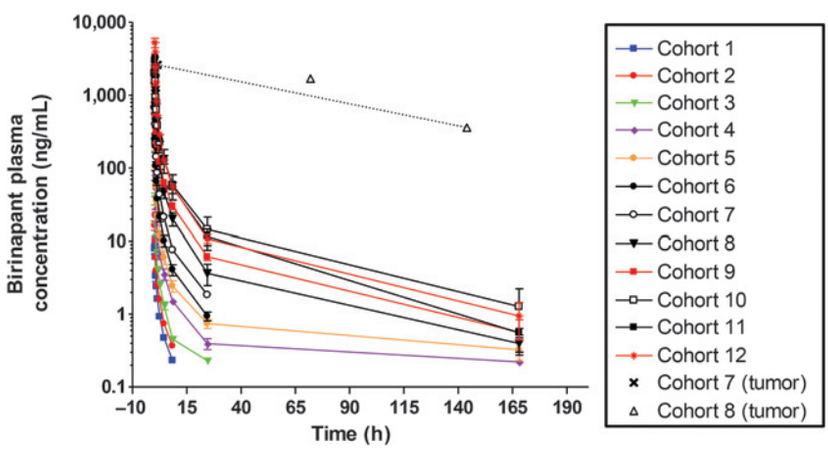

Figure 1.
Mean birinapant PK profiles in plasma at doses 0.18 to 63 mg/m^2 following a 30-minute i.v. infusion on day 1 (0–24 h). Note samples labeled as cohort 7 (tumor) and cohort 8 (tumor) represent results from tumor biopsies; x, level of birinapant from a tumor biopsy taken from a patient in cohort 7 (11.5 mg/m^2) 1.5 hours after infusion of birinapant with the dose level adjusted to that of cohort 8 (17 mg/m^2). Open triangles, level of birinapant in tumor biopsies obtained from 2 patients in cohort 8 (17 mg/m^2) taken at 72 and 144 hours.

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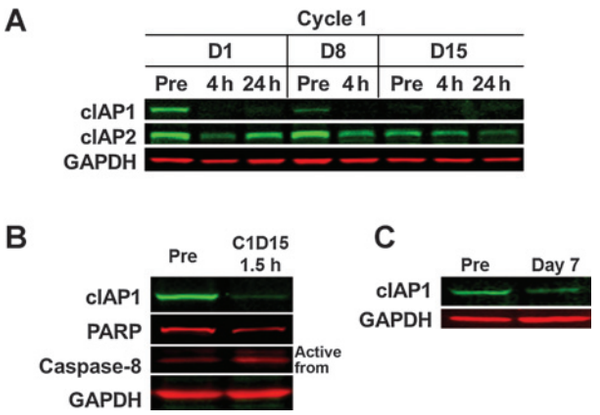

Figure 2.
A, Western blot analysis in PBMC of cIAP1, the principal target of birinapant, and cIAP2. GAPDH loading is shown as a control. Samples were taken prior to (Pre) and 4 hours and 24 hours posttreatment on days 1 and 15, and prior to and 4 hours posttreatment on day 8 during the first treatment cycle. B, Western blot analysis for paired tumor biopsy samples from a patient at dose level 11.5 mg/m^2 is shown. One biopsy was taken prior to drug administration (Pre), and the second was taken 1.5 hours after drug infusion on cycle 1 day 15 (C1D15 1.5 hours). These samples were analyzed for cIAP1, PARP (note decreased band intensity for both), caspase-8, and GAPDH (loading control). C, Western blot analysis for paired tumor biopsy samples from a patient at dose level 17.2 mg/m^2 is shown. One biopsy was taken prior to drug administration (Pre), and the second was taken 7 days after drug administration. These samples were analyzed for cIAP1 and GAPDH (loading control).

cancer treated with birinapant demonstrated radiographic evidence of tumor shrinkage.

Discussion

The results of this first-in-human, open-label, dose escalation study of birinapant demonstrated that birinapant was generally well tolerated at doses ranging from 0.18 to 47 mg/m^2. The most common AEs included fatigue, lymphopenia, nausea and vomiting, hypotension, headache, decreased appetite, and diarrhea.

A majority of AEs observed with birinapant at doses of 18 to 35 mg/m^2 were grade 1, 2, or 3 in severity and generally clinically manageable, and no DLTs were seen at these doses. DLTs at doses higher than this included hypophospatemia, macular rash, and amylase and lipase elevation in patients treated with 47 mg/m^2 and headache, nausea, and reversible cytokine release syndrome in patients treated with 63 mg/m^2. Bell's palsy was also reported in 2 patients treated with 63 mg/m^2 of birinapant.

The etiology of Bell's palsy following administration of birinapant is unclear. Bell's palsy may result from reactivation of VZV or HSV through an unknown mechanism (27, 28). VZV DNA is sometimes present in the blood of patients who develop Bell's palsy and in some asymptomatic patients with altered immune function. However, neither HSV nor VZV DNA was detected in the blood from the 2 patients who developed Bell's palsy in this study.

Despite presumptive diagnosis by study site investigators of transient cytokine release syndrome in several patients, there was no detectable increase in circulating blood cytokine levels nor was there evidence of a systemic inflammatory response in birinapant-treated patients. However, it is important to point out that the so-called transient cytokine release syndrome was primarily observed in patients treated with 63 mg/m^2, which exceed the MTD calculated for birinapant in this study. Thus, the etiology of Bell's palsy and "cytokine-release syndrome" in patients treated with high doses of birinapant is unknown and additional studies may be warranted.

PK analysis of birinapant demonstrated dose proportionality over the range of 0.18 to 63 mg/m^2. PK analysis suggests that birinapant is likely to behave predictably across patients in a dose-dependent manner. There was evidence of high volume of birinapant distribution that is indicative of extensive tissue uptake. This was confirmed by birinapant accumulation in tumor biopsies.

Birinapant at 11.5 and 17.2 mg/m^2 resulted in a greater than 75% reduction in cIAP1 in PBMC samples, and from biopsies taken from three different tumors (Fig. 2). The level of cIAP1 suppression is consistent with that observed previously in preclinical xenograft studies (13). Furthermore, suppression of cIAP1 was sustained in both PBMC and tumor tissue for at least 7 days after birinapant administration. Also, there was evidence of cell-death pathway activation (caspase-8 activation) and apoptosis (evidenced by PARP cleavage; Fig. 2). Together, these data support the on-mechanism and effect of birinapant as an IAP1 inhibitor.

The highest dose administered in the dose escalation portion of this study exceeded the anticipated dosing regimen based on the preclinical toxicology data and allometric scaling analysis. This was especially encouraging because of the concerns related to hypovolemic shock raised by dog toxicology studies. It is clear that dogs are more sensitive to IAP antagonists than humans (23, 29). There are several possible explanations for the safety and tolerability profiles exhibited by birinapant in this study as compared with other SMAC mimetics. First, birinapant is not a pan-IAP inhibitor, but is relatively selective for cIAP1 versus cIAP2. As such, it is more analogous to the endogenous molecule SMAC. Second, although birinapant effectively blocks caspase-inhibitory activity of XIAP *in vitro* (caspase de-repression), the ubiqutin ligase activity of XIAP appears to be spared (15). The sparing of either cIAP2 and/or XIAP likely contributes to the positive tolerability profile of birinapant (30). The tolerability profile is important as the antitumor activity of birinapant in preclinical models has been found to be increased when administered in combination with conventional chemotherapy or TNF-mediated cancer treatments. These observations, coupled with the results of this study, have provided the framework for a number of ongoing clinical trials evaluating the effectiveness of combinations of birinapant and other cancer therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R.J. Schilder, L.P. Martin, D.E. Weng, A.A. Adjei
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.K. Amaravadi, R.J. Schilder, L.P. Martin, M.A. Graham, D.E. Weng, A.A. Adjei
Writing, review, and/or revision of the manuscript: R.K. Amaravadi, R.J. Schilder, L.P. Martin, M. Levin, M.A. Graham, D.E. Weng, A.A. Adjei

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Other (interpretation of virologic data): M. Levin

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References

1. Hunter AM, LaCasse EC, Korneluk RG. The inhibitors of apoptosis (IAPs) as cancer targets. Apoptosis 2007;12:1543–68.
2. LaCasse EC, Mahoney DJ, Cheung HH, Plenchette S, Baird S, Korneluk RG. IAP-targeted therapies for cancer. Oncogene 2008;27:6252–75.
3. Ebert G, Allison C, Preston S, Cooney J, Toe JG, Stutz MD, et al. Eliminating hepatitis B by antagonizing cellular inhibitors of apoptosis. Proc Natl Acad Sci U S A 2015;112:5803–8.
4. Gyrd-Hansen M, Meier P. IAPs: from caspase inhibitors to modulators of NF-kappaB, inflammation and cancer. Nat Rev Cancer 2010;10:561–74.
5. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012;483:603–7.
6. Beug ST, Cheung HH, LaCasse EC, Korneluk RG. Modulation of immune signalling by inhibitors of apoptosis. Trends Immunol 2012;33:535–45.
7. Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer 2005;5:876–85.
8. Bertrand MJ, Milutinovic S, Dickson KM, Ho WC, Boudreault A, Durkin J, et al. cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Mol Cell 2008;30:689–700.
9. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, et al. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 2000;102:43–53.
10. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000;102:33–42.
11. Vince JE, Wong WW, Khan N, Feltham R, Chau D, Ahmed AU, et al. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell 2007;131:682–93.
12. SM C. The discovery and development of SMAC mimetics – small molecule antagonists of the inhibitor of apoptosis proteins. In:Macor JA, editor. Annual reports in medicinal chemistry 2011. Chapter 13. Washington, DC: American Chemistry Society; 2011.
13. Gaither A, Porter D, Yao Y, Borawski J, Yang G, Donovan J, et al. A Smac mimetic rescue screen reveals roles for inhibitor of apoptosis proteins in tumor necrosis factor-alpha signaling. Cancer Res 2007;67:11493–8.
14. Varfolomeev E, Blankenship JW, Wayson SM, Fedorova AV, Kayagaki N, Garg P, et al. IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. Cell 2007;131:669–81.
15. Condon SM, Mitsuuchi Y, Deng Y, LaPorte MG, Rippin SR, Haimowitz T, et al. Birinapant, a smac-mimetic with improved tolerability for the treatment of solid tumors and hematological malignancies. J Med Chem 2014;57:3666–77.
16. Fulda S. Smac mimetics as IAP antagonists. Semin Cell Dev Biol 2015;39:132–8.
17. Bai L, Smith DC, Wang S. Small-molecule SMAC mimetics as new cancer therapeutics. Pharmacol Ther. 2014;144:82–95.
18. Benetatos CA, Mitsuuchi Y, Burns JM, Neiman EM, Condon SM, Yu G, et al. Birinapant (TL32711), a bivalent SMAC mimetic, targets TRAF2-associated cIAPs, abrogates TNF-induced NF-kappaB activation, and is active in patient-derived xenograft models. Mol Cancer Ther 2014;13:867–79.
19. Carter BZ, Mak PY, Mak DH, Shi Y, Qiu Y, Bogenberger JM, et al. Synergistic targeting of AML stem/progenitor cells with IAP antagonist birinapant and demethylating agents. J Natl Cancer Inst 2014;106:djt440.
20. Carter BZ, Mak DH, Schober WD, Koller E, Pinilla C, Vassilev LT, et al. Simultaneous activation of p53 and inhibition of XIAP enhance the activation of apoptosis signaling pathways in AML. Blood 2010;115:306–14.
21. Krepler C, Chunduru SK, Halloran MB, He X, Xiao M, Vultur A, et al. The novel SMAC mimetic birinapant exhibits potent activity against human melanoma cells. Clin Cancer Res 2013;19:1784–94.
22. Simon R, Freidlin B, Rubinstein L, Arbuck SG, Collins J, Christian MC. Accelerated titration designs for phase I clinical trials in oncology. J Natl Cancer Inst 1997;89:1138–47.
23. Wong H, Budha NR, West K, Blackwood E, Ware JA, Yu R, et al. Dogs are more sensitive to antagonists of inhibitor of apoptosis proteins than rats and humans: a translational toxicokinetic/toxicodynamic analysis. Toxicol Sci 2012;130:205–13.
24. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
25. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, et al. Revised response criteria for malignant lymphoma. J Clin Oncol 2007;25:579–86.
26. Bonate PL. Pharmacokinetic-pharmacodynamic modeling and simulation. 2nd ed. New York/Dordrecht/Heidelberg/London: Springer; 2011. p. 157–86.
27. Levin MJ, Cai GY, Manchak MD, Pizer LI. Varicella-zoster virus DNA in cells isolated from human trigeminal ganglia. J Virol 2003;77:6979–87.
28. Levin MJ, Oxman MN, Zhang JH, Johnson GR, Stanley H, Hayward AR, et al. Varicella-zoster virus-specific immune responses in elderly recipients of a herpes zoster vaccine. J Infect Dis 2008;197:825–35.
29. Wong WW, Vince JE, Lalaoui N, Lawlor KE, Chau D, Bankovacki A, et al. cIAPs and XIAP regulate myelopoiesis through cytokine production in an RIPK1- and RIPK3-dependent manner. Blood 2014;123:2562–72.
30. Moulin M, Anderton H, Voss AK, Thomas T, Wong WW, Bankovacki A, et al. IAPs limit activation of RIP kinases by TNF receptor 1 during development. EMBO J 2012;31:1679–91.

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