A Phase I Study of ASTX660, an Antagonist of Inhibitors of Apoptosis Proteins, in Adults with Advanced Cancers or Lymphoma

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ABSTRACT

Purpose: This first-in-human, phase I study evaluated ASTX660, an oral, small-molecule antagonist of cellular/X-linked inhibitors of apoptosis proteins in patients with advanced solid tumors or lymphoma.

Patients and Methods: ASTX660 was administered orally once daily on a 7-day-on/7-day-off schedule in a 28-day cycle. Dose escalation followed a standard 3+3 design to determine the MTD and recommended phase II dose (RP2D). Dose expansion was conducted at the RP2D.

Results: Forty-five patients received ASTX660 (range 15–270 mg/day). Dose-limiting toxicity of grade 3 increased lipase with or without increased amylase occurred in 3 patients at 270 mg/day and 1 patient at 210 mg/day. The MTD was determined to be 210 mg/day and the RP2D 180 mg/day. Common treatment-related adverse events included fatigue (33%), vomiting (31%), and nausea (27%). Grade ≥3 treatment-related adverse events occurred in 7 patients, most commonly anemia (13%), increased lipase (11%), and lymphopenia (9%). ASTX660 was rapidly absorbed, with maximum concentration achieved at approximately 0.5–1.0 hour. An approximately 2-fold accumulation in AUC exposures was observed on day 7 versus 1. ASTX660 suppressed cellular inhibitor of apoptosis protein-1 in peripheral blood mononuclear cells, which was maintained into the second cycle beyond the off-therapy week at the 180-mg/day RP2D and above. Clinical activity was seen in a patient with cutaneous T-cell lymphoma.

Conclusions: ASTX660 demonstrated a manageable safety profile and exhibited evidence of pharmacodynamic and preliminary clinical activity at the 180-mg/day RP2D. The phase II part of the study is ongoing.

Introduction

The evasion of apoptosis is one of the hallmarks of cancer, with dysregulation of programmed cell death found in many tumor types (1). Inhibitors of apoptosis proteins (IAPs), such as cellular IAP (cIAP)-1 and -2, and X-linked IAP (XIAP), are key regulators of antiapoptotic and prosurvival signaling pathways. X-linked IAP directly inhibits caspases 3, 7, and 9, whereas cIAPs prevent formation of proapoptotic signaling complexes (2, 3). Thus, IAPs lead to suppression of apoptosis through both the extrinsic and intrinsic apoptotic pathways (4, 5). Deregulation of IAPs through amplification, overexpression, or loss of endogenous antagonists is often found in solid tumors and hematologic malignancies, and has been associated with tumor growth and progression, poor prognosis, and treatment resistance (2, 3, 6). As a result, IAPs are considered attractive therapeutic targets for anticancer treatment.

ASTX660 is a novel, potent, nonpeptidomimetic, small-molecule antagonist of both cIAP1/2 and XIAP, which was discovered using fragment-based drug design (7–10). ASTX660 inhibited intracellular degradation of cIAP1 in human breast cancer MDA-MB-231 cells with an IC_{50} of 0.22 nmol/L and blocked XIAP in a cellular XIAP-caspase 9 immunoprecipitation assay with an IC_{50} of 2.8 nmol/L (8). ASTX660 inhibited proliferation and induced apoptosis in multiple cancer cell lines; these effects were dependent on the presence of an inflammatory stimulus (10–12). Preclinical pharmacokinetic (PK) studies showed that ASTX660 was orally bioavailable and distributed into tumor xenografts, with detectable levels maintained in these tumors for up to 7 days after a single oral dose (10). ASTX660 inhibited tumor growth in mice bearing human MDA-MB-231 and A375 tumors over a range of oral doses and schedules, including an intermittent dosing schedule of 7 days on and 7 days off treatment. In all models, ASTX660 appeared to be well tolerated, with no evidence of excessive weight loss or significant adverse effects.

A first-in-human phase I–II study is evaluating ASTX660 in patients with advanced solid tumors or lymphoma. Herein, we present results from the phase I portion of the study. The phase II portion is currently enrolling.

Patients and Methods

Patients

Men or women ages ≥18 years were eligible if they had histologically or cytologically confirmed advanced solid tumors or lymphoma that was metastatic or unresectable and for whom standard life-prolonging measures were unavailable. Eligible patients also had to

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Translational Relevance

This first-in-human study of ASTX660, an orally bioavailable, fragment-derived, small-molecule antagonist of inhibitors of apoptosis proteins, demonstrates that dosing using a 7-day-on/7-day-off schedule is feasible in heavily pretreated patients with advanced solid tumors or lymphoma. At the recommended phase II dose of 180 mg/day, ASTX660 had a manageable safety profile, achieved exposure in the biologically active target range associated with activity in preclinical xenograft models, produced sustained biologic activity as measured by cellular inhibitor of apoptosis 1 suppression in peripheral blood mononuclear cells, and exhibited evidence of clinical activity in a patient with cutaneous T-cell lymphoma.

have Eastern Cooperative Oncology Group performance status 0–2 and acceptable organ function (defined as alanine aminotransferase and aspartate aminotransferase $\leq$2 times the upper limit of normal [ULN], total serum bilirubin $\leq$1.5 times ULN, absolute neutrophil count $\geq$1,500 cells/mm^3, platelet count $\geq$100,000 cells/mm^3, and serum creatinine $\leq$1.5 times ULN or creatinine clearance $\geq$50 mL/min). Women of childbearing potential and men with female partners of childbearing potential agreed to use two highly effective contraceptive measures during the study and for $\geq$3 months after completing treatment.

Patients with life-threatening illness, poor medical risk, or significant organ system dysfunction (in addition to the qualifying malignancy under investigation) were excluded, as were those with history of heart disease, grade $\geq$2 neuropathy, brain metastases (unless stable or previously treated), known mental illness or substance abuse, known history of HIV infection, seropositive results consistent with active hepatitis B or C virus infection, or left ventricular ejection fraction <50%. Women who were pregnant or breastfeeding were also excluded. Prior treatment with cytotoxic chemotherapy or radiotherapy within 3 weeks, mAbs within 4 weeks, or investigational drugs within 2 weeks (or 5 half-lives) was not allowed. Resolution of all toxicities related to prior treatment to grade $\leq$1 was required.

Study design

This open-label, 2-part (dose-escalation and -expansion) phase I study of ASTX660 was conducted at 4 U.S. centers from July 14, 2015 to February 6, 2018 (data cutoff date). The study was conducted in accordance with ethical principles originating in the Declaration of Helsinki, and in compliance with International Conference on Harmonisation Good Clinical Practice guidelines and applicable regulatory requirements. The study protocol was approved by an institutional review board or independent ethics committee prior to initiating the study. All patients provided written informed consent before participating in any study-related procedures. The study was registered with ClinicalTrials.gov, number NCT02503423.

Approximately 54 patients were planned for enrollment in this study. The dose-escalation portion of the study used a standard 3+3 dose-escalation design. Patients were screened for eligibility up to 21 days before starting ASTX660 treatment and then received ASTX660 once daily for 7 consecutive days every other week (i.e., days 1–7 and 15–21) of each 28-day cycle. The starting dose was 15 mg/day, which was escalated in a stepwise manner until the recommended phase II dose (RP2D) was determined. A data and safety review committee (DSRC) evaluated emerging data, discussed any safety concerns, and made recommendations to enhance patient safety and maximize chances for the study to meet its objectives. Decisions on dose escalation were made by the DSRC based on the occurrence of dose-limiting toxicities (DLT) during the first cycle at each dose level. If additional safety data were needed to better inform dose-escalation decisions, the DSRC was allowed to expand cohort size to six evaluable patients each until the RP2D was determined. Dose-limiting toxicities were defined as adverse events (AEs) causally related to study treatment that occurred in the first cycle, and included grade 4 thrombocytopenia (or grade 3 with clinically significant bleeding), febrile neutropenia, grade 4 neutropenia lasting >7 days, grade $\geq$3 cytokine release syndrome, liver-associated abnormalities, and any other grade $\geq$3 nonhematologic or grade 4 hematologic AE (except grade 3 nausea, vomiting, or diarrhea lasting <48 hours). The second half of the study was a dose-expansion phase in which an additional 6–9 patients were added to the cohort receiving the RP2D to supplement safety, PK, and pharmacodynamic (PD) data.

Patients continued to receive ASTX660 until disease progression, unacceptable toxicity, or withdrawn consent. Visits and assessments were frequent during cycle 1 (days 1, 2, 3, 7, 8, 9, 15, 16, and 22) and cycle 2 (days 1, 2, 3, 7, 8, 9, and 15), and less frequent thereafter (days 1, 8, and 15 of cycle 3, and days 1 and 15 of subsequent cycles). On permanent discontinuation of study treatment, patients underwent safety and efficacy evaluations at the treatment discontinuation visit and were encouraged to return for a 30-day safety follow-up visit.

During the dose-escalation portion of the study, two dosage forms of ASTX660 were used. Initially, a powder-in-bottle (PiB) formulation was used in which the contents of the bottle (ASTX660 400 mg) were reconstituted in SyrSpend SF (Fagron, Inc.) by the study center pharmacist. The reconstituted drug product was then divided into aliquots in single-use oral syringes and used within 14 days after reconstitution. The PiB formulation was used for cohorts at dose levels of 15, 30, 60, 120, and 180 mg/day. When available, a capsule formulation was introduced in a bridging cohort at 180 mg/day. Once sufficient and satisfactory PK, PD, and safety data had been obtained, the PiB formulation was abandoned and the capsule formulation (30 or 180 mg) was used in subsequent cohorts. The capsule formulation was used for cohorts at dose levels of 180, 210, and 270 mg/day, and for the dose-expansion cohort at 180 mg/day.

Endpoints

The primary endpoint was the incidence of DLTs and other AEs used to identify the MTD and RP2D. Secondary endpoints included PK parameters, duration of clinical response, progression-free survival, and percent degradation of cIAP1 in peripheral blood mononuclear cells (PBMCs) from baseline. Changes in circulating soluble markers of inflammation were evaluated as exploratory endpoints.

Safety

Safety was assessed throughout the study by patient-reported and investigator-observed AEs, clinical laboratory tests, vital signs, and 12-lead electrocardiograms; an echocardiogram or multigated acquisition scan was obtained at screening and the termination visit. Adverse events were captured from the time of the first dose until 30 days after the last dose or the start of an alternative treatment, whichever occurred first. Severity of AEs was graded according to NCI Common Terminology Criteria for Adverse Events 4.03 and the relationship of AEs to study treatment was judged by the investigator.

Pharmacokinetics

Whole blood (for plasma) was collected before, and 0.5, 1, 2, 3, 4, 5, 6, and 8 hours after dosing on days 1 and 7 of cycles 1 and 2. Additional samples were collected on days 2 and 3 (predose), and 8 and 9 (nondosing days), corresponding to 24- and 48-hour time points (trough levels). Patients fasted for 2 hours before and after ingesting study drug, but were allowed access to clear liquids. ASTX660 plasma samples were analyzed using a validated LC/MS-MS method with a dynamic range of 1–500 ng/mL. Pharmacokinetic parameters were estimated with standard noncompartmental methods using Phoenix WinNonlin 6.4 (Pharsight Corporation); the statistical analyses were performed via R version 3.5.1.

Pharmacodynamics

Whole blood was collected before, as well as 4 and 6 hours after, dosing on days 1 and 7 of cycles 1 and 2 to explore cIAP1 modulation, and release of cytokines and other inflammatory markers. Additional samples were collected on days 2 and 3 (predose), and 8 and 9 (nondosing days). For assessment of cIAP1, protein extracts were obtained from PBMC lysates and quantified using the bicinchoninic acid assay. A Meso Scale Diagnostics customized assay plate was developed and validated to quantify cIAP1 and assess relative change in protein concentration from baseline. For assessment of circulating markers of inflammation, plasma samples were evaluated for 46 analytes, including TNFα, IL6 and -8, and C-reactive protein, with Inflammation MAP v. 1.0 (Myriad RBM).

Efficacy

Antitumor response was assessed using computed tomography scan, MRI scan, or other appropriate disease evaluation methods. Overall response rate was determined by the investigator according to Response Evaluation Criteria in Solid Tumors v1.1 (RECIST1.1; ref. 13); confirmation of complete response (CR) or partial response (PR) was not required. Best response was defined as the best clinical response across all time points. Duration of response was calculated from the date of earliest response (CR or PR) until relapse or death, whichever occurred earlier. Duration of stable disease was calculated from the date of the first dose of ASTX660 until disease progression or death, whichever occurred earlier.

Statistics

Safety was assessed in all patients who received any dose of study drug. Efficacy was evaluated in all patients who received study drug, and had tumor evaluations at baseline and $\geq$1 postbaseline visit. For the PK and PD analyses, patients were included if they received study drug and their blood samples were collected and successfully analyzed. In the primary safety analysis, MTD was defined as the preceding dose level below which >33% of patients experienced a DLT; RP2D was defined as either the MTD or a dose below the MTD that the DSRC agreed showed adequate pharmacologic evidence of target engagement or clinical activity. Adverse events were coded according to the Medical Dictionary for Regulatory Activities Version 21.0 (MedDRA MSSO) and analyzed using descriptive statistics. Other safety parameters, as well as PK and PD parameters, were also analyzed descriptively. Objective response rate was calculated as the percentage of evaluable patients with best responses of CR or PR; disease control rate was calculated as the percentage with CR, PR, or stable disease. For both these rates, 95% Clopper–Pearson exact confidence intervals (CIs) were determined. Survival parameters were analyzed using the Kaplan–Meier method. SAS Version 9.4 (SAS Institute Inc.) was used to create tables and listings.

Results

Patient demographics and clinical characteristics

Forty-five patients received ASTX660 (37 in the dose-escalation cohorts and 8 in the dose-expansion cohort). At the time of the data cutoff, all patients had discontinued study treatment, mostly due to disease progression ($n = 34$; 76%). Two patients (4%) discontinued due to AEs, 3 (7%) withdrew consent, and the remaining 6 (13%) discontinued for other reasons. At the time of data cutoff, 31 patients (69%) had died, including 28 due to disease progression and 3 due to unknown causes.

Median age was 63 years (range 36–77; **Table 1**). Most patients were women (60%) and white (91%), and had an Eastern Cooperative Oncology Group performance status of 1 (76%). The majority of patients had solid tumors ($n = 43$; 96%), most commonly colorectal cancer (18%), head and neck cancer (13%), and gynecologic malignancy (ovarian or cervical cancer; 11%); 2 patients had lymphoma [follicular lymphoma and cutaneous T-cell lymphoma (CTCL; folliculotropic mycosis fungoides), respectively]. The patient with CTCL was assessed by the investigator through clinical examination and computed tomographic scans, and was found to not have measurable nodal or visceral disease at study entry. The study population was heavily pretreated; the median number of prior anticancer agents was 5 (range 1–15), with 31 patients (69%) having received $\geq$4 prior anticancer agents. Most patients had received prior radiotherapy (64%).

Table 1. Baseline patient demographics and disease characteristics.

Characteristic	RP2D (180 mg) cohort ($n = 17$)	Phase I total ($N = 45$)
Median age, years (range)	66 (36–77)	63 (36–77)
Sex, *n* (%)		
Male	9 (53)	18 (40)
Female	8 (47)	27 (60)
Race, *n* (%)[a]		
White	16 (94)	41 (91)
Asian	1 (6)	3 (7)
Multiple	0	1 (2)
Ethnicity, *n* (%)[b]		
Hispanic or Latino	5 (29)	8 (18)
Not Hispanic or Latino	12 (71)	36 (80)
Unknown	0	1 (2)
ECOG performance status, *n* (%)		
0	2 (12)	8 (18)
1	15 (88)	34 (76)
2	0 (0)	3 (7)
Tumor type, *n* (%)		
Colorectal cancer	1 (6)	8 (18)
Head and neck cancer	3 (18)	6 (13)
Gynecologic malignancy	1 (6)	5 (11)
Other	12 (71)	26 (58)
Prior anticancer agents, *n* (%)		
0–3	6 (35)	14 (31)
$\geq$4	11 (65)	31 (69)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
[a]One patient in 270-mg cohort was multiracial.
[b]Ethnicity unknown for 1 patient in 30-mg cohort.

Dose-limiting toxicities and MTD

The dose of ASTX660 was escalated in sequential cohorts from 15 to 180 mg/day using the PiB formulation and then from 180 to 270 mg/day using the capsule formulation (Supplementary Table S1). Overall, 16 patients received the PiB formulation and 29 received capsules. Dose-limiting toxicities occurred in 4 patients; all were laboratory abnormalities. At the 270-mg/day dose level, grade 3 DLTs of asymptomatic pancreatic enzyme elevation (increased lipase or hyperlipasemia with or without increased amylase) occurred in 3 of 6 patients. As a result, the dose was de-escalated to 210 mg/day for the next cohort, with a DLT of asymptomatic grade 3 increased lipase occurring in 1 of 9 patients. The lipase elevations recovered, however, to grade $\leq$1 with dose interruption, and 2 of the patients were successfully rechallenged with a lower dose. A total of 17 patients received ASTX660 at 180 mg/day (3 patients as PiB and 14 as capsules) with no DLTs reported. On the basis of the DLTs, 210 mg/day was determined to be the MTD and 180 mg/day was selected for the dose expansion cohort.

Safety

The median number of ASTX660 cycles received was 2 (range 1–15). Treatment delays were reported in 1 of 36 cycles at 180 mg/day and in 4 of 42 cycles at $\geq$210 mg/day. All 45 patients had AEs (Supplementary Table S2), and 37 (82%) had treatment-related AEs (**Table 2**). Overall, the most common AEs regardless of relationship to study treatment were fatigue (33%), vomiting (31%), and nausea (27%; Supplementary Table S2). The most common treatment-related AEs were nausea (22%), pruritus (18%), and vomiting (18%; **Table 2**). Mild–moderate treatment-related nausea and vomiting were reported commonly in the study. These AEs were managed with supportive and prophylactic measures [including the use of Listerine lozenges (Johnson & Johnson Consumer, Inc.) to ease the aftertaste of the PIB], and no cases were grade $\geq$3 or dose-limiting. Grade $\geq$3 AEs were reported in 27 patients (60%), most commonly anemia (13%), increased lipase (11%), and lymphopenia (9%; Supplementary Table S3). Seven patients (16%) reported treatment-related grade $\geq$3 AEs, the most common of which was increased lipase [$n = 4$ (7%)], followed by increased amylase, anemia, hyperlipasemia, hyponatremia, hypophosphatemia, and lymphopenia [$n = 1$ each (2%)]. One patient permanently discontinued ASTX660 due to a treatment-related AE (grade 3 lipase elevation in the 270-mg/day cohort). Two other patients discontinued due to AEs unrelated to study treatment (one had metastases to the brain, and the other had AEs of cough, dyspnea, acute respiratory failure, sepsis, and thoracic vertebral fracture). Serious AEs were reported in 17 patients, but none of these AEs was considered related to study drug. Two patients died as a result of a serious AE (large intestinal obstruction and sepsis, respectively) not considered related to study treatment.

Overall, 17 patients received ASTX660 at 180 mg/day, including 14 who received the capsule formulation. Grade $\geq$3 AEs were reported in 7 of these patients (41%), most commonly anemia (18%; Supplementary Table S3). The most common treatment-related AEs were fatigue (18%), pruritus (18%), dry mouth (12%), nausea (12%), and vomiting (12%; **Table 2**).

Tolerability as a measure of grade $\geq$3 AEs showed that treatment-related AEs were all laboratory abnormalities. At the ASXT660 60-, 120-, and 180-mg doses, there were no treatment-related grade $\geq$3 AEs.

Pharmacokinetics

Forty-three patients were included in the PK analyses. ASTX660 was rapidly absorbed following oral administration, with maximum concentration (C_{max}) achieved at approximately 0.5–1.0 hour. Mean plasma ASTX660 concentration–time curve exhibited a biphasic profile, however, with a secondary peak observed in some cohorts that was more prominent on day 7 than on day 1 (**Fig. 1**). The cause and nature of this secondary peak are not known at this time. There were no noticeable differences in PK profile between the PiB and capsule formulations at 180 mg/day, although data for the PiB at this dose were limited to 3 patients only.

Mild accumulation in drug levels based on AUC over 24 hours ($AUC_{0\text{-}24}$; but not on C_{max}) was observed on day 7 compared with day 1 of the first cycle (median accumulation ratio 2.07; **Table 3**). Elimination half-life was longer on day 7 versus 1, which was consistent

Table 2. Treatment-related adverse events occurring in $\geq$5% of total phase I population.

AE[a]	15 mg: PiB ($n = 3$)	30 mg: PiB ($n = 3$)	60 mg: PiB ($n = 4$)	120 mg: PiB ($n = 3$)	180 mg: PiB ($n = 3$)	180 mg: Cap ($n = 14$)	180 mg: PiB + Cap (total: $n = 17$)	270 mg: Cap ($n = 6$)	210 mg: Cap ($n = 9$)	Phase I total ($N = 45$)
Total treatment-related AEs, n	13	4	5	7	4	23	27	35	21	112
Patients who reported $\geq$1 treatment-related AE, *n* (%)	3 (100)	3 (100)	3 (75)	3 (100)	2 (67)	9 (64)	11 (65)	6 (100)	8 (89)	37 (82)
Nausea	1 (33)	0	1 (25)	0	0	2 (14)	2 (12)	2 (33)	4 (44)	10 (22)
Pruritus	0	0	1 (25)	2 (67)	0	3 (21)	3 (18)	0	2 (22)	8 (18)
Vomiting	3 (100)	0	0	0	1 (33)	1 (7)	2 (12)	2 (33)	1 (11)	8 (18)
Fatigue	0	0	1 (25)	0	0	3 (21)	3 (18)	1 (17)	1 (11)	6 (13)
Maculopapular rash	0	0	0	1 (33)	0	1 (7)	1 (6)	3 (50)	0	5 (11)
Anemia	0	0	1 (25)	0	0	1 (7)	1 (6)	1 (17)	1 (11)	4 (9)
Diarrhea	1 (33)	1 (33)	0	0	0	1 (7)	1 (6)	0	1 (11)	4 (9)
Increased lipase	0	0	0	0	0	0	0	2 (33)	2 (22)	4 (9)
Stomatitis	0	0	0	2 (67)	0	0	0	2 (33)	0	4 (9)
Dry mouth	0	0	0	0	1 (33)	1 (7)	2 (12)	1 (17)	0	3 (7)

Abbreviation: Cap, capsule.
[a]Patients are counted only once for each AE-preferred term.

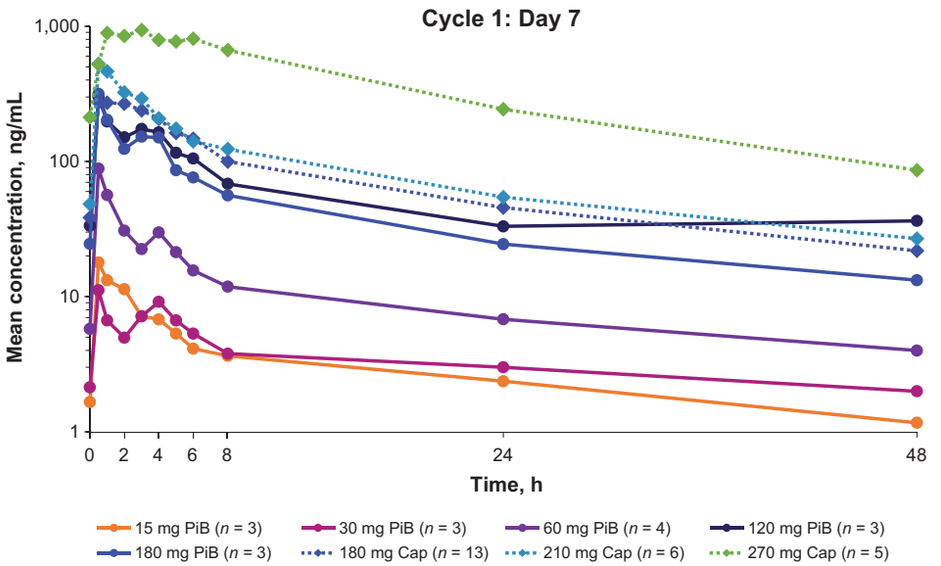

Figure 1.
PK profile of ASTX660: mean plasma ASTX660 concentrations in cycle 1: day 7. Cap, capsule.

with the accumulation observed for $AUC_{0\text{-}24}$. On the basis of a power model analysis (Supplementary Fig. S1; ref. 14), exposure to ASTX660 as measured by $AUC_{0\text{-}24}$ and C_{max} increased in a dose-proportional manner over the dose range from 15 to 180 mg/day (slopes 1.24 and 1.43, respectively) and supraproportionally from 180 to 270 mg/day (slopes 3.68 and 3.03, respectively). Consistent with this profile, apparent clearance and apparent volume of distribution decreased at the higher ASTX660 doses.

At the RP2D of 180 mg/day with the capsule formulation, mean $AUC_{0\text{-}24}$ values were 1,690 h·ng/mL on day 1 and 2,960 h·ng/mL on day 7; the corresponding mean C_{max} values were 468 and 482 ng/mL. Elimination half-life increased from 9.3 hours on day 1 to 18.0 hours on day 7. Pharmacokinetic parameters on days 1 and 7 of cycle 2 (Supplementary Table S4) were comparable with those seen in cycle 1, suggesting no continuous accumulation in exposures cycle over cycle. The accumulation in the linear range (doses from 15 to 180 mg) on day 7 versus 1 was consistent with the observed terminal half-life. At dose levels in the nonlinear/supraproportional range, there was evidence of decreased clearance, which was likely due to a saturation in metabolic/excretion pathways for ASTX660. This is currently being investigated. At the 180-mg/day dose, ASTX660 $AUC_{0\text{-}24}$ exposure reached the target active range based on data from preclinical models.

Pharmacodynamics

Cellular IAP1 levels were measured at baseline and cycle 1 for 37 patients, and through cycle 2 for 30 patients. Rapid and sustained cIAP1 degradation was observed following treatment with ASTX660, with the suppression maintained during cycle 2 at doses ≥180 mg/day (**Fig. 2**). Inflammatory analytes, including cytokines and other inflammatory markers such as TNFα, IL6 and -8, and C-reactive protein, were measured at screening and following treatment in plasma. Many cytokines and other inflammatory markers were undetectable (below the limit of quantitation of the assay) in plasma, and of those detected, no consistent trend from baseline was observed with increasing dose levels of ASTX660 (Supplementary Figs. S2–S5).

Efficacy

Of 35 evaluable patients, none had an objective response and 10 (29%) had a best response of stable disease. Median duration of stable disease was 150.5 days (95% CI, 49–349); 1 patient with chondrosarcoma who was treated with ASTX660 210 mg/day had stable disease lasting 418 days. Of 5 patients not evaluable by RECIST1.1, the 1 patient diagnosed with CTCL showed a clinically meaningful improvement in skin lesions through two cycles of treatment with the ASTX660 180-mg/day capsule formulation as assessed by the investigator (**Fig. 3**). For the entire phase I cohort, median

Table 3. Pharmacokinetic parameters on day 7 of cycle 1.

Dose (mg/day)	Formulation	Mean $AUC_{0\text{-}24}$, h·ng/mL (SD)	Mean C_{max}, ng/mL (SD)	Median T_{max}, h (range)	$t_{1/2}$, hours (SD)	Mean accumulation ratio for AUC (SD)
15 ($n = 3$)	PiB	107 (38.8)	18.5 (7.34)	0.50 (0.48–2.02)	26.1 (9.37)	3.25 (1.91)
30 ($n = 3$)	PiB	104 (33.6)	14.9 (3.42)	0.52 (0.50–3.98)	34.7 (7.70)	1.57 (1.10)
60 ($n = 4$)	PiB	378 (92.9)	88.6 (34.1)	0.50 (0.45–0.65)	23.1 (4.83)	2.24 (0.96)
120 ($n = 3$)	PiB	1930 (1260)	302 (152)	0.55 (0.50–0.58)	21.8 (2.76)[a]	2.09 (0.42)
180 ($n = 3$)	PiB	1650 (1360)	388 (378)	1.00 (0.50–3.13)	20.9 (5.02)	2.05 (0.58)
180 ($n = 13$)	Capsule	2960 (1720)	482 (307)	1.83 (0.50–4.30)	18.0 (4.25)	1.69 (0.68)
210 ($n = 6$)	Capsule	3470 (897)	696 (406)	1.03 (0.47–7.53)	19.1 (5.27)	2.37 (0.86)
270 ($n = 5$)	Capsule	13,400 (11,800)	1180 (526)	3.00 (1.00–6.07)	13.6 (3.54)	2.64 (1.00)

Abbreviations: $t_{1/2}$, elimination half-life; T_{max}, time to C_{max}.
[a]n = 2

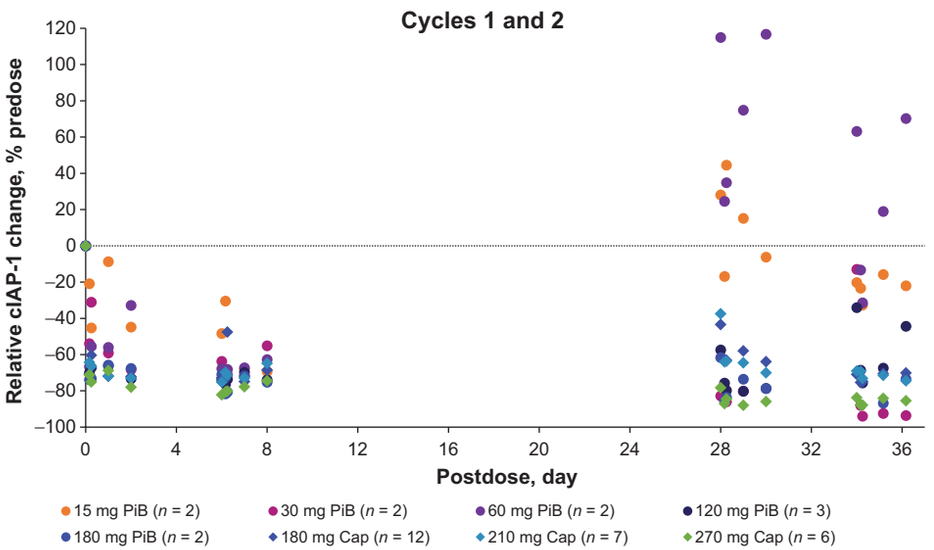

Figure 2.
Relative changes in cIAP1 protein levels in PBMCs in cycles 1 and 2 at the indicated doses. Cap, capsule.

progression-free survival was 55 days (95% CI, 54–56) and median overall survival was 265 days (95% CI, 174–389) at a median follow-up of 496 days.

Discussion

Inhibitors of apoptosis proteins have attracted significant interest as potential anticancer therapies, because of their roles in the evasion of apoptosis. Antagonists of IAPs have the potential to switch prosurvival signaling pathways in cancer cells toward cell death. Various second mitochondrial-derived activators of caspases (SMAC)-peptidomimetics with inherent cIAP1 selectivity have been tested clinically (4, 5, 15). ASTX660 is a potent, nonpeptidomimetic antagonist of cIAP1/2 and XIAP discovered using fragment-based drug design (7–10). A more balanced antagonism of cIAP1/2 and XIAP may offer advantages and a different profile compared with first-generation SMAC-mimetics, mainly by countering to some extent the redundancy of the activity of different IAPs in cancer cells.

The phase I portion of this study was designed to determine the MTD and RP2D, and assess the safety, PK, and PD of ASXT660 given orally on a 7-day-on/7-day-off schedule in patients with advanced solid tumors and lymphoma. The intermittent schedule was selected on the basis of preclinical mouse xenograft data demonstrating the 7-day-on/7-day-off schedule had similar efficacy to continuous daily dosing, with an improved therapeutic window. In the dose-escalation portion of the study, ASTX660 was well tolerated at doses $\leq$180 mg/day. The most common treatment-related AEs were fatigue, vomiting, and nausea. Overall, nausea and vomiting were manageable with supportive and prophylactic measures, and no cases of grade $\geq$3 were reported in the study. No treatment-related serious AEs or treatment-related deaths were reported.

Small-molecule antagonists of IAPs may induce proinflammatory changes secondary to their mechanism of action. Inhibition of IAPs leads to activation of the NF-κB pathway and, subsequently, to increased production of inflammatory cytokines (2, 3). The ensuing systemic inflammation may present a potential for inflammatory liver injury and other symptoms of an inflammatory response, such as cytokine release syndrome (15, 16). ASTX660 given in an intermittent 7-day-on/7 day-off schedule optimized during preclinical development resulted in a manageable safety profile. Liver injury, cytokine release syndrome, and Bell's palsy that were reported to be dose limiting in phase I studies of monovalent and bivalent SMAC mimetic compounds were not reported in this study (4). Dose-limiting toxicities in this study all consisted of laboratory abnormalities (grade 3 increased lipase with or without increased amylase). Amylase and lipase elevations were also reported with other agents in this class (4),

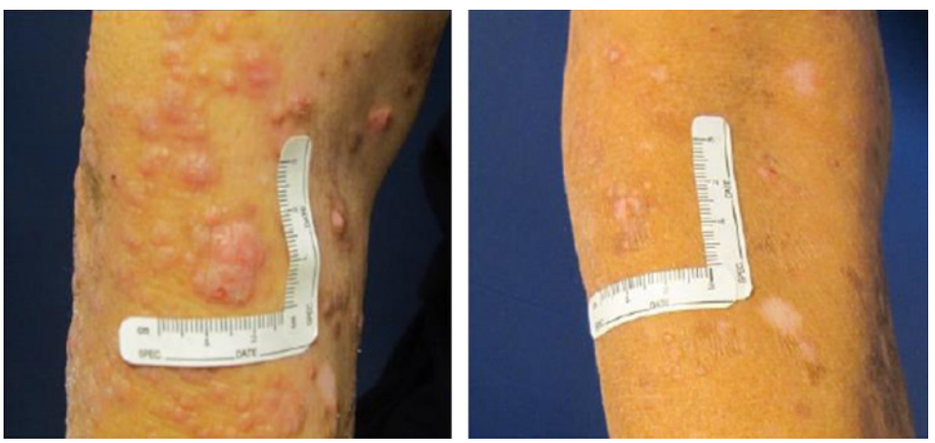

Figure 3.
Skin photographs of patient with CTCL at baseline (left) and after 2 cycles of treatment with ASTX660 180 mg/day (right).

and were expected on the basis of nonclinical toxicology findings with ASTX660. No AEs of clinical pancreatitis were reported in this study.

The PK of ASTX660 was characterized by rapid absorption, dose proportionality over the dose range of 15–180 mg/day, and approximately 2-fold accumulation based on $AUC_{0\text{-}24}$ on day 7 versus 1. At doses >180 mg, exposures increased in a greater than dose-proportional manner. No further accumulation was evident when the PK on day 7 of cycle 2 was compared with day 7 of cycle 1. Importantly, ASTX660 exposure levels at the 180 mg/day dose reached the target therapeutic exposure range that had been established previously in murine xenograft models (10). A capsule formulation of ASXT660 was introduced in a bridging cohort during the dose-escalation stage of the study. The solid dosage formulation had an acceptable bioavailability, safety, and PK/PD profile, and thus was chosen for continued development.

The PD of ASTX660 was characterized by a rapid and robust degradation of cIAP1 in PBMCs, which was maintained during the week off treatment and during cycle 2 at doses $\geq$180 mg/d. This finding demonstrates the ability of ASTX660 to engage the target cIAP1 and maintain suppression of cIAP1 beyond the week on treatment. No consistent trends or substantial changes from baseline were seen in systemic cytokine levels or other systemic inflammatory markers. Target engagement and proinflammatory changes in tissue will be explored in pre- and on-treatment tumor biopsies collected in the phase II portion of the study.

The rationale for developing ASTX660 was based on its novel profile and preclinical single-agent efficacy in various tumor models (8, 10, 12). Although no objective responses were seen in patients with solid tumors in this phase I study, a clinical response was observed in a patient with CTCL who did not have measurable disease by RECIST1.1 and stable disease was achieved in 10 of 35 evaluable patients with solid tumors. Two patients with lymphoma were enrolled in the phase I portion of the study: 1 with CTCL at the 180-mg/day dose and 1 with follicular lymphoma at the 270-mg/day dose.

On the basis of the available data from this phase I study, it was concluded that ASTX660 180 mg/day demonstrated an acceptable tolerability profile, a satisfactory PK profile with systemic exposure levels that reached the target efficacious therapeutic exposure range observed in nonclinical models, and rapid and sustained on-target PD effects with preliminary evidence of clinical activity in a patient with CTCL. Taken together, these data supported the selection of ASTX660 180 mg/day dosed on a 7-day-on/7-day-off schedule as the RP2D for solid tumors and lymphomas. The phase II portion of this study is ongoing, and preliminary evidence of clinical activity has been demonstrated in patients with relapsed/refractory peripheral T-cell lymphoma and CTCL (17). Enrollment into these two phase II cohorts continues.

In summary, the manageable safety profile, achievement of sustained target engagement, and preliminary evidence of clinical activity support further clinical development of ASTX660 in patients with solid tumors and lymphomas. In addition, the ability of ASTX660 to augment activity of other anticancer therapies in numerous preclinical models suggests that this agent may have clinical utility when combined with other treatment modalities (10, 18–22).

Disclosure of Potential Conflicts of Interest

P. M. LoRusso is an unpaid consultant/advisory board member for AbbVie, Agios, Five Prime, Genmab, Halozyme, Roche-Genentech, CytomX, Takeda, SOTIO, Cybrexa, Agenus, Tyme, IQVIA, TRIGR, Pfizer, I-Mab, ImmunoMet, Black Diamond, Salarius, GlaxoSmithKline, QED, AstraZeneca, EMD Serono, Astellas, Silverback, and MacroGenics. A. C. Mita reports receiving speakers bureau honoraria from Genentech. R. Ferraldeschi is an employee of Astex Pharmaceuticals. H. Keer is an employee of Astex Pharmaceuticals. A. Oganesian is an employee of Astex Pharmaceuticals. S. Jueliger is an employee of Astex Pharmaceuticals. A. W. Tolcher is a paid consultant or advisory board member for AbbVie, AbGenomics, ADC, Adagene, Agenus, Aerobiotix, Ascentage, AxImmune, Bayer, BioInvent, Boston BioProducts, Cello Health, EMD Serono, Elucida, Forbius, Roche-Genentech, Gilde, HBM, Immunome, ImmunoMet, Ignyta, Mekanistic, Menarini, Mirati, Nanobiotix, NBE, Nuvalent, OSI, Partner, Pelican, Pfizer, Pieris Pharmaceuticals, Pierre Fabre, Seattle Genetics, Sesen, Symphogen, and TFS. No potential conflicts of interest were disclosed by the other authors.

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References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144:646–74.
2. Dubrez L, Berthelet J, Glorian V. IAP proteins as targets for drug development in oncology. OncoTargets Ther 2013;9:1285–304.
3. Fulda S, Vucic D. Targeting IAP proteins for therapeutic intervention in cancer. Nat Rev Drug Disc 2012;11:109–24.
4. Bai L, Smith DC, Wang S. Small-molecule SMAC mimetics as new cancer therapeutics. Pharmacol Ther 2014;144:82–95.
5. Derakhshan A, Chen Z, Van Waes C. Therapeutic small molecules target inhibitor of apoptosis proteins in cancer with deregulation of extrinsic and intrinsic cell death pathways. Clin Cancer Res 2017;23:1379–87.
6. Holcik M, Yeh C, Korneluk RG, Chow T. Translational upregulation of X-linked inhibitor of apoptosis (XIAP) increases resistance to radiation-induced cell death. Oncogene 2000;19:4174–7.
7. Chessari G, Buck IM, Day JE, Day PJ, Iqbal A, Johnson CN, et al. Fragment-based drug discovery targeting inhibitor of apoptosis proteins: discovery of a non-alanine lead series with dual activity against cIAP1 and XIAP. J Med Chem 2015; 58:6574–88.
8. Johnson CN, Ahn JS, Buck IM, Chiarparin E, Day JEH, Hopkins A, et al. A fragment-derived clinical candidate for antagonism of X-linked and cellular inhibitor of apoptosis proteins: 1-(6-[(4-fluorophenyl)methyl]-5-(hydroxymethyl)-3,3-dimethyl-1H,2H,3H-pyrrolo[3,2-b]pyridine-1-yl)-2-[(2R,5R)-5-

methyl-2([(3R)-3-methylmorpholin-4-yl]methyl)piperazin-1-yl]ethan-1-one (ASTX660). J Med Chem 2018;61:7314–29.
9. Tamanini E, Buck IM, Chessari G, Chiarparin E, Day JEH, Frederickson M, et al. Discovery of a potent nonpeptidomimetic, small-molecule antagonist of cellular inhibitor of apoptosis protein 1 (cIAP1) and X-linked inhibitor of apoptosis protein (XIAP). J Med Chem 2017;60:4611–25.
10. Ward GA, Lewis EJ, Ahn AS, Johnson CN, Lyons JF, Martins V, et al. ASTX660, a novel non-peptidomimetic antagonist of cIAP1/2 and XIAP, potently induces TNFa-dependent apoptosis in cancer cell lines and inhibits tumor growth. Mol Cancer Ther 2018;17:1381–91.
11. Mita M, LoRusso P, Gordon M, Oganesian A, Zhang X, Ferraldeschi R, et al. Abstract A091: Phase 1 study of the IAP inhibitor ASTX660 in adults with advanced cancers and lymphomas. Mol Cancer Ther 2018;17:A091.
12. Ward G, Chessari G, Johnson CN, Lewis J, Rich S, Thompson N, et al. Induction of apoptosis with a novel dual cIAP1/XIAP antagonists in models of melanoma. Eur J Cancer 2014;50:122.
13. 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.
14. Smith BP, Vandenhende FR, DeSante KA, Farid NA, Welch PA, Callaghan JT, et al. Confidence interval criteria for assessment of dose proportionality. Pharm Res 2000;17:1278–83.
15. Amaravaldi RK, Schilder RJ, Martin LP, Levin M, Graham MA, Weng DE, et al. A phase 1 study of the SMAC-mimetic birinapant in adults with refractory solid tumors or lymphoma. Mol Cell Ther 2015;14:2569–75.
16. Infante JR, Dees EC, Olszanski AJ, Dhuria SV, Sen S, Cameron S, et al. Phase 1 dose-escalation study of LCL161, an oral inhibitor of apoptosis proteins inhibitor, in patients with advanced solid tumors. J Clin Oncol 2014;32:3103–10.
17. Mehta A, Hollebecque A, Foss F, Lister J, Mita M, Wagner-Johnston N, et al. Preliminary results of ASTX660, a novel non-peptidomimetic cIAP1/2 and XIAP antagonist, in relapsed/refractory peripheral T-cell lymphoma and cutaneous T-cell lymphoma [poster]. Available from: https://astx.com/wp-content/uploads/2019/06/2019_ASTX660_poster_EHA_abst-PS1073_Mehta.pdf.
18. Crawford NT, Stott K, Smyth T, Lyons J, Ferraldeschi R, Ward G, et al. Entinostat sensitizes colorectal cancer cell lines to the IAP antagonist, ASTX660 by down-regulating FLIP expression [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14-18; Chicago, IL. Abstract nr 4399.
19. Ward G, Miura A, Smyth T, Muraoka H, Lyons J, Hashimoto A, et al. Combining NAE inhibition and IAP antagonism leads to apoptosis through enhanced NF-kB inhibition in DLBCL cells and demonstrates potent anti-tumor activity in a preclinical DLBCL model. Eur J Cancer 2018;103S1:e38.
20. Smyth T, Nakatsuru Y, Fujita R, Ward G, Bevan L, Lewis J, et al. The dual IAP antagonist, ASTX660, increases the anti-tumor activity of paclitaxel in preclinical models of triple-negative breast cancer *in vivo* [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research 2016; 2016 Apr 16-20; New Orleans, LA. Philadelphia (PA): AACR; 2016. Abstract nr 1287.
21. Smyth T, Tsuji S, Tanaka G, Nakatsuru Y, Lyons J, Thompson N. ASTX660, a dual XIAP and cIAP antagonist, potentiates the anti-PD-L1 antibody therapy in mouse tumor models [abstract]. In: Proceedings of the AACR Special Conference on Tumor Immunology and Immunotherapy; 2016 Oct 20-23; Boston, MA. Philadelphia (PA): AACR; 2017. Abstract nr A33.
22. Xiao R, Allen CT, Tran L, Patel P, Park S-J, Chen Z, et al. Antagonist of cIAP1/2 and XIAP enhances antitumor immunity when combined with radiation and PD-1 blockade in a syngeneic model of head and neck cancer. OncoImmunology 2018;7:e1471440.