Oral Poly(ADP-Ribose) Polymerase-1 Inhibitor BSI-401 Has Antitumor Activity and Synergizes with Oxaliplatin against Pancreatic Cancer, Preventing Acute Neurotoxicity

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Abstract

Purpose: Development of novel agents and drug combinations are urgently needed for treatment of pancreatic cancer. Oxaliplatin belongs to an important class of DNA-damaging organoplatinum agents, useful in pancreatic cancer therapy. However, increased ability of cancer cells to recognize and repair DNA damage enables resistance to these agents. Poly (ADP ribose) polymerase-1 is a sensor of DNA damage with key roles in DNA repair. Here, we report the therapeutic activity of the poly (ADP ribose) polymerase-1 inhibitor BSI-401, as a single agent and in combination with oxaliplatin in orthotopic nude mouse models of pancreatic cancer, and its effect on oxaliplatin-induced acute neurotoxicity.

Experimental Design: We determined in vitro the effect of BSI-401 and its synergism with oxaliplatin on the growth of pancreatic cancer cells. Activity of different dosages of parenteral and oral BSI-401, alone and in combination with oxaliplatin, was evaluated in orthotopic nude mouse models with luciferase-expressing pancreatic cancer cells. The effect of BSI-401 in preventing oxaliplatin-induced acute cold allodynia was measured in rats using a temperature-controlled plate.

Results: BSI-401 alone and in synergism with oxaliplatin significantly inhibited the growth of pancreatic cancer cells in vitro. In nude mice, i.p. [200 mg/kg once a week (QW) × 4] and oral [400 mg/kg days 1-5 of each week (QD5 + R2) × 4] administration of BSI-401 significantly reduced tumor burden and prolonged survival (46 versus 144 days, \( P = 0.0018 \); 73 versus 194 days, \( P = 0.0017 \)) compared with no treatment. BSI-401 combined with oxaliplatin had potent synergistic antitumor activity (46 versus 132 days, \( P = 0.0063 \)), and significantly (\( P = 0.0148 \)) prevented acute oxaliplatin-induced neurotoxicity.

Conclusions: BSI-401, alone or in combination with oxaliplatin, is a promising new therapeutic agent that warrants further evaluation for treatment of pancreatic cancer.

Pancreatic adenocarcinoma is the fourth leading cause of adult cancer mortality in the United States. The 5-year survival rate is 1% to 3%, and median survival duration after diagnosis is <6 months (1). Single-agent gemcitabine is the standard treatment for advanced pancreatic cancer, but offers only a modest advantage in tumor-related symptoms and survival advantage (2). Thus, pancreatic cancer represents one of the greatest challenges in cancer treatment.

Oxaliplatin, a member of the organoplatinum compound family, is a useful drug in pancreatic cancer therapy. Like all platinum drugs, including cisplatin, oxaliplatin forms interstrand...
and intrastand platinum-DNA adducts or cross-links, inhibiting DNA replication and transcription. In addition, oxaliplatin induces a remarkably higher proportion of single-strand DNA breaks than cisplatin (3).

The increased ability of cancer cells to recognize and repair DNA damage is an important mechanism of resistance to DNA-damaging agents (4). Base excision repair is the primary DNA repair pathway that controls and corrects base lesions induced by oxidative damage, alkylation, deamination, depurination, and depyrimidination due to chemotherapeutic treatment, and poly (ADP ribose; PAR) polymerase (PARP-1) plays a key role in this process (5). PARP-1 is a chromatin-associated DNA-binding enzyme that uses NAD as a substrate to catalyze covalent transfer of ADP-ribose to a variety of protein acceptors. PARP-1 functions as a DNA damage sensor for both single- and double-stranded DNA breaks, and through its physical association with or by the poly(ADP-riboseyl)ation of partner proteins, it converts DNA damage into intracellular signals leading to DNA repair by the base excision repair pathway or cell death (6). Growing evidence has shown a role for PARP-1 in chromatin structure modulation, regulation of transcription, cell proliferation, and energy metabolism (7). Enhanced PARP-1 expression and activity have been found in several hematologic and solid tumors (8). Elevated levels of PARP-1 in cancer cells compared with normal cells are associated with drug resistance and overall ability to survive genotoxic stress (9). PARP-1 knockdown mice are hypersensitive to ionizing radiation and alkylating agents (10, 11). Overexpression of dominant-negative PARP-1 interferes with tumor formation as a result of tumor cell apoptosis in nude mice (12).

Numerous preclinical studies have shown that PARP-1 inhibitors can potentiate the in vitro and in vivo antitumor effects of chemotherapeutic agents and radiation (6), thus prompting interest in their clinical evaluation in treatment of several cancers (13). Interestingly, inhibition of PARP-1 activity has also been reported to protect against some side effects of cancer chemotherapeutic drugs, such as doxorubicin-induced cardiotoxicity (14, 15) and cisplatin-induced nephrotoxicity (16). Neurotoxicity is one of the major side effects and a common dose-limiting toxicity associated with oxaliplatin. An acute neurotoxicity that is unique to oxaliplatin manifests as paraesthesia and dysesthesia in the extremities, which are induced or exacerbated by exposure to the cold (17). Whether or not PARP-1 inhibition can antagonize oxaliplatin-induced acute neurotoxicities has not previously been described.

BSI-401 is a derivative of 6-iodo-5-amino-1,2-benzopyrone, a noncovalently binding PARP-1 inhibitor (18). Interestingly, 6-iodo-5-amino-1,2-benzopyrone also protects spinal cord neurons from the toxic effects of peroxynitrite, a key mediator in neuronal damage from spinal cord injury (19).

In this study, we showed the antitumor activity and therapeutic efficacy of BSI-401 as a single agent or in combination with oxaliplatin in vitro and in an orthotopic nude mouse model of pancreatic cancer. We present novel findings of PARP-1 potentiation of oxaliplatin-induced antitumor effects and inhibition of oxaliplatin-induced acute neurotoxicity.

Materials and Methods

Cell lines, culture methods, and reagents. The human pancreatic cancer cell lines COLO357FG and L3.6pl have been previously described (20). MiaPaCa-2, AsPC-1, and Panc28 human pancreatic cancer cell lines were purchased from the American Type Culture Collection. All cells were maintained as previously described (20). COLO357FG, L3.6pl, and MiaPaCa-2 cell lines expressing both Firefly luciferase and green fluorescence protein (GFP) were generated by infection with a modified lentiviral vector encoding Firefly luciferase and GFP as described previously (20). Firefly luciferase- and GFP-positive cells were selected by repeated flow cytometry sorting using a FACSort flow cytometer (BD Biosciences). A12 PARP-/- and A16 PARP-1+/+ murine embryonic fibroblasts (MEF) were obtained from Dr. Ya Wang (Thomas Jefferson University, Philadelphia, PA) and maintained as previously described (21).

BSI-401 was provided by BiPar Sciences, Inc. For in vitro assays, BSI-401 was dissolved in 100% DMSO at a stock concentration of 10 mmol/L. The concentration of DMSO did not exceed 0.1% in any assay. For in vitro studies, BSI-401 was dissolved in 100% DMSO or the SX-1292 oral vehicle (1% sodium carboxymethylcellulose, 0.5% sodium laurel sulfate, and 0.05% antifoam; Eli Lilly) and administered orally.

We used the cell proliferation enzyme-linked immunosorbent assay, BrdUrd colorimetric immunonassay (Roche) to obtain relative variable cell numbers. Synergy was assessed according to the method described by Chou and Talalay (22) using CalcuSyn software (Biosoft).

The activity of PARP in human pancreatic cancer cell lines and MEFs was measured using a universal colorimetric PARP assay kit ( Trevigen, Inc.).

Apoptosis. The extent of apoptosis was determined by fragmented DNA detection as previously described by Matassov et al. (23).

Western blot analysis. Human pancreatic cancer cell lines and MEFs were washed twice with cold PBS and lysed at 4°C into radiolabeled precipitation assay buffer [50 mmol/L Tris-HCl (pH 8), 150 mmol/L NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS]. The lysates were cleared by centrifugation. Each lysate (20 μg of protein) was separated by 8% SDS-PAGE and probed with a monoclonal mouse antibody against PARP-1, or a monoclonal mouse antibody against β-actin (Santa Cruz Biotechnology, Inc.). Immunoreactive bands were visualized using an enhanced chemiluminescence kit (Amersham Biosciences) according to manufacturer's instructions.

Translational Relevance

Because of the lack of effective therapies, only 1% to 4% of patients with pancreatic cancer will be alive 5 years after diagnosis. The DNA-damaging agent oxaliplatin has been shown to be useful but not effective in pancreatic cancer therapy. Recent findings suggest that pancreatic cancer is an attractive candidate for therapeutic strategies that target poly (ADP ribose) polymerase (PARP)-1, an enzyme with key roles in the repair of DNA damages such as those caused by oxaliplatin.

We provide an extensive preclinical characterization of BSI-401, a novel PARP-1 inhibitor with both oral bioavailability and strong antitumor potency. Our study is the first to show in vivo chemopotentiation of oxaliplatin through PARP inhibition. Moreover, we show the ability of BSI-401 to prevent cold allodynia, the dose-limiting toxicity associated with oxaliplatin.

These findings provide a strong support for the clinical development of BSI-401 as pancreatic cancer treatment, either as monotherapy or in combination with oxaliplatin. Prevention of the oxaliplatin-induced neurotoxicity could further improve the therapeutic index of this combination.
proteins were visualized with Lumi-Light Western blotting substrate (Roche) according to the manufacturer's instructions.

**Soft-agar colony formation assay.** On day 0, COLO357FG and MiaPaCa-2 cells (1.0 × 10^6 cells per well) were suspended in 0.5 mL of 0.6% Difco Bacto agar (Becton Dickinson) supplemented with complete culture medium. This suspension was layered over 0.5 mL of a 0.8% agar medium base layer in 24-well plates and treated on day 1. After 14 d, photographs of fluorescent colonies were taken under a Leica MZ16 stereoscopic fluorescence microscope (Leica Microsystems) equipped with a Hamamatsu Orca ER (C4742-95-12EB) cooled charge-coupled device digital camera (Hamamatsu Photonics) or with a cryogenically cooled IVIS 100 imaging system coupled to a data acquisition computer running Living Image software (Xenogen).

**Nude mouse orthotopic xenograft model.** A total of 151 female athymic nude mice (Ncr-nu) were purchased from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research Facility. The mice were housed and maintained in specific pathogen-free conditions. The facilities were approved by the Association for Assessment and Accreditation of Laboratory Animal Care and met all current regulations and standards of the U.S. Departments of Agriculture and Health and Human Services and the NIH. The mice were used in accordance with institutional guidelines when they were 6- to 8-wk-old. To produce pancreatic tumors, COLO357FG or I3.6pl cells were harvested from subconfluent cultures by brief exposure to 0.25% trypsin and 0.02% EDTA. Trypsinization was stopped with medium containing 10% fetal bovine serum, and the cells were washed once in serum-free medium and resuspended in serum-free HBSS. Only suspensions consisting of single cells with >90% viability were used for the injections.

The mice were anesthetized with a 1.5% isoﬂurane-air mixture. A small incision in the left abdominal flank was made, and the spleen was exteriorized. Tumor cells (1.0 × 10^6 cells in 50 μL of HBSS) were injected subcapsularly in a region of the pancreas just beneath the spleen. A 30-gauge needle, 1-mL disposable syringe, and calibrated, push button–controlled dispensing device (Hamilton Syringe) were used to inject the tumor cell suspension. A successful subcapsular intrapancreatic injection of tumor cells was identiﬁed by the appearance of a ﬂuid bleb without i.p. leakage. To prevent such leakage, a cotton swab was held for 1 min over the injection site. One layer of the abdominal wound was closed with wound clips (Auto-clip; Clay Adams). The animals tolerated the surgical procedure well, and no anesthesia-related deaths occurred.

All mice were weighed weekly and observed for tumor growth. Bulky disease was considered to be present when the tumor burden was prominent in the mouse abdomen (tumor volume, >2,000 mm^3). When at least three of ﬁve mice in a treatment group presented with bulky disease, the median survival duration for that group was considered to have been reached. At the median survival duration of the control group, the tumor growth in mice in all groups was evaluated using the bioluminescence emitted by the tumor cells. Bioluminescence imaging was conducted using a cryogenically cooled IVIS 100 imaging system coupled to a data acquisition computer running Living Image software (Xenogen). The mice were euthanized by carbon dioxide inhalation when evidence of advanced bulky disease was present; this was considered the day of death for survival evaluation.

**Analysis of PAR polymer formation in vivo.** Tumor samples were snap frozen in liquid nitrogen and stored at -80°C until homogenized, in preparation for use in the assay for the PAR formation as previously described (24). After sample analysis, tumor homogenates were assayed for total soluble protein content. The results of the PAR formation assay were then quantiﬁed as pmols PAR formed per microgram total soluble protein.

**Behavioral test.** Experiments were done on male Sprague-Dawley rats (Charles River), initially weighing 150 grams. Animals were housed three per cage under a 12-h light/dark cycle with water and food ad libitum. All efforts were made to minimize the number of animals used and their suffering. Cold allodynia was assessed using a water-cooled temperature-controlled plate equipped with a Plexiglas box to contain test animals (25–27). Rats were habituated to handling by the investigator and to the testing procedures during the week before the experiment. All tests were done before drug administration to assess baselines. The temperature of the cold plate was set at 4°C and allowed to stabilize for 20 min. The animal was then placed onto the cold plate, and the time to the ﬁrst brisk lift of the ipsilateral hindpaw was recorded. Locomotor movements were quite distinct, involving coordinate movement of all four limbs, and these were excluded. We interpreted the time to the brisk response as the latency for cold pain withdrawal. A maximum cutoff time of 300 s was used to prevent tissue damage. The researchers performing the behavioral studies were blinded to the treatment administered.

**Statistical analysis.** The results of in vitro proliferation and colony formation were expressed as means and SEMs for at least three independent experiments done in triplicate, and their statistical significance was determined by ANOVA.

The statistical signiﬁcance of differences in tumor growth was determined by one-way ANOVA and Dunnett's multiple comparison posttest; differences in survival duration were determined using a log-rank test. All statistical tests were two-sided, and a P value of <0.05 was used to indicate statistical signiﬁcance. All statistical analyses were done using GraphPad Prism software version 4.0c for Macintosh (GraphPad Software).

## Results

**BSI-401 preferentially inhibits in vitro growth of PARP-1-expressing cells.** To evaluate the role of PARP-1 expression on the in vitro cytotoxic activity of its inhibitor, BSI-401, we evaluated the growth rate of A12 (PARP−/−) and A16 (PARP-1+/+) MEF lines treated with escalating doses of BSI-401. Whereas the A16 PARP-1+/+ cells were sensitive to the cytotoxic activity of BSI-401, the A12 PARP−/− cells were twice as resistant, demonstrating a signiﬁcantly higher inhibitory concentration 50%, as indicated in Fig. 1A. These results suggest that PARP-1 protein is a preferential target for BSI-401.

**PARP-1 is expressed and overactivated in pancreatic cancer cell lines.** Single-agent BSI-401 shows antitumor activity in vitro and inhibits low-anchor tumor cell colony formation. To validate the target of BSI-401, we analyzed ﬁve pancreatic cancer cell lines and A12 PARP−/− and A16 PARP-1+/+ MEF lines for PARP-1 protein expression and constitutive poly(ADP-ribosyl)ating activity. As shown in Fig. 1B and C, the pancreatic cancer cell lines and the A16 MEF line showed similar levels of PARP-1 protein expression, but the pancreatic cancer cell lines had signiﬁcantly (P = 0.0009) higher levels of poly(ADP-ribosyl)ating activity than did the normal PARP-1+/+ fibroblasts.

To determine the cytotoxic activity of BSI-401 as a single agent in vitro, we treated each of the ﬁve pancreatic cancer cell lines for 24 hours with escalating doses of BSI-401. BSI-401 had potent cytotoxic activity on pancreatic cancer cell growth as monolayers (Fig. 1D).

To conﬁrm these results in an in vitro model that better represents the in vivo growth of pancreatic cancer cells, we evaluated the ability of GFP-labeled COLO357FG and MiaPaCa-2 pancreatic cancer cells to form colonies in soft agar in the presence of escalating doses of BSI-401. As shown in Fig. 1E, doses of 2.5 to 5 μmol/L completely suppressed the growth of pancreatic cancer cells in the presence of soft agar. These data showed that PARP-1 is an important target for which BSI-401 is effective in suppressing pancreatic cancer cell growth in vitro.
in vivo setting, we used two orthotopic nude mouse models with different metastatic growth patterns. Thirty mice \((n = 5\) per group) were orthotopically injected with COLO357FG (Fig. 2A-C) or L3.6pl (Fig. 2D-F) pancreatic cancer cells and randomly assigned to receive 25 or 100 mg/kg i.p. of BSI-401 on days 2 and 5 of each week. A vehicle only group (100% DMSO, i.p.) served as the control. Treatments were continued for 4 weeks. At the time of median survival duration of the control group (COLO357FG, 49 days; L3.6pl, 27 days), 25 mg/kg of i.p. BSI-401 had minimal activity on the growth of COLO357FG tumors (Fig. 2A); only 100 mg/kg resulted in significant antitumor activity in COLO357FG \((P = 0.0277; \text{Fig. } 2A)\) and L3.6pl \((P = 0.0168; \text{Fig. } 2D)\) pancreatic cancer cell models, prolonging the median survival durations to 99 (Fig. 2C) and 57 days (Fig. 2F), respectively.

We next determined the effect of different administration schedules on the antitumor efficacy of i.p. BSI-401 as a single agent, using the same weekly cumulative dose of 200 mg/kg. Twenty mice with orthotopic COLO357FG pancreatic tumors were randomly assigned to four groups \((n = 5\) per group) to receive on a weekly schedule: (1) 40 mg/kg of i.p. BSI 401 (days 1-5), (2) 100 mg/kg (days 2 and 5), (3) 200 mg/kg once weekly, or (4) a vehicle only control. Treatments were continued for 4 weeks. On day 46, the fractionated schedules (groups 1 and 2) showed strong antitumor activity, confirming previous results. The once-a-week bolus schedule (group 3) showed stronger antitumor activity (Fig. 3A), completely suppressing pancreatic tumor growth in all mice treated (Fig. 3B). This activity translated into a significant prolongation of the median survival duration, from 46 to 144 days \((P = 0.0018; \text{Fig. } 3C)\). Treatment with single-agent i.p. BSI-401 was well tolerated; no weight loss or other signs of acute or delayed toxicity were observed.

To determine the ability of BSI-401 to inhibit PARP activity in vivo, we analyzed the concentration of PAR polymer in COLO357FG pancreatic tumor xenografts harvested at different time points after a single injection with 200 mg/kg of i.p. BSI 401. Consistently with preliminary pharmacokinetic studies (data not shown), we observed a reduction at 4 hours, and a significant \((P = 0.0213)\) suppression of PAR polymer formation at 8 hours after the treatment with BSI-401, indicating that BSI-401 is able to inhibit the activity of PARP in vivo (Fig. 3D). A normal activity was restored at 24 hours.

BSI-401 administered orally as a single agent is safe and effective at decreasing the growth of pancreatic tumors in vivo. To determine the efficacy of BSI-401 when administered orally as a single agent, we first investigated its tolerability in the
COLO357FG model. Twenty-one mice (n = 3 per group) bearing orthotopic COLO357FG pancreatic tumors were randomly assigned to receive oral BSI-401 at increasing doses according to a Fibonacci-modified series, from 50 to 444 mg/kg on days 1 to 5, or its oral vehicle. Treatments were continued for 4 weeks. Oral BSI-401 was well tolerated in all groups (Supplementary Fig. S1). No weight loss or other signs of acute or delayed toxicity were observed, even at the highest doses.

Fig. 2. Effect of parenteral BSI-401 on the tumor growth and survival of athymic mice bearing orthotopic COLO357FG or L3.6pl/GLT pancreatic tumors. Fifteen mice (n = 5) bearing tumors were randomly assigned to be treated with BSI-401 i.p. as indicated, or DMSO as control. At the median survival duration of the control group, the tumor growth in the mice of all groups was evaluated on the basis of the bioluminescence emitted by the tumor cells. A and D, tumor volume following treatment, quantified as the sum of all detected photons within the region of the tumor per second. Columns, means; bars, SEM. *, P < 0.05 treatment versus control by one-way ANOVA and Dunnett’s multiple comparison posttest. B and E, mice bearing luciferase-expressing tumors. A digital grayscale image of each mouse was acquired, which was followed by the acquisition and overlay of a pseudocolor image representing the spatial distribution of detected photons emerging from active luciferase within the mouse. C and F, survival curve following treatment. Mice were sacrificed by carbon dioxide inhalation when evidence of advanced bulky disease was present. The day of sacrifice was considered the day of death for survival evaluation.
On the basis of these results, we next determined the in vivo antitumor activity of BSI-401 administrated orally at two doses and on three schedules. Forty mice with developed orthotopic COLO357FG pancreatic tumors were randomly assigned into eight groups (n = 5 per group) to receive oral BSI-401 on a weekly schedule (1) 200 mg/kg weekly, (2) 200 mg/kg (days 2 and 5), or (3) 200 mg/kg (days 1 to 5), (4) 400 mg/kg weekly, (5) 400 mg/kg (days 2 and 5), or (6) 400 mg/kg (days 1 to 5). As controls, two groups of mice were treated with (7) a clinically relevant dose of gemcitabine [25 mg/kg (days 2 and 5)] or (8) a vehicle only control (days 1-5). Treatments were continued for 4 weeks. At the median survival duration of mice in the control group (day 73), gemcitabine and low-dose BSI-401 had resulted in minor decreases in tumor volume; only mice treated with 400 mg/kg of oral BSI-401 2 or 5 days a week (groups 5 and 6) experienced a significant reduction in tumor burden (P = 0.0219 and 0.0171, respectively; Fig. 4A and B). Accordingly, only the mice in these two groups showed significantly prolonged median survival of up to 132 and 194 days in mice treated 2 or 5 days a week, respectively (P = 0.0198 and 0.0017, respectively; Fig. 4C). All regimens were well tolerated, and no weight loss or other signs of acute or delayed toxicity were observed (Supplementary Fig. S2).

**BSI-401 synergistically enhances oxaliplatin antitumor activity.** Chemopotentiation of DNA-damaging agents is a distinct property of several PARP-1 inhibitors. Therefore, we determined whether BSI-401 resulted in synergistic or additive antitumor in vitro activity in COLO357FG and MiaPaCa-2 pancreatic cancer cells in vitro when combined with the organoplatinum-compound oxaliplatin. We performed a combination analysis of BSI-401 and oxaliplatin at their equipotent ratio and generated a combination index-effect plot, according to the methods described by Chou and Talalay (22). In this mathematical model, combination index values of <1, 1, and >1 indicate synergy, additivity, and antagonism, respectively. In both COLO357FG (Fig. 5A and B) and MiaPaCa-2 (Fig. 5D and E) pancreatic cancer cells, BSI-401 synergized with oxaliplatin, especially at the higher doses used.

As compared with control untreated cells, a synergistic pro-apoptotic effect was observed when BSI-401 and oxaliplatin were combined at a dose of 5 μmol/L in COLO357FG (Fig. 5C), and in MiaPaCa-2 cells (Fig. 5F).

To further confirm these findings, we evaluated the ability of luciferase-labeled COLO357FG and MiaPaCa-2 cells to form colonies in soft agar in the presence of escalating doses of BSI-401, oxaliplatin, or both. As shown in Supplementary Fig. S3, combined doses of BSI-401 and oxaliplatin, ranging from 2.5 to 5 μmol/L, significantly (P < 0.0005) suppressed the growth of pancreatic cancer cells in low-anchorage conditions.

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**Fig. 3.** Effect of different schedules of administration on the efficacy of BSI-401 i.p. as single agent. Twenty athymic mice bearing orthotopic COLO357FG pancreatic tumors were randomly allocated (n = 5) to be treated with BSI-401 i.p. as indicated, or DMSO as control. A and B, tumor volume following treatment, quantified as the sum of all detected photons within the region of the tumor per second. Columns, means; bars, SEM. C, survival curves following treatment. Mice were sacrificed by carbon dioxide inhalation when evidence of advanced bulky disease was present. The day of sacrifice was considered the day of death for survival evaluation. D, in vivo levels of total PARP. Ten athymic mice bearing orthotopic COLO357FG pancreatic tumors were treated with a single injection of BSI 401 i.p. 200 mg/kg. Tumors from two mice were harvested at any time point as indicated. Each sample was analyzed in four replicates. Relative concentrations (dots) and means (lines) are shown. *, P < 0.05 treatment versus control by one-way ANOVA and Dunnett’s multiple comparison posttest.
Oral BSI-401 and oxaliplatin cooperate in inhibiting COLO357FG pancreatic tumor growth in vivo orthotopic xenografts. To determine the in vivo therapeutic potential of oral BSI-401 in combination with i.p. oxaliplatin, 20 mice with orthotopic COLO357FG pancreatic tumors were randomly assigned into four groups (n = 5 per group) to receive on a weekly schedule: (1) 400 mg/kg of oral BSI-401 (days 1-5), (2) 10 mg/kg (a clinically relevant dose) of i.p. oxaliplatin (day 3), (3) combination [BSI-401 (400 mg/kg; oral; days 1-5] and oxaliplatin (10 mg/kg; i.p.; day 3)], or (4) vehicles only as control. Treatments were continued for 4 weeks. At the median survival duration of mice in the control group (day 46), both oral BSI-401 and oxaliplatin resulted in measurably and similarly decreased pancreatic tumor volumes (Fig. 6A and B); however, only mice treated with oral BSI-401 experienced a significantly longer median survival duration (P = 0.0191; Fig. 6C).

A significant antitumor effect was observed in mice treated with both BSI-401 and oxaliplatin (P = 0.0088; Fig. 6B); pancreatic tumor growth was almost completely suppressed in all mice (Fig. 6A). This activity resulted in a significant prolongation of the median survival duration, from 46 to 132 days (P = 0.0063; Fig. 6C). This treatment was well tolerated; no weight loss or other signs of acute or delayed toxicity were observed.

BSI-401 prevents oxaliplatin-induced acute cold allodynia. To determine the effect of BSI-401 on oxaliplatin-induced cold allodynia, 12 rats were randomly assigned into four groups (n = 3 per group) to receive single doses of (1) BSI-401 (200 mg/kg i.p.), (2) oxaliplatin (5 mg/kg i.p.), (3) BSI-401 (200 mg/kg i.p.) and oxaliplatin (5 mg/kg i.p.), or (4) vehicles only as control. The latency for cold pain hind paw withdrawal was measured on days 0 and 6. Before treatment, all rats had shown withdrawal latency longer than the maximum cutoff time of 300 seconds (data not shown). As expected, oxaliplatin significantly
Fig. 5. Synergistic in vitro cytotoxic effect of BSI-401 and oxaliplatin in combination on COLO357FG and MiaPaCa-2 pancreatic cancer cells. A and D, relative effect of BSI-401, oxaliplatin, or both on tumor cells growth. On day 0, COLO357FG and MiaPaCa-2 cell lines were seeded at a density of 1.0 × 10^3 cells per well. On the following day, the cells were treated with increasing doses of BSI-401, oxaliplatin, or their combination. On day 2, the medium containing drugs was removed, the cells were washed twice with PBS, and fresh medium was added. After 5 d of incubation, the relative variable cell numbers was determined. DMSO-treated cells were assigned a value of 0%. Means and SEM of three independent experiments performed in triplicate are shown. B and E, combination index plot, representing a quantitative measure of the degree of drug interaction for a given end point of the effect measurement. The combination index (CI) values of <1, 1, and >1 indicate synergy, additivity, and antagonism, respectively. Points, mean of at least tree different replicate experiments. C and F, measurement of apoptosis by DNA fragmentation. On day 0, COLO357FG and MiaPaCa-2 cell lines were seeded at a density of 5 × 10^6 cells per dish. On the following day, the cells were treated with 5 μmol/L BSI-401, 5 μmol/L oxaliplatin, their combination, or DMSO as control. On day 2, the cells were collected, the DNA was extracted, and the fragmented DNA detected by agarose gel electrophoresis.
reduced the response latency to cold, starting 2 hours after the injection ($P = 0.0027$) and lasting until day 6 ($P = 0.0048$; Fig. 6D). BSI-401 alone did not induce cold allodynia, but significantly prevented both early (2 hours, $P = 0.0148$) and late (6 days, $P = 0.0048$) oxaliplatin-induced neurotoxicity when administered before oxaliplatin, restoring withdrawal latency to that of rats in the control group.

Discussion

Current therapies for managing pancreatic cancer lack efficacy, and an urgent need remains for the development of novel therapies. Our current study shows antitumor activity and therapeutic efficacy of the novel PARP-1 inhibitor BSI-401 as a single agent, both in vitro and in pancreatic cancer orthotopic nude mouse models.

Two strategies for the clinical development of PARP inhibitors as cancer therapy have emerged based on recent findings of molecular mechanisms of DNA repair (8). PARP inhibitors were initially developed only in combination with ionizing radiation or other anticancer agents, but some PARP-1 inhibitors have recently been reported to have single-agent activity against tumors with defects in homologous recombination (HR) repair due to deficiencies in BRCA-1 and BRCA-2 functions (28–30). These results have led to the evaluation of several PARP-1 inhibitors as single agents for the treatment of BRCA-deficient tumors (30, 31). However, it was recently shown that increased sensitivity to PARP inhibitors is not defined only by a BRCA-1 or BRCA-2 defects but also by deficiencies of several other proteins involved in HR repair (32), indicating that PARP inhibition is a useful strategy for the treatment of a wide range of tumors with defects in HR repair pathways. A recent genetic analysis of pancreatic cancer showed that this disease results from the genetic alteration of a large number of genes that can be clustered in a limited number of pathways and processes, including the ability to repair genomic damage. In particular,
genetic alterations in at least one gene involved in DNA damage control were found in 83% of pancreatic tumors, with the most representative genes involved in HR repair, such as ERCC4, RANBP2 (33), and TP53 (34). These findings suggest that pancreatic cancer is an attractive candidate for the development of advanced therapeutic strategies that target PARP-1.

We found that the novel PARP-1 inhibitor BSI-401, when used as a single agent, had a potent antitumor effect on five pancreatic cancer cell lines, both in monolayers and in low-anchorage conditions. We confirmed these results in vivo, clearly demonstrating that BSI-401 is a potent antitumor drug in two different clinically relevant orthotopic models of pancreatic cancer. In these models, the minimum dosage of i.p. BSI-401 that resulted in consistent and significant antitumor activity was 100 mg/kg twice a week. We divided this cumulative dose into three different schedules; the bolus schedule (200 mg/kg one weekly) resulted in impressive antitumor activity, with no signs of toxicity. At this dose, we showed a significant suppression of PARP activity in vivo after 8 hours from the treatment.

We next determined the efficacy of oral administration. Evaluation of two different doses and three different schedules of oral BSI-401 showed that the lowest effective oral dose was 400 mg/kg, twice weekly. BSI-401 was safe up to doses of 444 mg/kg/day, and no toxic or lethal doses were reached in the escalation study. On the basis of its efficacy, we propose the use of 400 mg/kg oral BSI-401, 5 days a week for 4 weeks, in further development of this drug.

The other and most typical setting for the development of PARP-1 inhibitors is the chemopotentiation of classic DNA-damaging agents. After the approval of gemcitabine in 1997 as the reference treatment for advanced pancreatic cancer (2), many large phase III trials were conducted on the use of cytotoxic-targeted agents alone and in combination with gemcitabine. Only combinations of gemcitabine and the tyrosine kinase erlotinib (35) and the orally administered precursor of 5-fluorouracil capecitabine (36) were shown to be superior to single-agent gemcitabine. Despite the encouraging results of a phase II trial, the gemcitabine-oxaliplatin combination did not show a statistically significant advantage in terms of overall survival compared with gemcitabine alone (37). First results from the Eastern Cooperative Oncology Group E6201 trial confirmed that neither the gemcitabine + oxaliplatin combination nor a fixed-dose rate infusion of gemcitabine induced significantly longer overall survival than standard gemcitabine (38). Therefore, novel agents and combination approaches are needed to increase the activity of oxaliplatin in pancreatic cancer therapy. Potentiation of chemotherapy by PARP-1 inhibitors has been shown for those agents that damage DNA by diverse mechanisms, including the DNA alkylating agent temozolomide (39–43), the topoisomerase I inhibitors topotecan and irinotecan (39–44), and the first-generation organoplatinum compounds cisplatin and carboplatin (43, 45, 46), but not the thymidylate synthase inhibitors or nucleoside analogues, such as gemcitabine (47).

To our knowledge, our present study was the first to evaluate the effect of PARP-1 inhibition on the efficacy of the third-generation platinum-based drug oxaliplatin. Our results show that BSI-401 strongly synergized with oxaliplatin to inhibit the growth of pancreatic cancer cell lines in vitro, both in monolayers and in low-anchorage conditions. Further evaluation of BSI-401 in an in vivo setting showed that oral BSI-401 administered on 5 days a week for 4 weeks potentiated the efficacy of oxaliplatin, administered at a dosage equivalent to that used in humans. The overall safety of parenteral and oral BSI-401 was also shown, both alone and with oxaliplatin.

It has been suggested that oxaliplatin affects neuronal voltage-gated Na+ channels (48), but its mechanisms of neurotoxicity are not fully understood. Increased PAR synthesis after acute neuronal injuries plays a crucial role in neurodegenera- tion (49); hence, the use of PARP-1 inhibitors has been proposed as an attractive strategy to decrease neuronal cell death in several models of acute and chronic neurodegenerative dis- eases (50). Following this rationale, we investigated the effect of BSI-401 on oxaliplatin-induced acute neurotoxicity and showed that BSI-401 prevented oxaliplatin-induced acute cold allodynia up to 6 days after treatment. This effect could further improve the therapeutic index of the BSI-401/oxaliplatin combination.

In conclusion, we provide an extensive preclinical characterization of BSI-401, a novel PARP inhibitor with both oral bioavailability and strong antitumor potency. We showed its antitumor efficacy as a single agent both in in vitro and in vivo pancreatic cancer models. To our knowledge, our study is the first to show in vivo chemopotentiation of oxaliplatin through PARP inhibition. Moreover, we showed the safety of BSI-401 and oxaliplatin and the ability of BSI-401 to prevent oxaliplatin-induced acute neurotoxicity. Our study shows that BSI-401 is a potent cancer therapeutic agent that warrants further development as pancreatic cancer treatment, either as monotherapy or in combination with oxaliplatin.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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2. Burris HA III, Moore MJ, Andersen J, et al. Im- portance of chemotherapy by PARP-1 inhibitors has been shown for those pancreatic cancer cell lines, both in monolayers and in low-an- chorage conditions. We confirmed these results in vivo, clearly demonstrating that BSI-401 is a potent antitumor drug in two different clinically relevant orthotopic models of pancreatic cancer. In these models, the minimum dosage of i.p. BSI-401 that resulted in consistent and significant antitumor activity was 100 mg/kg twice a week. We divided this cumulative dose into three different schedules; the bolus schedule (200 mg/kg one weekly) resulted in impressive antitumor activity, with no signs of toxicity. At this dose, we showed a significant suppression of PARP activity in vivo after 8 hours from the treatment.

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Oral Poly(ADP-Ribose) Polymerase-1 Inhibitor BSI-401 Has Antitumor Activity and Synergizes with Oxaliplatin against Pancreatic Cancer, Preventing Acute Neurotoxicity

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