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Excipient-free nanodispersion of 7-ethyl-10-hydroxycamptothecin exerts potent therapeutic effects against pancreatic cancer cell lines and patient-derived xenografts

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ABSTRACT

Irinotecan (CPT-11) is an anti-tumor drug and formulated as nanomedicines to reduce side effects and improve efficacy. *In vivo*, CPT-11 must be hydrolyzed by carboxylesterase to its active form 7-ethyl-10-hydroxycamptothecin (SN-38) to exert anti-tumor activity, but the lack of this enzyme in humans causes inefficient generation of SN-38. Thus, direct delivery of SN-38, not relying on carboxylesterase, will potentially achieve higher efficacy. However, it is difficult to effectively formulate SN-38 using current excipients due to its hydrophobicity and tendency to crystallize. Herein, we report the nanodispersion of SN-38 with its amphiphilic prodrug, CPT-11, as an effective treatment for pancreatic cancer (PC). SN-38 and CPT-11 formed stable nanoparticles without any other excipients, and showed potent cytotoxicity against PC cells *in vitro*, slowed tumor growth *in vivo*, namely subcutaneously and orthotopically xenografted mice, with minimal adverse effects, and prolonged their overall survival. Even in clinically-relevant patient-derived xenograft (PDX) models, the nanodispersion showed greater anti-tumor efficacy than CPT-11. Importantly, the nanodispersion directly released SN-38, resulting in carboxylesterase-independent anti-tumor activity, in contrast to carboxylesterase-dependent CPT-11. These characteristics may enable the excipient-free nanodispersion to exert potent therapeutic effects in patients.

1. Introduction

Pancreatic cancer is one of the most highly malignant and fatal cancers, with an extremely low 5-year survival rates largely because most patients are diagnosed at a relatively late stage [1,2]. A chemotherapy regimen consisting of a combination of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) is the primary treatment for pancreatic cancer. However, this treatment strategy results in a median overall survival of only 11.1 months, and is accompanied by severe side effects [3]. Thus, novel drugs that will improve the efficacy and reduce side effects in patients are urgently needed.

Nanomedicines were developed in an attempt to mitigate the

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adverse effects and enhance the efficacy of cancer treatments [4,5]. Irinotecan liposome (ONIVYDE injection), the first irinotecan nanomedicine to be approved for the treatment of pancreatic cancer in 2015, exerts slightly improved anti-tumor responses and shows reduced toxicity compared to free irinotecan, even though ONIVYDE delivers irinotecan more efficiently to tumors [6–8]. The primary reason for this is that irinotecan (CPT-11) is a prodrug of 7-ethyl-10-hydroxycamptothecin (SN-38) and thus must be hydrolyzed, via carboxylase-mediated catalysis to its active form SN-38, in order to exert its anti-tumor activity. However, humans, and particularly tumor cells, lack this enzyme, resulting in limited SN-38 generation and efficacy [9,10].

Thus, the direct delivery of free SN-38 would overcome the carboxylase dilemma in the clinic [11]. However, SN-38 is very difficult to formulate using existing excipients because of its strong hydrophobicity and tendency to crystallize. Many new materials and nanocarriers have been developed for SN-38 delivery, but these are considered new clinical entities and require extensive efforts and clinical trials prior to being approved for clinical use [12].

CPT-11 is a [1,4′-bipiperidine]-1′-carboxylate of SN-38 and thus has a strong affinity for SN-38 via π-π stacking [13]. Thus, amphiphilic CPT-11 can serve as a surfactant and help in the formulation of SN-38 into stable nanoparticles. The greatest advantage of this formulation is that it does not require new excipient materials, which facilitates clinical translation, and it has also been shown to be potent in colon cancer model [14]. Herein, we aim to investigate the therapeutic effects of CPT-11 against the challenging pancreatic cancer. In particular, we focused on clinically relevant orthotopic murine model of pancreatic cancer and patient-derived xenografts (PDX), which closely mirror the heterogeneity and anatomical histology of human tumors [15–17], with the goal of developing a more effective treatment for pancreatic cancer in the clinic.

2. Materials and methods

2.1. Nanoparticle synthesis and characterization

The SN-38/CPT-11 nanodispersion was produced as previously reported, at a 1:1 (w/w) ratio, and was denoted S1C1 [14]. Briefly, SN-38 and CPT-11 were dissolved in dimethyl sulfoxide (DMSO) with warming, followed by the addition of 5% glucose with ultrasonication. 10 mg/ml S1C1 was confirmed to be stable at 4 °C storage for several months. Hepes buffers with adjusted pH values using HCl and NaOH were applied to test the stability of S1C1 (1 mg/ml) under different pH conditions at room temperature.

2.2. Cell culture

Pancreatic cancer cell lines AsPC-1, BxPC-3, L3.6 pl, MIA PaCa-2, and PANC-1 were obtained from our institute. AsPC-1 and BxPC-3 cells were cultured in RPMI-1640 (Gibco 22400-089); PANC-1 and MIA PaCa-2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco 10569-010); L3.6 pl cells were cultured in Minimum Essential Medium α (MEM α, Gibco 12561-056). All culture media were supplemented with 10% fetal bovine serum (FBS, Gibco 16000-044) and antibiotics (100 IU/mL penicillin, 100 μg/mL streptomycin). All cells were grown in a humidified incubator with 5% CO_2 at 37 °C.

2.3. Immunohistochemistry

Standard protocols for immunohistochemistry were followed, as described previously [18]. Resected tissue was fixed in 4% formaldehyde solution, then embedded in paraffin. Sections underwent hematoxylin and eosin (H&E) staining to observe morphology. Anti-Ki67 (Abcam, ab15580) was used as a primary antibody at a final concentration of 2 μg/mL to evaluate cell proliferation.

2.4. Cell transfection

A lentivirus encoding luciferase was constructed by GeneChem, China. L3.6 pl cells were treated with the lentivirus at a multiplicity of infection (MOI) of 50, with 5 μg/mL polybrene, according to the manufacturer's instructions. After 48 h, puromycin was added to the culture medium to screen for stably transfected cells. The remaining cells were referred to as L3.6 pl-luc.

2.5. Cell viability assays

Drug cytotoxicity and drug-related effects on cell proliferation were determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) uptake method. All experiments were performed in triplicate. Briefly, 2000 cells per well were seeded onto 96 well-plates. Cells were exposed to increasing concentrations of S1C1 or CPT-11 for 48 h at 37 °C. MTT solution was added to each well and incubated for 30 min, followed by addition of extraction buffer (DMSO). Absorbance was measured at 570 nm. Half-maximal inhibitory concentration values (IC_{50}) were obtained after adjusting the data to log (inhibitor) vs. response curve using GraphPad Prism software.

2.6. Apoptosis assay

Cells in early and late apoptosis were quantified using flow cytometry (BD FACSCANTO II) following staining with Annexin V and propidium iodide (Annexin V, FITC Apoptosis Detection Kit, DOJINDO, AD10) according to the manufacturer's instructions. Analysis was performed using FlowJo v10 software.

2.7. Establishment of subcutaneous cell line-derived xenografts (CDX) in mice

To induce subcutaneous tumorigenesis, L3.6 pl cells (1×10^6 in 100 μL PBS) were injected subcutaneously into the upper right flank of nu/nu mice (4-6-week-old males). Tumor size was measured every 3 days using a Vernier caliper and volumes were calculated using the following formula: Volume = (Length × Width^2)/2. Diarrhea was graded as follows: Grade 0, normal stool; Grade 1, soft or wet stool; Grade 2, watery diarrhea without blood or mucus; Grade 3, watery diarrhea with mucus but without blood; and Grade 4, severe diarrhea with blood and mucus and no recovery [14,19].

2.8. Establishment of orthotopic CDX in mice

Nu/nu mice were anesthetized and placed in a right lateral position. A 5-mm incision was carefully made in the left abdomen using scissors. The spleen was pulled gently through the incision to fully expose the pancreas tail near the hilum of the spleen. L3.6 pl-luc cells (5×10^5 cells) in 50 μL media containing 20% Matrigel (Corning, 354234) were injected into the subcapsular region of the pancreas tail using sterile cotton swabs and ultrafine needles. After confirming that no leakage occurred at the injection site, the exposed spleen and pancreas tail were gently returned to the abdominal cavity, and the incision was sutured in one layer [20–23]. Mice were monitored continuously until they regained consciousness. To track tumor growth *in vivo*, anesthesia was induced via inhalation, and D-luciferin (Yeasen, 40901ES01) was injected *i. p.* 10 min prior to imaging.

2.9. Establishment of PDX in mice

Surgically resected pancreatic cancer tissue was harvested from patients and temporarily stored in Tissue Storage Solution (Miltenyi, 130-100-008). Patient consent was acquired in compliance with ethics committee guidance at our hospital. Tissues were cut into 1 mm pieces, then implanted subcutaneously into the upper right flank of nu/nu mice

(4-6-week-old males). Initiation and propagation were induced as previously described [24]. The generation harboring the patient-derived material was termed F0, with subsequent generations numbered consecutively (F1, F2, F3, F4, etc.). Passage of xenografts from one generation to the next was variable and could take several months. Generally, F3 and later generations were able to be expanded for use in evaluating drug treatments [25,26].

2.10. Drug treatment in murine models and monitoring

Mice with subcutaneous and orthotopic CDXs were treated identically to evaluate the effects of SN-38. Doses of S1C1 and CPT-11 used *in vivo* were equivalent to 15 mg/kg SN-38. For mice bearing pancreatic cancer CDXs, the first injection was given on the 9th day, and subsequent injections given every 3 days, with administration of the final dose on the 21st day. Bis-*p*-nitrophenyl phosphate (BNPP), a specific inhibitor of carboxylesterase used to achieve carboxylesterase inhibition, was injected 30 min before administration of S1C1 or CPT-11 [27–29]. All mice were monitored for 1 month, during which body weight and diarrhea score were recorded. After the final observation, all mice were sacrificed. For mice bearing PDXs, the first injection was given when the largest xenograft reached 1 cm in diameter, and subsequent injections were given every 3 days, with administration of the final dose on the 13th day. Animals were observed until the 28th day. At the end of the study, all mice were sacrificed by cervical vertebral dislocation. Tumors were isolated and frozen in liquid nitrogen or fixed in 4% formaldehyde solution for further analysis.

2.11. Statistical analysis

Results are presented as mean ± SEM. All statistical analyses were performed using GraphPad Prism v7. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Pancreatic cancer cells were more sensitive to S1C1 nanodispersion compared to free CPT-11 in vitro

The Z-average size of S1C1 (10 mg/ml) measured by dynamic light scattering (DLS) was 79.81 ± 7.03 nm with a polydispersity index (PDI) of 0.19 ± 0.01 (Fig. 1A). Transmission Electron Microscope (TEM) and Atomic Force Microscope (AFM) results further confirmed the formation of S1C1 nanoparticles (Fig. 1B), which is also consistent with our previous findings [14]. The pH value of S1C1 nanodispersion ranges from 6.0 to 7.0 when fabricated, which is acidic and close to commercial CPT-11 solution due to the existence of hydrochloric acid. When applied to Hepes buffers with different pH values (pH = 5, 6, 7, 8, respectively) at room temperature, S1C1 (1 mg/ml) showed stable size distribution at the buffers with pH about 6 and 7 (Fig. 1C). However, when pH went down to 5 or up to 8, size of nanoparticles became increasingly larger (Fig. 1C). *In vitro*, the cytotoxicity of S1C1 compared to that of CPT-11 was estimated in five pancreatic cancer cell lines (AsPC-1, BxPC-3, L3.6 pl, MIA PaCa-2 and PANC-1) using MTT assays (Fig. 1D–H). The IC50 values are summarized in Table 1 with their equivalence to SN-38. Both S1C1 and CPT-11 inhibited pancreatic cancer cell proliferation in a dose-dependent manner. The IC_{50} of S1C1 for AsPC-1 was 0.68 ± 0.31 μg/mL, whereas that of CPT-11 was 43.20 ± 9.52 μg/mL, which is 47 times more cytotoxic than SN-38. S1C1 was also 110-, 404-, and 150- fold more effective than CPT-11 against BxPC-3, L3.6 pl, and MIA PaCa-2 cells, while PANC-1 cells had only 4-fold better efficacy.

Cell apoptosis induced by S1C1 and CPT-11 was analyzed by flow cytometry and the quantified results are shown in Fig. 1I. S1C1 was found to be 3–6 times more effective at inducing apoptosis than CPT-11. These results demonstrate that S1C1 exhibits a better anti-tumor efficacy than CPT-11 *in vitro*, which led us to further investigate the efficacy of S1C1 *in vivo*.

3.2. S1C1 efficiently inhibited growth of subcutaneous and orthotopic pancreatic cancer CDXs

To further characterize S1C1, we compared the effects of S1C1 and CPT-11 on tumor xenografts *in vivo*. S1C1 showed a significant therapeutic effect against subcutaneous (*s.c.*) xenografts (Fig. 2A and B). Importantly, the administration route of S1C1 was a significant factor in determining its efficacy. *S. c.* injection was used as a positive control. Intraperitoneal injection (*i.p.*) produced similar results as *s. c.* administration, whereas intravenous (*i.v.*) injection was less effective. An *i. p.* injection of S1C1 reduced the tumor volume by 90.42%, whereas CPT-11 reduced it by 73.00%. Furthermore, *i. p.* S1C1 caused milder side effects, such as less weight loss and less severe diarrhea, than *i. p.* CPT-11 (Fig. 2C and D). As demonstrated in a previous study, unlike CPT-11, S1C1 did not induce any obvious organ damage [14].

Next, we established orthotopic xenografts by injecting pancreatic cancer cells into the pancreas tail to better mimic the complex microenvironment of pancreatic tumors [20,21]. The representative images in Fig. 3A show cancerous tissue originating from the pancreas tail (red arrow) near the spleen (green arrow) 9 days after inoculation. Furthermore, the injected cells were all located beneath the capsule of the pancreas, as shown by H&E staining, and had formed local tumors without visible metastasis. After 5 injections every 3 days starting from the 9th day, we performed imaging using D-luciferin and found that both CPT-11 and S1C1 significantly inhibited tumor growth, resulting in significantly smaller residual lesions compared to the negative control (Fig. 3B and C). Gemcitabine, a classic pancreatic cancer drug, was used as an additional control [30]. Quantitative imaging showed that *i. p.* injection of S1C1 inhibited tumor growth to a greater extent than *i. v.* injection (Fig. 3C), suggesting that *i. p.* administration may be the preferred route for S1C1. Immunohistochemical analysis of isolated xenografts showed that CPT-11 treatment resulted in greater residual lesions than S1C1 treatment, as demonstrated by Ki-67 staining (Fig. 3D). In addition, *i. p.* S1C1 caused milder diarrhea and less weight loss in orthotopically xenografted mice than *i. p.* CPT-11 or *i. v.* S1C1 (Fig. 3E and F).

3.3. Inhibition of carboxylesterase attenuated the therapeutic effect of CPT-11 but not S1C1

Although CPT-11 shows good therapeutic efficacy in treating tumors in murine models, this efficacy does not translate to patients because humans lack carboxylesterases. This means that even after 24 h, only 2–8% of the administered CPT-11 is converted to SN-38 [13]. More clinically relevant animal models are therefore needed in order to test whether S1C1 can potentially overcome this problem of insufficient carboxylesterase.

BNPP, a specific inhibitor of carboxylesterase, was administered to mice to mimic the human carboxylesterase insufficiency [28,29]. BNPP in the range of 25–200 mg/kg was shown to be safe and cause dose-dependent inhibition of CPT-11 activity in mice [31]. Tumor growth inhibition in subcutaneous xenografts (Fig. 4A) in response to *i. p.* CPT-11 was reduced by 13.67%, from 73.00% (Fig. 2B) to 59.33% (Fig. 4B) when 50 mg/kg BNPP was administered. In contrast, treatment with BNPP did not alter tumor growth inhibition following *i. p.* S1C1 (90.73% with (Figs. 4B) and 90.42% without (Fig. 2B) carboxylesterase inhibition). Similarly, tumor growth inhibition in orthotopic xenografts (Fig. 3B) in response to *i. p.* CPT-11 was partially attenuated by carboxylesterase inhibition (Fig. 4C). Moreover, mice treated with CPT-11 and BNPP were more likely to experience tumor relapse by the 27th day than those treated with S1C1 and BNPP, as demonstrated by quantified imaging every 6 days (Fig. 4D). This is similar to the greater residual lesions seen after treatment (Fig. 3D).

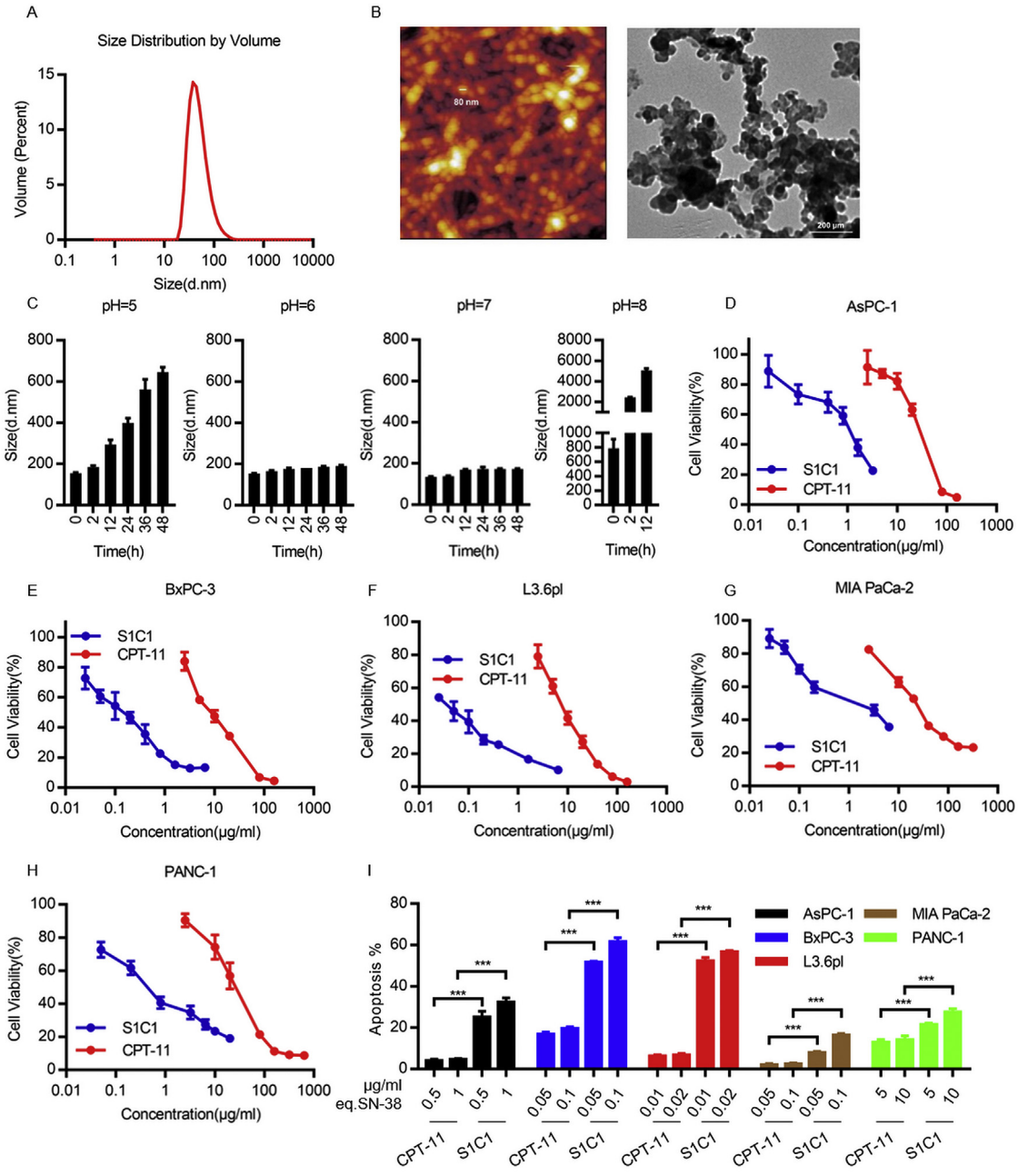

Fig. 1. Production, cytotoxicity, and proapoptotic effects of S1C1 on pancreatic cancer cell lines compared to CPT-11. (**A**) Particle size distribution of S1C1 as measured by dynamic light scattering (DLS). (**B**) Typical AFM (Atomic Force Microscope, left) and TEM (Transmission Electron Microscope, right) images of S1C1. (**C**) Particle size distribution of S1C1 (1 mg/ml) as measured by DLS in Hepes solution with different pH values, at room temperature. (**D-H**) MTT assay of pancreatic cancer cell lines AsPC-1, BxPC-3, L3.6 pl, MIA PaCa-2, and PANC-1 treated with S1C1 or CPT-11 (n = 3). (**I**) Proportion of apoptotic cells in pancreatic cancer cell lines (AsPC-1, BxPC-3, L3.6 pl, MIA PaCa-2, and PANC-) treated with S1C1 or CPT-11 (n = 3). *** $p < 0.001$.

Table 1
In vitro cytotoxicity comparison of CPT-11 and S1C1 nanodispersion on five pancreatic cancer cell lines. The cells were treated with drugs for 48 h before analysis by MTT assay. (mean ± SD, n = 3).

IC50(μg/ml)	CPT-11			CPT-11			S1C1			S1C1		
Pancreatic Cancer Cell Line				eq.SN-38						eq.SN-38		
AsPC-1	43.20	±	9.52	25.03	±	5.52	0.68	±	0.31	0.53	±	0.25
BxPC-3	9.62	±	0.47	5.58	±	0.27	0.07	±	0.05	0.05	±	0.04
PANC-1	44.84	±	6.70	25.99	±	3.88	7.01	±	3.22	5.53	±	2.54
L3.6 pl	6.98	±	0.63	4.04	±	0.36	0.02	±	0.01	0.01	±	0.01
MIA PaCa-2	15.57	±	8.80	9.03	±	5.10	0.08	±	0.02	0.06	±	0.01

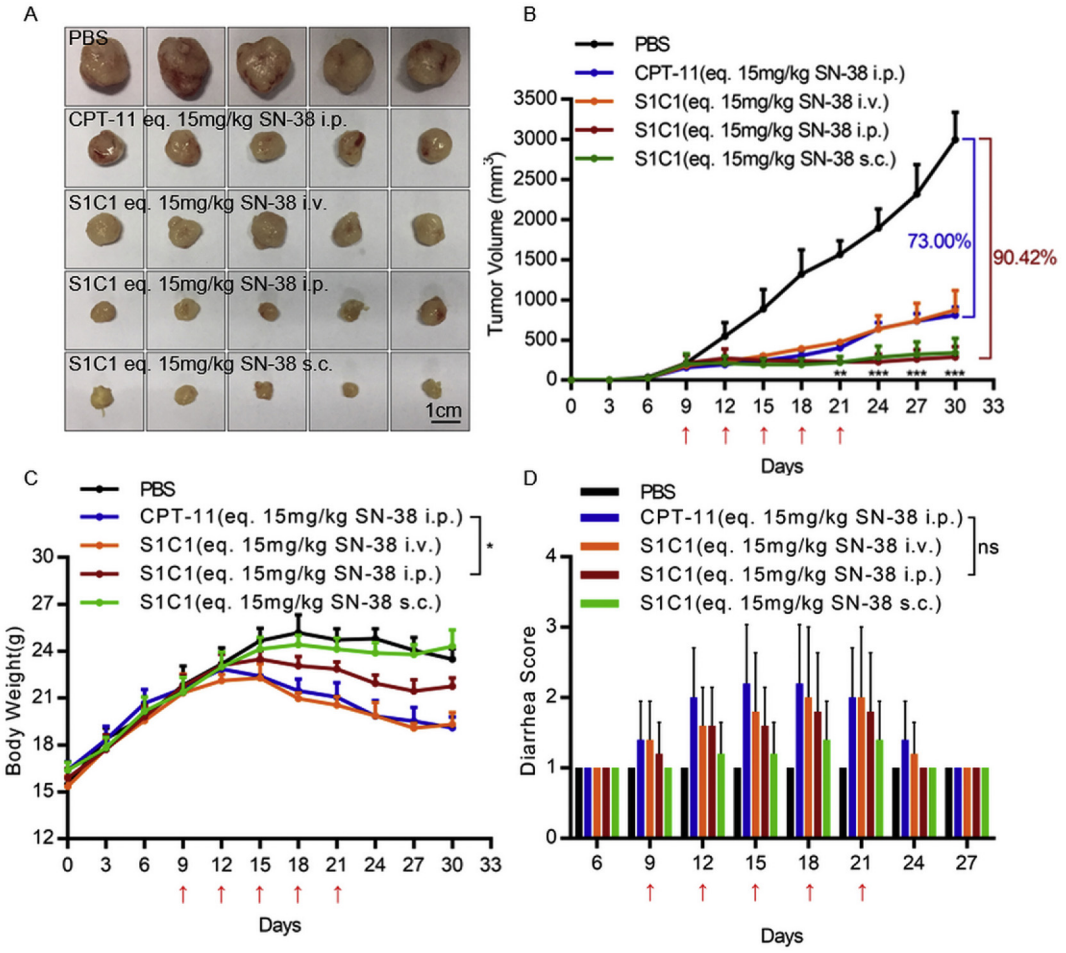

Fig. 2. Anti-tumor efficacy and associated side effects of S1C1 versus CPT-11 in subcutaneously xenografted mice with pancreatic cancer CDX. (**A**) Xenografts were resected from mice at the end the treatment (n = 5). Scale bar: 1 cm. (**B**) L3.6 pl xenografted tumor volume as a function of time after xenografted mice were treated with CPT-11 or S1C1, as indicated by red arrows (q3d × 5, n = 5). (**C**) Body weight and (**D**) diarrhea score of mice bearing subcutaneous xenografts during S1C1 and CPT-11 treatment (n = 5). * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

3.4. S1C1 improved the long-term outcome of mice with orthotopic pancreatic cancer

Having shown that treatment with CPT-11 and BNPP resulted in a reduced efficacy (Fig. 4A–C) and an increase in the potential for tumor relapse (Fig. 4D), we increased the number of mice and the duration of observation to determine if S1C1 could provide long-term benefits to orthotopically xenografted mice. Compared to a median survival of 43 days in the negative control group, the median survival time reached 86 days in the S1C1 group with carboxylesterase inhibition, and 61 days in the CPT-11 group (Fig. 4E). Six of fifteen mice in S1C1 group were still alive and in good physical condition on the 98th day, whereas the longest survival time in the CPT-11 group was 68 days. This shows that S1C1 provides better long-term outcomes in orthotopically xenografted mice than CPT-11.

As most patients with pancreatic cancer are diagnosed at relatively late stages [1], we also established murine models to determine if S1C1 might represent an effective treatment strategy for late-stage pancreatic cancer. Based on the median survival of 43 days in the negative control group (Fig. 4E), we implanted xenografts orthotopically into the pancreas tail on the first day and then gave the first injection of S1C1 on the 40th day, followed by four additional injections every 3 days thereafter. Due to advanced tumor stages, we chose to use S1C1 as a monotherapy to reduce the drug metabolism burden. Four of the six mice showed obvious tumor reduction (Fig. 4F), suggesting that S1C1 could also be effective in the treatment of late-stage pancreatic cancer.

3.5. S1C1 suppressed tumor growth of PDXs to a greater extent than CPT-11 in mice

In addition to differential carboxylesterase abundance, another reason for the generally high failure rate of new agents in the treatment of pancreatic cancer is the lack of pre-clinical models that recapitulate the heterogeneity of tumors in patients [15,32–34]. To test the effectiveness of S1C1 in a more clinically relevant model, we established a PDX murine model. As shown in Fig. 5A, F4 PDX mice perfectly mirrored the structures of pancreatic cancer as observed in the original patients, including the cavity (green arrow) and tumor stroma (red arrow), demonstrating an improvement of this model over pancreatic cancer CDX. The female patient was pathologically diagnosed with pancreatic adenocarcinoma in the pancreas head, as shown in Table 2. Due to the heterogeneity of xenografts, tissue blocks used for implantation from different parts of the original xenograft would inevitably result in variable features such as tumor growth rate [24]. After individual observation and normalization of tumor volume to the volume recorded on the first injection day, we showed that S1C1 suppressed tumor growth to a greater extent than CPT-11 with equivalent SN-38 (Fig. 5B and C). Furthermore, PDX mice treated with S1C1 experienced less weight loss (Fig. 5D) and milder diarrhea (Fig. 5E) than those that received CPT-11 injections. These findings suggest that S1C1 could be more clinically effective and cause fewer side effects than CPT-11 in the treatment of patients with pancreatic cancer.

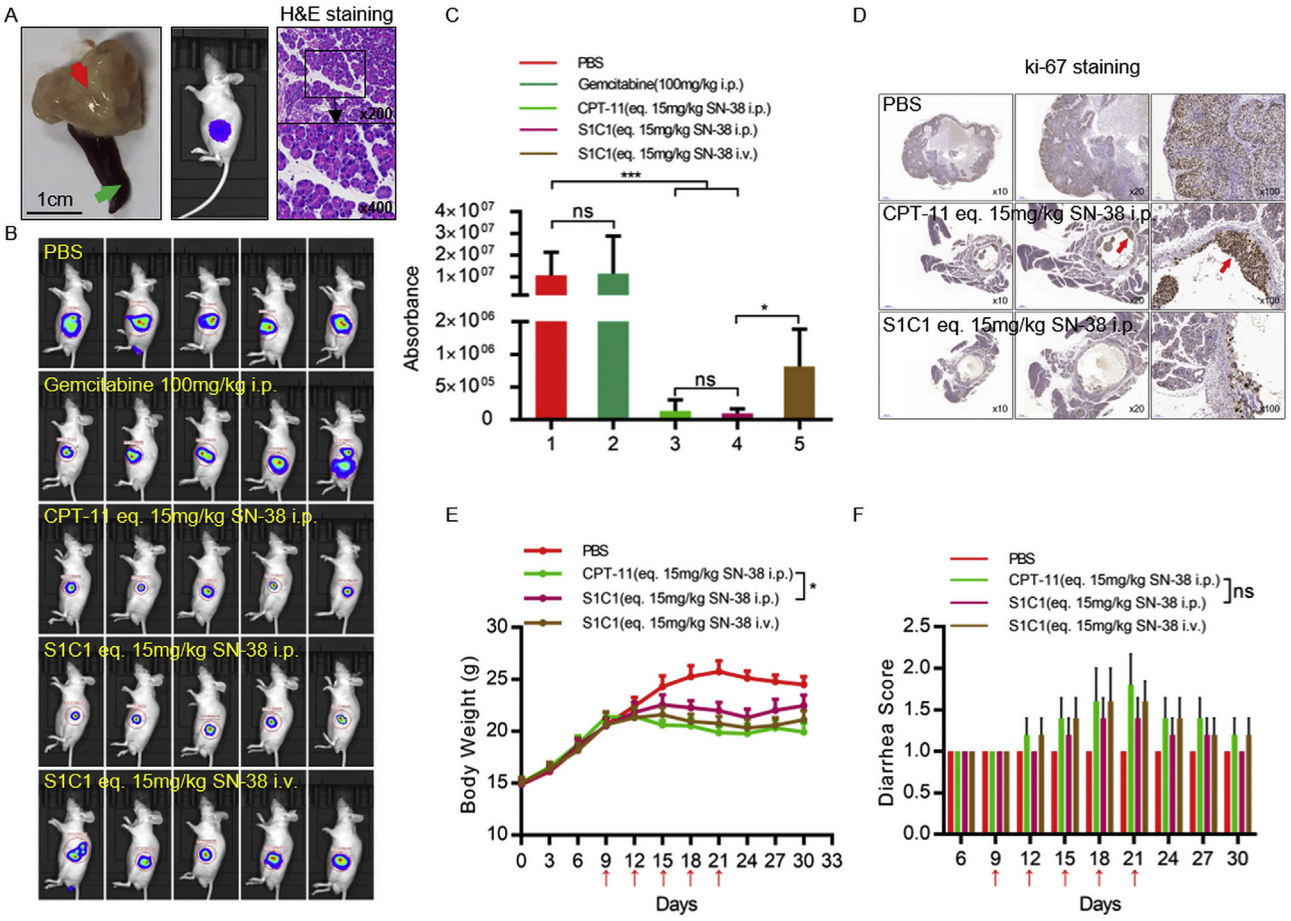

Fig. 3. Anti-tumor efficacy and associated side effects of S1C1 versus CPT-11 in mice bearing orthotopic pancreatic cancer CDX. (**A**) Establishment of orthotopically xenografted nu/nu mice. (**B**) *In vivo* imaging at the end the treatment (n = 5). (**C**) Quantified absorbance of L3.6 pl-luc xenografted tumor after nu/nu mice were treated with gemcitabine, CPT-11, or S1C1 (q3d × 5, n = 5). (**D**) Ki-67 staining of residual lesions (red arrows) after treatment with S1C1 or CPT-11. (**E**) Body weight and (**F**) diarrhea score of mice bearing subcutaneous xenografts during S1C1 and CPT-11 treatment (n = 5). * $p < 0.05$. *** $p < 0.001$.

4. Discussion

Pancreatic cancer is a highly malignant and fatal type of cancer, with the highest ratio of mortality to morbidity among all cancers [1,2]. The development of novel drugs is essential if the prognosis of pancreatic cancer is to improve. SN-38 is insoluble in most pharmaceutically acceptable solvents and excipients, making it difficult for a formulation to be achieved [11]. Excipient-free nanomedicines have shown potential for increasing the efficacy of drugs, reducing side effects, and allowing for better transition from animal studies to the clinic [4,12]. In the present study, we demonstrated that S1C1 excipient-free nanodispersion exerted potent therapeutic effects against pancreatic cancer *in vitro*. S1C1 showed tens or hundreds of fold higher efficacy in AsPC-1, BxPC-3, L3.6 pl and MIA PaCa-2 cells, but only 4-fold more in PANC-1 cells, an investigated chemo-resistant pancreatic cancer cell line [35,36]. Moreover, S1C1 inhibited tumor growth in both subcutaneously and orthotopically xenografted mice. Since S1C1 is comprised of equal masses of CPT-11 and SN-38, SN-38 is readily released when the nanoparticles reach the tumor site.

Subcutaneously xenografted mice are the primary model for the preclinical evaluation of new drugs. However, it lacks a pancreatic microenvironment, which is a significant limitation because stromal cells are known to influence tumor growth and treatment efficacy [21,37,38]. In orthotopically xenografted mice, S1C1 significantly slowed tumor growth, suggesting that this formulation could overcome stromal effects. CPT-11 was used as a control because it is currently used clinically and is also a component of S1C1. Despite being used as a control, CPT-11 is known to exhibit limited efficacy and is associated with severe side effects such as weight loss and intestinal damage [14]. The differential abundance of carboxylesterase activity between humans and mice is believed to be responsible for differences in CPT-11 efficacy [13]. Therefore, establishing a carboxylesterase inadequacy model in mice may provide a better prediction of the efficacy of S1C1 in human patients. To this end, we used BNPP, a carboxylesterase inhibitor, in mice to mimic carboxylesterase inadequacy in patients [28,29]. We found that S1C1 efficacy was relatively independent of carboxylesterase activity, whereas the efficacy of CPT-11 was significantly affected by carboxylesterase inhibition. Thus, S1C1 has the potential to overcome carboxylesterase inadequacy, which is currently a limiting factor for CPT-11 efficacy in patients.

The lack of pre-clinical models that recapitulate the heterogeneity of tumors in patients is another reason for the generally high failure rate of new therapeutic agents. We used PDX models in the current study and found that S1C1 suppressed tumor growth to a greater extent than CPT-11, indicating that S1C1 may be effective for the treatment of patients with pancreatic cancer, and may also facilitate clinical translation.

Clinical translation is an obstacle that all nanomedicines must overcome before they can be used clinically. Formulations containing carriers have a difficult path to approval due to a need for extensive validation [12]. As SN-38 is the active form of CPT-11, and CPT-11 is a drug that is already used clinically, approval for S1C1 was relatively straightforward because it does not contain any new components. Additional studies to evaluate greater quantities of PDX will be needed to

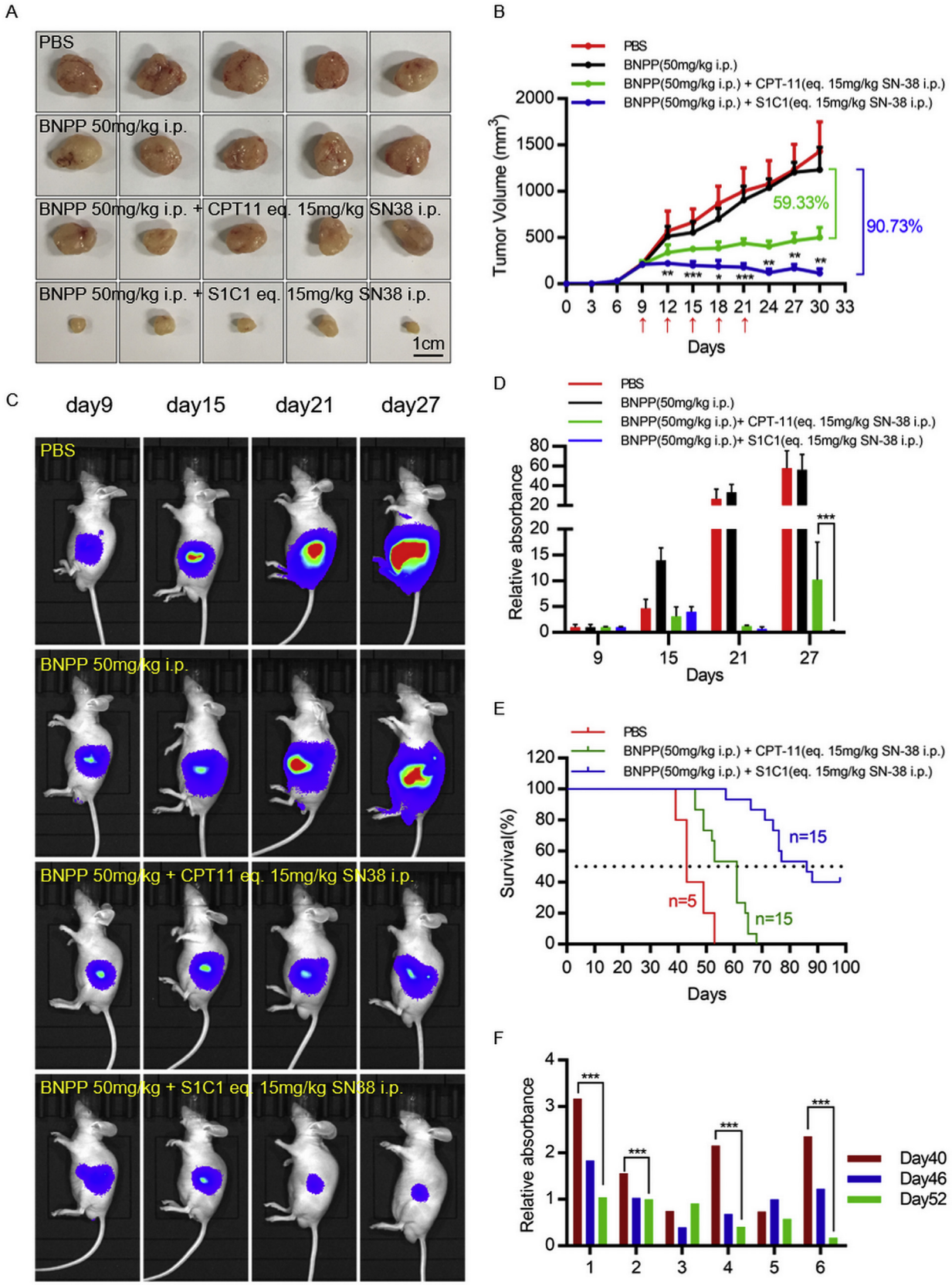

Fig. 4. Effects of carboxylesterase inhibition, which mimics human clinical carboxylesterase inadequacy, on mice bearing subcutaneous or orthotopic pancreatic cancer cell line-derived xenografts. (**A**) Xenografts were resected from mice after treatment courses had ended (n = 5). Scale bar: 1 cm. CPT-11 and S1C1 monotherapies are shown in Fig. 2. (**B**) L3.6 pl xenografted tumor volume as a function of time after mice were treated with BNPP and CPT-11 or S1C1, as indicated by red arrows (q3d × 5, n = 5). (**C**) Continuous *in vivo* imaging every 6 days. CPT-11 and S1C1 monotherapies are shown in Fig. 3. (**D**) Quantified absorbance of L3.6 pl-luc xenografted tumors after mice were treated with BNPP and CPT-11 or S1C1 (q3d × 5, n = 5). ***p < 0.001. (**E**) Survival after treatment with S1C1 or CPT-11 with concurrent carboxylesterase inhibition using 50 mg/kg BNPP. (**F**) Continuous *in vivo* imaging every 6 days of mice bearing orthotopic xenografts 40 days after inoculation. The first injection was given on the 40th day (q3d × 5). * p < 0.05. ** p < 0.01. *** p < 0.001.

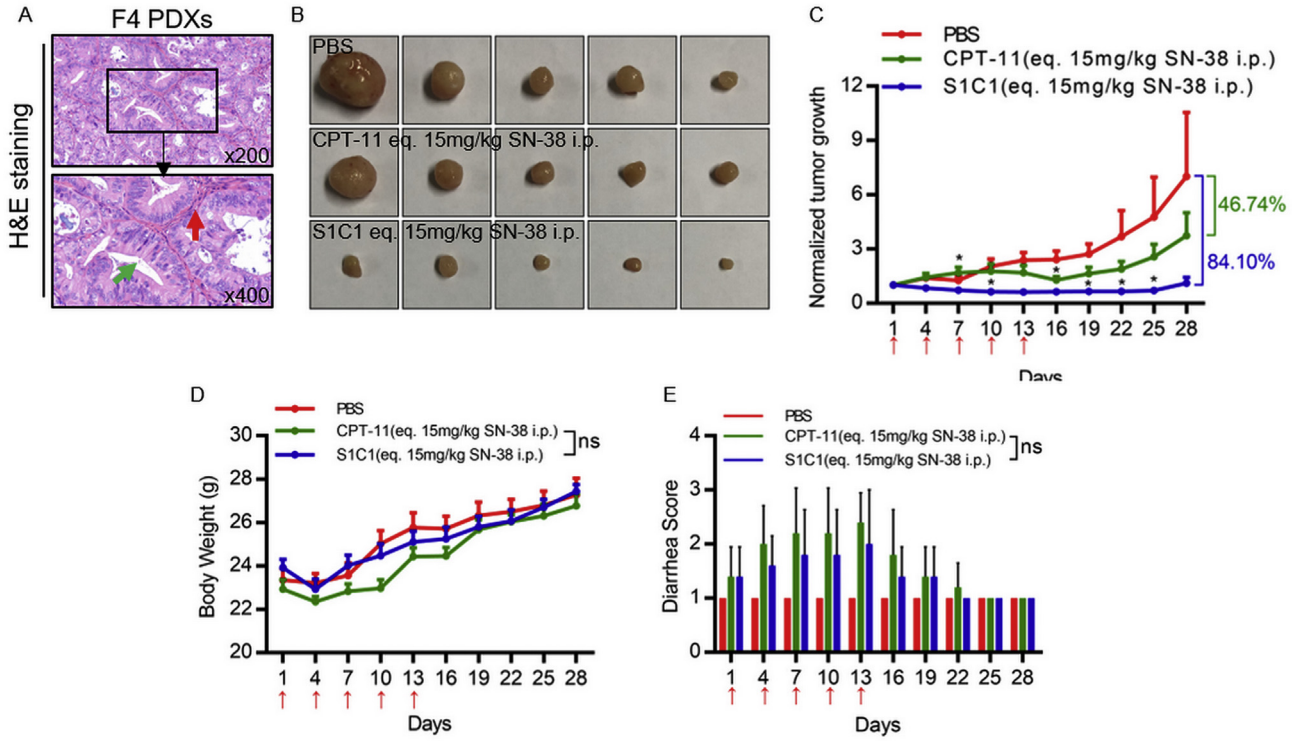

Fig. 5. Therapeutic effects of S1C1 in mice bearing PDXs. (**A**) H&E staining of passaged xenografts from F4 mice. (**B**) Xenografts were resected from mice after treatment (n = 5). (**C**) Tumor growth normalized as a function of time after treatment with CPT-11 or S1C1, as indicated by red arrows (q3d × 5, n = 5). (**D**) Body weight and (**E**) diarrhea score of mice bearing PDXs during treatment with S1C1 or CPT-11 (n = 5). * $p < 0.05$.

Table 2
Patient information.

Gender	Female
Pathological diagnosis	Pancreatic Adenocarcinoma (Head)
Tumor size (cm)	2*1*1
Tumor differentiation	Medium
Local invasion	Nerve. Bile Duct. Duodenum
Lymph node metastasis	1/16
CA19-9(U/mL)	1647.6

further characterize the general efficacy of S1C1 in the treatment of pancreatic cancer.

Collectively, the present study showed that S1C1 excipient-free nanodispersion exerts therapeutic effects against pancreatic cancer in cell lines and in pancreatic cancer CDX, both subcutaneously and orthotopically. Due to its relative carboxylesterase independence, S1C1 was found to function better than CPT-11 under BNPP-induced partial carboxylesterase inhibition, which was used to mimic the low abundance of carboxylesterase seen in patients with pancreatic cancer. Moreover, in our study, pancreatic cancer PDXs in mice provided a clinically promising early evaluation of S1C1. As CPT-11 is currently used clinically, and SN-38 is the metabolic product of CPT-11, this excipient-free nanodispersion may represent a promising nanomedicine for the treatment of pancreatic cancer, with an accelerated approval for clinical use.

Ethics approval

All animal experiments were performed in accordance with the animal ethics committee guidelines and were approved by the Ethical Committee at local authorities.

Conflicts of interest

None.

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