A Phase 1 Expansion Cohort Study Evaluating the Safety and Efficacy of the CHK1 Inhibitor LY2880070 with Low-Dose Gemcitabine in Metastatic Pancreatic Adenocarcinoma Patients

Brandon M. Huffman[1], Hanrong Feng[1], Kalindi Parmar, PhD[2,3], Junning Wang[1], Kevin S. Kapner[1], Bose Kochupurakkal[1,2], David B. Martignetti[2,3], Golbahar Sadatrezaei[2,3], Thomas A. Abrams[1], Leah H. Biller[1], Marios Giannakis[1,4], Kimmie Ng[1], Anuj K. Patel[1], Kimberly J. Perez[1], Harshabad Singh[1], Douglas A. Rubinson[1], Benjamin L. Schlechter[1], Elizabeth Andrews[1], Alison M. Hannigan[1], Stanley Dunwell[1], Zoe Getchell[1], Srivatsan Raghavan[1,4], Brian M. Wolpin[1], Caroline Fortier[5], Alan D. D'Andrea[2,3,*], Andrew J. Aguirre[1,4,*], Geoffrey I. Shapiro[1,2,*], James M. Cleary[1,*]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute/Harvard Medical School, Boston, MA 02215, USA

[2]Center for DNA Damage and Repair, Dana-Farber Cancer Institute, Boston, MA 02215, USA

[3]Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA 02215, USA

[4]The Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA

[5]Esperas Pharma, Montreal, Canada

Abstract

Correspondence: James M. Cleary, MD, PhD, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215. Tel: 617-632-6073; Fax: 617-632-5370, james_cleary@dfci.harvard.edu.
*These authors are co-senior authors.

Background: Combining gemcitabine with CHK1 inhibition has shown promise in preclinical models of pancreatic ductal adenocarcinoma (PDAC). Here, we report the findings from a phase I expansion cohort study (NCT02632448) investigating low-dose gemcitabine combined with the CHK1 inhibitor LY2880070 in patients with previously treated advanced PDAC.

Methods: Patients with metastatic PDAC were treated with gemcitabine intravenously at 100 mg/m^2 on days 1, 8, and 15, and LY2880070 50 mg orally twice daily on days 2–6, 9–13, and 16–20 of each 21-day cycle. Pre-treatment tumor biopsies were obtained from each patient for correlative studies and generation of organoid cultures for drug sensitivity testing and biomarker analyses.

Results: Eleven patients with PDAC were enrolled in the expansion cohort between August 27, 2020 and July 30, 2021. Four patients (36%) experienced drug-related grade 3 adverse events. No objective radiological responses were observed, and all patients discontinued the trial by 3.2 months. In contrast to the lack of efficacy observed in patients, organoid cultures derived from biopsies procured from two patients demonstrated strong sensitivity to the gemcitabine/ LY2880070 combination and showed treatment-induced upregulation of replication stress and DNA damage biomarkers, including pKAP1, pRPA32, and γ-H2AX, as well as induction of replication fork instability.

Conclusions: No evidence of clinical activity was observed for combined low dose gemcitabine and LY2880070 in this treatment refractory PDAC cohort. However, the gemcitabine/LY2880070 combination showed *in vitro* efficacy, suggesting that drug sensitivity for this combination in organoid cultures may not predict clinical benefit in patients.

Keywords

pancreatic adenocarcinoma; gemcitabine; CHK1 inhibitor; replication stress; DNA damage

INTRODUCTION

Pancreatic adenocarcinoma (PDAC) is an aggressive malignancy, and treatment options for most patients are limited to cytotoxic chemotherapy (1–3). More than 90% of PDACs harbor a *KRAS* mutation; moreover, *TP53*, *CDKN2A*, and *SMAD4* alterations are also frequently observed (4–6). Efforts to incorporate molecularly targeted therapy into the PDAC treatment paradigm have been stymied by the limited number of actionable genomic targets in most tumors, until the recent advent of novel *KRAS* inhibitors (7,8). At present, targeting tumors with precision medicine is reserved for rare PDAC subtypes, such as patients with *BRCA2/ BRCA1/PALB2* mutations with PARP inhibition, mismatch repair deficient PDAC with PD-1 inhibition, or rare gene rearrangements, such as *NTRK* or *RET*, with targeted kinase inhibitors (7,9–11). Since a majority of patients with PDAC do not have a targeted therapy option, identification of biomarker-driven targeted therapy strategies is urgently needed to improve the care of these patients (7,12).

Rapidly proliferating malignancies, such as PDAC, generate enormous stress on the cell's replication and mitotic machinery (13–15). This replication stress results in DNA replication fork stalling and instability. Several cell cycle regulatory proteins, including the serine/ threonine kinase CHK1, compensate for replication stress by activating the G1/S, S, and

G2/M checkpoints, thereby halting the cell cycle and allowing for DNA damage repair and replication fork stabilization. The critical role that CHK1 plays in compensating for replication stress makes it a vulnerability in the highly proliferative cells of PDAC (16,17).

Gemcitabine, an irreversible ribonucleotide reductase inhibitor, induces replication stress and consequently further primes PDAC cells to respond to CHK1 inhibition (18–21). Multiple groups have demonstrated that combined gemcitabine/CHK1 inhibition has synergistic cytotoxicity in *in vitro* and *in vivo* PDAC models (16,19,22–25). Despite these promising preclinical data, a phase II clinical trial combining standard-dose gemcitabine (1000 mg/m^2) with the CHK1 inhibitor rabusertib (LY2603618) failed to show an improvement in efficacy beyond that of gemcitabine monotherapy (26). The disappointing results from this study and other efforts to combine gemcitabine with CHK1 inhibitors in other malignancies have prompted efforts to develop improved strategies for combining these two agents (27–33).

Therapy-induced toxicity has been a major challenge in combining gemcitabine with CHK1 inhibitors because both drugs are myelosuppressive. Preclinical studies have indicated that low dose gemcitabine is sufficient to induce replication stress and cause synergistic cytotoxicity with CHK1 inhibition (19,34). Building upon these observations in a recent phase I clinical trial, combining the CHK1 inhibitor SRA737 with gemcitabine at 250 mg/m^2 achieved six partial responses in advanced ovarian, anogenital, rectal and small cell lung cancers, whereas no responses were observed in a separate trial of SRA737 monotherapy (35–37).

LY2880070 is a highly potent CHK1 inhibitor with half maximal inhibitory concentration (IC$_{50}$) of 0.5 nM. A phase 1 trial of LY2880070 monotherapy demonstrated that it was well tolerated with myelosuppression being the major toxicity (38). To augment the efficacy of LY2880070, it was combined with lose dose gemcitabine in a phase 1 trial. During the dose escalation portion of the trial, the combination of LY2880070 and low dose gemcitabine was well tolerated with encouraging signs of activity, including one confirmed partial response in ovarian cancer (39).

Challenges in drug development, particularly in cancers as aggressive as PDAC, create a great need for model systems that can assess novel therapeutic strategies. Tumor-derived organoid cultures, three dimensional culture systems that morphologically resemble tumor structures, are a promising model system that enable high-throughput evaluation of drug combinations at lower costs than animal model experimentation (40). Organoid cultures of PDAC may predict chemotherapy sensitivity in patients and can serve as a platform for drug discovery (29).

Here, we report the clinical results of treatment with gemcitabine combined with the CHK1 inhibitor LY2880070 in a phase I expansion cohort comprised of patients with previously treated metastatic PDAC. We additionally report the results of parallel correlative drug testing of organoids derived from pre-treatment tumor biopsies.

METHODS

Ethics approval and consent to participate

The Dana-Farber Cancer Institute institutional review board approved the study (NCT02632448), and participants provided written informed consent before study enrollment. The study was monitored by the Data Safety Monitoring Committee at Dana-Farber/Harvard Cancer Center and was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Study population

Enrolled patients had histologically confirmed, metastatic PDAC, and had progressed after treatment with at least one prior line of prior chemotherapy. Prior gemcitabine was permitted. Patients were required to be 18 years of age or older, to have measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) (version 1.1), to have adequate organ function, and to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Key exclusion criteria included receipt of any major surgery within 4 weeks of enrollment, history of major organ transplantation, QTc interval prolongation, and brain metastases at the time of enrollment.

Study design and treatment administration

The PDAC expansion cohort study was performed at a single institution (Dana-Farber Cancer Institute). Patients were treated at the established recommended phase 2 dose (RP2D) determined in the phase I clinical trial with gemcitabine intravenously at 100 mg/m^2 on days 1, 8, and 15 of each 21-day cycle and LY2880070 50 mg orally twice daily on days 2–6, 9–13, and 16–20 of each 21-day cycle. The day 15 gemcitabine dose could be withheld at the investigator's discretion. The planned sample size of the PDAC expansion cohort was 15 patients. However, due to limited clinical activity of the trial combination, the trial was stopped after eleven patients were enrolled.

Efficacy and safety assessments

The primary endpoint of the study was confirmation of the safety and tolerability of the study drug combination in the advanced PDAC population. The secondary endpoints were ORR and progression-free survival (PFS). Evaluation of adverse reactions was based on Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0 criteria. The ORR was determined according to RECIST version 1.1 criteria (41). PFS was defined as the time from registration to cancer progression or death, whichever occurred first. Overall survival (OS) was defined as the time from registration to death. Radiological assessments occurred every 6 weeks.

Generation of organoid cultures and drug sensitivity testing

OncoPanel targeted next-generation sequencing was performed on archival or pre-treatment tumor specimens (42). Pre-treatment tumor biopsies were also used for organoid culture generation. Organoid cultures were established and maintained according to previously described techniques (29). Biopsies were dissociated, and 5000 single cells per well were

seeded in 150 μl of 10% growth factor-reduced Matrigel (Corning, 356231, Glendale, AZ) and 90% human organoid feeding medium into poly-(2-hydroxyethyl methacrylate)-coated clear flat-bottom 96-well plates (Corning, 3903). The wells on the plate's perimeter were filled with 1× phosphate buffered saline. For drug sensitivity experiments, organoids were treated 2 days after seeding with dimethyl sulfoxide (DMSO) control, LY2880070, gemcitabine, or a combination thereof, administered with a Tecan D300e drug dispenser (Tecan, Männedorf, Switzerland). Ten days after drug treatment, organoid viability was assessed with Cell-TiterGlo 3D Cell Viability Assays (Promega, G9683, Madison, WI) on a Clariostar Plate Reader (BMG Labtech, NC) according to the manufacturer's protocol. Cells were seeded in triplicate for each drug concentration, and the mean cell viability reading for each dose was normalized to the mean reading for the DMSO control wells to create a 12-point dose curve, from which values of the area under the curve were extrapolated. To determine whether the combination of gemcitabine and LY2880070 was synergistic or antagonistic, both the Highest Single Agent model and Bliss model were used. Combenefit software was used for the Bliss model (43).

Immunoblotting for DNA damage and replication stress biomarkers

Organoid cultures were assessed for markers of DNA damage and replication stress by western blotting of lysates generated after 24-hour exposure to DMSO, gemcitabine, LY2880070, or a combination of gemcitabine plus LY2880070. Organoids were dissociated, and 6.7×10^5 single cells were seeded in one 10 cm Ultra Low Culture Dish (Corning, 3262) with 10 ml of 10% growth-factor-reduced Matrigel (Corning, 356231, Glendale, AZ) and 90% human organoid feeding medium, which were the same conditions used in drug testing. At 24 hours after drug treatment, organoid cells were resuspended in Cell Recovery Solution (Corning, 354253) for 60 minutes and washed two or three times with ice-cold phosphate buffered saline to remove the Matrigel. The cell pellets were then flash frozen and stored at −80 degrees Celsius for western blots. For western blots, the organoid cells were lysed in lysis buffer (Cell Signaling Technology, 9803S) containing 20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L Na_2EDTA, 1 mmol/L EGTA, 1% Triton, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L beta-glycerophosphate, 1 mmol/L sodium vanadate, 1 μg/mL leupeptin, and 1 mmol/L PMSF (Cell Signaling Technology, 8553S). Western blots were performed according to standard protocols with antibodies to CHK1 (G-4; Santa Cruz Biotechnology, SC-8408, RRID:AB_627257), phospho-CHK1 [Ser345] (Cell Signaling Technology, 2348S, RRID:AB_331212), phospho-histone H2AX [S139] (EMD/Millipore Sigma, 05–636, RRID:AB_309864), phospho-RPA32 [S4/S8] (pRPA32) (Bethyl, A300–245A, RRID:AB_210547), RPA32 (Bethyl, A300–244A, RRID:AB_185548), phospho-KAP1 [S824] (pKAP1) (Abcam, ab70369, RRID: AB_1209417), KAP1 (Abcam, ab10484, RRID:AB_297223), and vinculin (Santa Cruz Biotechnology, SC-25336, RRID:AB_628438).

Immunohistochemical assessment of replication stress markers

Formalin fixed paraffin embedded sections of archival and pre-treatment biopsy specimens were used to assess replication stress biomarkers namely, pRPA32 and pKAP1 based on previously standardized protocols (44). Organoid cultures were harvested, fixed in normal buffered saline, paraffin embedded and sectioned. The staining was performed on the Leica

BOND auto-stainer using standard reagents. Stained slides were imaged and analyzed using the Aperio image analysis platform. Cut-offs for 1+, 2+ and 3+ staining intensity established previously were used to calculate the H-score based on the formula: H-score = [(3 X % cells with 3+ intensity) + (2 X % cells with 2+ intensity) + (% cells with 1+ intensity)].

Pharmacodynamic assessment of replication fork stability

Replication combing assay was performed using the FiberComb machine (Genomic Vision) as previously described (45). Briefly, cells were treated with CldU and IdU for 30 minutes each, then DMSO, hydroxyurea (HU), gemcitabine (GEM), LY2880070 (CHK1i), or GEM + CHK1i with 3 PBS washes between each treatment. Organoid cultures were then trypsinized, counted and embedded in low melting point agarose plugs, then treated with proteinase k overnight. Agarose plugs were then washed and digested with agarase overnight. Agarase-treated samples were then poured into FiberComb wells and combed onto silanized coverslips (Genomic Vision COV-002) using the Molecular Combing System from Genomic Vision (Genomic Vision, MCS-001). Coverslips were probed with Rat anti-CldU (Abcam ab6326, RRID:AB_305426), Mouse anti-IdU (BD Biosciences 347580, RRID:AB_400326), followed by staining with fluorochrome labelled appropriate secondary antibodies and visualized by fluorescence microscopy. Pictures were taken of 200 fibers per condition. DNA fibers were measured with ImageJ (RRID:SCR_003070) and graphed using GraphPad Prism (RRID:SCR_002798) software. Each experiment was repeated at least three times.

Statistical analyses

PFS and OS were calculated using Kaplan-Meier analysis using R Project for Statistical Computing (RRID:SCR_001905) or Stata (RRID:SCR_012763). Comparisons of replication fork stability were calculated using GraphPad Prism (RRID:SCR_002798) software by t-test. P value < 0.05 was considered significant.

Data availability statement

The data generated in this study are not publicly available due to patient privacy requirements but are available upon reasonable request from the corresponding author.

RESULTS

Patient disposition and characteristics

A total of 11 patients with PDAC were enrolled in the expansion cohort between August 27, 2020, and July 30, 2021. All patients received at least one study drug treatment. Each of the 11 patients was included in the safety and efficacy evaluations. All patients had discontinued study drug treatment at the time of data cutoff (April 15, 2022). The median age was 65 years, and most patients were male (72.7%) and white (90.9%) (Table 1). All patients were heavily pretreated with systemic chemotherapy. Before enrollment in the trial, 100% of patients had received FOLFIRINOX, and eight patients (72.7%) had received gemcitabine plus nab-paclitaxel. One patient had received a PARP inhibitor and a separate patient had received immune checkpoint inhibitor therapy. All patients had received at least two prior therapies prior to enrollment, and five patients (45.5%) had received three or more

lines of therapy. Most patients (54.5%) had an ECOG performance status of 1 (Table 1). Representativeness of the study participants is described in Supplementary Table S1 (46,47).

Targeted next-generation sequencing, using the Oncopanel platform, was performed on 9 of the 11 trial participants. Two of the 11 trial patients had insufficient tumor to perform the Oncopanel sequencing assay. The genomic profiles of trial subjects was similar to the molecular profiles to those reported in several large scale PDAC sequencing efforts (Figure 1) (4,6,48,49). The most frequent oncogenic alterations observed were in the genes *KRAS* (9/9, 100%), *TP53* (5/9, 56%), *CDKN2A* (5/9, 56%), and *SMAD4* (2/9, 11%). In our cohort, there was one patient with an oncogenic *BRCA2* missense mutation and three patients with *BRCA1* low-level amplifications. Three patients (27%) had low level *MYC* amplifications with copy numbers between 3 and 6. Other genomic alterations associated with increased replication stress and sensitivity to CHK1 inhibition, such as inactivating *RB1* and *FBXW7* alterations, as well as *CCNE1* amplifications, are rare in PDAC (prevalence< 5%) and were not present in patients enrolled in the trial (4,6,48–50).

Antitumor activity

All eleven patients were evaluable for response. Patients were treated with the study drugs for a median of two cycles, and the median time on trial was 45 days (range: 15–106 days). No partial responses were observed, and nine patients were found to have progressive disease on the first restaging scan. Two patients had stable disease and remained on therapy for 2.3 months (PT-9) or 3.2 months (PT-3) before discontinuing the study for disease progression. For the entire 11-patient study population, the median PFS was 1.2 months (95% CI: 0.92–1.61), and the median OS was 4.4 months (95% CI: 2.6–7.2).

Adverse events

All patients experienced at least one grade 1 treatment-related adverse event (Table 2). Myelosuppression was common with any grade neutropenia (5/11, 45%), anemia (7/11, 64%), or thrombocytopenia (6/11, 54%). Eight patients (73%) experienced grade 3 adverse events. Eight patients experienced gemcitabine dose interruptions for adverse events, ranging from one to three total interruptions (most often holding of day 15 gemcitabine infusion and LY2880070 on days 16–20 because of myelosuppression). LY2880070 administration was interrupted in one patient because of an adverse event (grade 3 alkaline phosphatase at provider discretion). Two patients (18%) required treatment discontinuation because of adverse events related to gemcitabine and LY2880070 (grade 2 diarrhea in one patient; grade 3 elevated alanine aminotransferase in another patient). No grade 4 or 5 related adverse events were observed in this expansion cohort.

Patient-derived organoid modeling of therapeutic response

In parallel with the clinical trial, we attempted to derive organoid models from all 11 patients enrolled. Of the five pre-treatment biopsies with adequate viable tissue for an attempt at organoid culture generation, two biopsies (PT-4 and PT-6) yielded successful organoid cultures, and these were subsequently used for drug sensitivity testing of gemcitabine and LY2880070. Both patients showed progressive disease at the first restaging, and biopsies were obtained from metastatic liver lesions that had subsequently increased in

size during treatment. In the PT-6 organoid, the IC_{50} of gemcitabine was 56 nM, and that of LY2880070 was 91 nM. In the PT-4 organoid, the IC_{50} of gemcitabine was 15 nM, and that of LY2880070 was 38 nM. Although both organoid cultures were sensitive to gemcitabine and LY2880070 monotherapy, this sensitivity was enhanced when the drugs were used in combination. The Highest Single Agent (HSA) score showed that the PT-6 organoid achieved maximal synergistic efficacy with 20 nM gemcitabine + 80 nM LY2880070, whereas the PT-4 organoid showed maximal synergistic efficacy with 20 nM gemcitabine + 30 nM LY2880070 (Figure 2A–B). Synergy between gemcitabine and LY2880070 was also confirmed with Bliss synergy plots and Highest Single Agent scores (Figure 2C–F).

To evaluate the hypothesis that the gemcitabine/LY2880070 combination induces replication stress, we performed immunoblotting on lysates of organoid cultures that were treated with DMSO, gemcitabine, LY2880070 or the combination (Figure 3A). Treatments of the organoids with gemcitabine or LY2880070 monotherapy resulted in a modest induction of DNA damage (γ-H2AX) and replication stress (pKAP1, pCHK1, and pRPA32) protein expression. However, the gemcitabine/LY2880070 combination markedly enhanced the levels of DNA damage (γ-H2AX) and replication stress markers (pKAP1, pCHK1, and pRPA32), consistent with the synergy we observed *in vitro* on drug sensitivity testing (Figure 3A).

We next assessed the functional consequence of high replication stress by performing DNA fiber assays (51,52). These assays allow interrogation of replication fork stability by sequentially pulsing cells with two nucleotide analogues, CldU and IdU, and measuring their uptake. Unstable replication forks cannot stably incorporate both nucleotides and subsequently have IdU/CIdU ratio <1. DNA fiber assays demonstrated that PT-6 had unstable replication forks after exposure to hydroxyurea, as well as in response to study drug treatment (Figure 3B). PT-4 had more stable replication forks, evidenced by an IdU/CldU ratio of approximately one after hydroxyurea exposure, which became unstable after treatment with gemcitabine or LY2880070 monotherapy, as well as with the gemcitabine/LY2880070 combination.

To explore the heterogeneity of baseline replication stress biomarkers in the patient tumor samples, we performed IHC testing of several tumor samples from patients enrolled on the study. We observed variation in H scores of pRPA32 and pKAP1 among the tumor cores obtained from the same patient at different time points along the disease course (Supplementary Figure S1A–B). For example, one of the biopsies from PT-7 had high pRPA32 (H-score: 32) and pKAP1 (H-score: 61) staining levels after progression on FOLFIRINOX, but the original diagnostic biopsy and the pretrial biopsy had lower pRPA32 (H-scores < 5) and pKAP1 (H-scores < 20) staining levels (Supplementary Figure S1A–B). Additionally, in the pre-treatment tumor biopsy from PT-4, that was also used to derive the organoid, there was intratumoral heterogeneity of pRPA32 immunostaining (Figure 4). The tumor biopsy from PT-4 had a pRPA32 H-score of 3, while the organoid culture had more uniform pRPA32 immunostaining with an H-score of 13.

While both organoid cultures were sensitive to gemcitabine and LY2880070, the PT-6 organoid culture had increased sensitivity to gemcitabine and LY2880070 compared to PT-4

organoid culture and displayed greater replication fork instability. Consistent with this, we found that there were higher baseline levels of replication stress biomarkers in PT-6 (H-scores: pKAP1 140.7; pRPA32 22) compared to PT-4 (H-scores: pKAP1 67.4; pRPA32 13) (Supplementary Figure S2).

Discussion

Treatment options for patients with metastatic PDAC are largely confined to cytotoxic chemotherapy, and targeted therapy options are greatly needed. Preclinical data from several groups have suggested that the combination of gemcitabine and CHK1 inhibition is a promising therapy in PDAC (19,22–24). To circumvent the clinical challenge of combining these myelosuppressive agents, we used a strategy of low dose gemcitabine combined with CHK1 inhibition. Despite its promising preclinical activity, we did not observe evidence of clinical activity of low dose gemcitabine combined with LY2880070 in our 11-patient PDAC cohort. Our results are consistent with those from a previous trial using gemcitabine at 1000 mg/m^2 with the CHK1 inhibitor rabusertib (LY2603618) (26). This disappointing outcome underscores the difficulties in therapeutic targeting of replication stress in PDAC and emphasizes the need to develop new strategies for approaching this vulnerability.

The results of this trial highlight the challenges of drug development in refractory PDAC populations, given that all patients in the trial had already received at least two lines of therapy. In the clinic, only approximately 19% of patients with PDAC receive third-line therapy, most of whom experience substantial disease-related symptoms (53). Hence, evaluating a therapy in a disease population characterized by rapidly declining performance status is fraught with difficulties. The aggressive disease biology observed in patients receiving third-line therapy for PDAC was clearly observed in the results of this trial, despite being tested in a highly selected population of fit patients (ECOG performance status 0–1 and median albumin 4.1 g/dL), because the median PFS was 1.2 months and median OS was 4.4 months. These results are similar to those from other studies, evaluating agents like immunotherapy or chemotherapy, in refractory PDAC (54,55). Counterbalancing the disappointing results in these third-line studies are more recent encouraging examples of trials that targeted essential oncogenes, such as *KRAS* G12C inhibitors, and allowed enrollment of patients with PDAC who had received more than two lines of therapy (56,57). Hence, while novel therapies targeting essential oncogenes can be successful in the third-line setting, investigators should be cautious in designing phase 2 and phase 3 clinical trials for PDAC in patients receiving their third-line of therapy (7,56,58).

Although all 11 patients showed rapid disease progression on the study drugs, the LY2880070/gemcitabine combination had *in vitro* efficacy in organoid cultures derived from two patients in the study. Organoid testing revealed that the LY2880070/gemcitabine combination was synergistic; moreover, in agreement with our hypothesis, the study drug combination greatly increased the amount of replication stress observed in these cultures. At the functional level, the degree of replication stress induced, even by the monotherapies, was adequate to destabilize replication forks, as measured in DNA fiber assays.

One possible explanation for the differential sensitivities observed in patients and organoid cultures is that cells selected during the organoid development process may be those that are highly replicative and therefore particularly susceptible to therapeutic targeting of replication stress. This may be a particular problem for PDAC organoid cultures, which are typically generated over multiple weeks of time (28,31,59,60). Consistent with this possibility, we observed substantial intrapatient heterogeneity in the levels of the replication stress biomarkers pRPA32 and pKAP1 among tumor cores from archival or pre-treatment biopsies. Because of this heterogeneity, the organoid cultures may not represent all of the tumor cell populations seen in the patient and could instead have arisen from a small subset of cells that were sensitive to the gemcitabine/LY2880070 combination (29,61). Tumor cells that had higher levels of replication stress may be more likely to grow in organoid culture, promoting the hypothesis that they would be more sensitive to gemcitabine/LY2880070 *in vitro*. In addition, most *in vitro* cell culture conditions are designed to promote rapid tumor cell proliferation, which may further predispose *in vitro* model systems to undergo replication stress and consequently exhibit greater sensitivity to therapies that target these pathways. Organoid culture systems are also lacking the tumor microenvironment, which includes stromal cells and immune cells (62), that is present *in vivo* and this could contribute to the differing *in vitro* and *in vivo* sensitivities. Another possibility is that the concentrations of drug used in patients might be insufficient for antitumor activity *in vivo*. However, a counterbalance to this view is that during dose escalation there was one partial response observed in a patient with ovarian cancer (39). Additionally, preclinical xenograft studies of gemcitabine combined with LY2880070 projected that the minimum dose of LY2880070 for tumor response would be between 32–64 mg daily in humans, and patients in this study were dosed with 50 mg twice daily (unpublished observation). Notably, the tumors that were biopsied in each patient increased in size on restaging imaging studies.

A limitation to this trial was that there were no on-treatment biopsies obtained on this trial. In subsequent trials, on-treatment biopsies will be useful to confirm drug delivery and expected intratumoral pharmacodynamic effects. Adequate tissue sampling presents a major challenge in PDAC trials and will be necessary for future translational work. On this trial, we were only able to generate organoid cultures from 2 of 11 patients. It is important to mention that the *in vitro* drug sensitivity data was from a small cohort of two organoids and that a larger sample size would be required for more definitive conclusions to be drawn from this work. A further limitation to this trial was its use of low-dose gemcitabine. Preclinical studies have demonstrated that low-dose gemcitabine can cause replicative stress but clinically defining the optimal dosage of low-dose gemcitabine is challenging (19,34,63). In our trial, we utilized gemcitabine $100 mg/m^2$ whereas another study, evaluating low-dose gemcitabine combined with the SRA-737 CHK1 inhibitor, utilized gemcitabine at $250 mg/m^2$. While low-dose gemcitabine is an attractive strategy in avoiding the myelosuppression seen with regimens combining DNA repair inhibitors and chemotherapy, further work is needed to more precisely define the clinically optimal dosage of low-dose gemcitabine.

In the clinical community, there has been great hope that therapeutic drug testing of organoid cultures could predict clinical responsiveness in patients. However, the inconsistency between the gemcitabine/LY2880070 results in the clinic versus those in

organoid cultures underscores the complexity of this type of testing. To accomplish the goal of predictive organoid testing, technology will need to be further refined to account for potentially confounding issues of tumor heterogeneity and *in vivo* impediments to drug delivery (28,31,59,60).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

References:

1. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364(19):1817–25 doi 10.1056/ NEJMoa1011923. [PubMed: 21561347]

2. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369(18):1691–703 doi 10.1056/NEJMoa1304369. [PubMed: 24131140]

3. Huffman BM, Basu Mallick A, Horick NK, Wang-Gillam A, Hosein PJ, Morse MA, et al. Effect of a MUC5AC Antibody (NPC-1C) Administered With Second-Line Gemcitabine and Nab-Paclitaxel on the Survival of Patients With Advanced Pancreatic Ductal Adenocarcinoma: A Randomized Clinical Trial. JAMA Netw Open 2023;6(1):e2249720 doi 10.1001/jamanetworkopen.2022.49720. [PubMed: 36602796]

4. Aguirre AJ, Nowak JA, Camarda ND, Moffitt RA, Ghazani AA, Hazar-Rethinam M, et al. Real-time Genomic Characterization of Advanced Pancreatic Cancer to Enable Precision Medicine. Cancer Discov 2018;8(9):1096–111 doi 10.1158/2159-8290.CD-18-0275. [PubMed: 29903880]

5. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321(5897):1801–6 doi 10.1126/science.1164368. [PubMed: 18772397]

6. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016;531(7592):47–52 doi 10.1038/nature16965. [PubMed: 26909576]

7. Huffman BM, Ellis H, Jordan AC, Freed-Pastor WA, Perez K, Rubinson DA, et al. Emerging Role of Targeted Therapy in Metastatic Pancreatic Adenocarcinoma. Cancers (Basel) 2022;14(24) doi 10.3390/cancers14246223.

8. Hosein AN, Dougan SK, Aguirre AJ, Maitra A. Translational advances in pancreatic ductal adenocarcinoma therapy. Nat Cancer 2022;3(3):272–86 doi 10.1038/s43018-022-00349-2. [PubMed: 35352061]

9. Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, et al. Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer. N Engl J Med 2019;381(4):317–27 doi 10.1056/NEJMoa1903387. [PubMed: 31157963]

10. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/ Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. J Clin Oncol 2020;38(1):1–10 doi 10.1200/JCO.19.02105. [PubMed: 31682550]

11. Reiss KA, Mick R, O'Hara MH, Teitelbaum U, Karasic TB, Schneider C, et al. Phase II Study of Maintenance Rucaparib in Patients With Platinum-Sensitive Advanced Pancreatic Cancer and a Pathogenic Germline or Somatic Variant in BRCA1, BRCA2, or PALB2. J Clin Oncol 2021;39(22):2497–505 doi 10.1200/JCO.21.00003. [PubMed: 33970687]

12. Cleary JM, Wolpin BM, Dougan SK, Raghavan S, Singh H, Huffman B, et al. Opportunities for Utilization of DNA Repair Inhibitors in Homologous Recombination Repair-Deficient and Proficient Pancreatic Adenocarcinoma. Clin Cancer Res 2021;27(24):6622–37 doi 10.1158/1078-0432.CCR-21-1367. [PubMed: 34285063]

13. Ubhi T, Brown GW. Exploiting DNA Replication Stress for Cancer Treatment. Cancer Res 2019;79(8):1730–9 doi 10.1158/0008-5472.CAN-18-3631. [PubMed: 30967400]

14. Berti M, Cortez D, Lopes M. The plasticity of DNA replication forks in response to clinically relevant genotoxic stress. Nat Rev Mol Cell Biol 2020;21(10):633–51 doi 10.1038/s41580-020-0257-5. [PubMed: 32612242]

15. Zhu H, Swami U, Preet R, Zhang J. Harnessing DNA Replication Stress for Novel Cancer Therapy. Genes (Basel) 2020;11() doi 10.3390/genes11090990. [PubMed: 33374124]

16. Dreyer SB, Upstill-Goddard R, Paulus-Hock V, Paris C, Lampraki EM, Dray E, et al. Targeting DNA Damage Response and Replication Stress in Pancreatic Cancer. Gastroenterology 2021;160(1):362–77 e13 doi 10.1053/j.gastro.2020.09.043. [PubMed: 33039466]

17. Dreyer SB, Upstill-Goddard R, Legrini A, Biankin AV, Glasgow Precision Oncology L, Jamieson NB, et al. Genomic and Molecular Analyses Identify Molecular Subtypes of Pancreatic Cancer Recurrence. Gastroenterology 2022;162(1):320–4 e4 doi 10.1053/j.gastro.2021.09.022. [PubMed: 34534536]

18. Wallez Y, Dunlop CR, Johnson TI, Koh SB, Fornari C, Yates JWT, et al. The ATR Inhibitor AZD6738 Synergizes with Gemcitabine In Vitro and In Vivo to Induce Pancreatic Ductal Adenocarcinoma Regression. Mol Cancer Ther 2018;17(8):1670–82 doi 10.1158/1535-7163.MCT-18-0010. [PubMed: 29891488]

19. Koh SB, Courtin A, Boyce RJ, Boyle RG, Richards FM, Jodrell DI. CHK1 Inhibition Synergizes with Gemcitabine Initially by Destabilizing the DNA Replication Apparatus. Cancer Res 2015;75(17):3583–95 doi 10.1158/0008-5472.CAN-14-3347. [PubMed: 26141863]

20. Aye Y, Li M, Long MJ, Weiss RS. Ribonucleotide reductase and cancer: biological mechanisms and targeted therapies. Oncogene 2015;34(16):2011–21 doi 10.1038/onc.2014.155. [PubMed: 24909171]

21. Parsels LA, Morgan MA, Tanska DM, Parsels JD, Palmer BD, Booth RJ, et al. Gemcitabine sensitization by checkpoint kinase 1 inhibition correlates with inhibition of a Rad51 DNA damage response in pancreatic cancer cells. Mol Cancer Ther 2009;8(1):45–54 doi 10.1158/1535-7163.MCT-08-0662. [PubMed: 19139112]

22. Morimoto Y, Takada K, Takeuchi O, Takagi A, Watanabe K, Hirohara M, et al. Prexasertib increases the sensitivity of pancreatic cancer cells to gemcitabine and S-1. Oncol Rep 2020;43(2):689–99 doi 10.3892/or.2019.7421. [PubMed: 31789403]

23. Chung S, Vail P, Witkiewicz AK, Knudsen ES. Coordinately Targeting Cell-Cycle Checkpoint Functions in Integrated Models of Pancreatic Cancer. Clin Cancer Res 2019;25(7):2290–304 doi 10.1158/1078-0432.CCR-18-1620. [PubMed: 30538111]

24. Koh SB, Wallez Y, Dunlop CR, Bernaldo de Quiros Fernandez S, Bapiro TE, Richards FM, et al. Mechanistic Distinctions between CHK1 and WEE1 Inhibition Guide the Scheduling of Triple Therapy with Gemcitabine. Cancer Res 2018;78(11):3054–66 doi 10.1158/0008-5472.CAN-17-3932. [PubMed: 29735549]

25. Italiano A, Infante JR, Shapiro GI, Moore KN, LoRusso PM, Hamilton E, et al. Phase I study of the checkpoint kinase 1 inhibitor GDC-0575 in combination with gemcitabine in patients with refractory solid tumors. Ann Oncol 2018;29(5):1304–11 doi 10.1093/annonc/mdy076. [PubMed: 29788155]

26. Laquente B, Lopez-Martin J, Richards D, Illerhaus G, Chang DZ, Kim G, et al. A phase II study to evaluate LY2603618 in combination with gemcitabine in pancreatic cancer patients. BMC Cancer 2017;17(1):137 doi 10.1186/s12885-017-3131-x. [PubMed: 28202004]

27. Boj SF, Hwang CI, Baker LA, Chio II, Engle DD, Corbo V, et al. Organoid models of human and mouse ductal pancreatic cancer. Cell 2015;160(1–2):324–38 doi 10.1016/j.cell.2014.12.021. [PubMed: 25557080]

28. Tiriac H, Belleau P, Engle DD, Plenker D, Deschenes A, Somerville TDD, et al. Organoid Profiling Identifies Common Responders to Chemotherapy in Pancreatic Cancer. Cancer Discov 2018;8(9):1112–29 doi 10.1158/2159-8290.CD-18-0349. [PubMed: 29853643]

29. Raghavan S, Winter PS, Navia AW, Williams HL, DenAdel A, Lowder KE, et al. Microenvironment drives cell state, plasticity, and drug response in pancreatic cancer. Cell 2021;184(25):6119–37 e26 doi 10.1016/j.cell.2021.11.017. [PubMed: 34890551]

30. Ravichandran M, Hu J, Cai C, Ward NP, Venida A, Foakes C, et al. Coordinated Transcriptional and Catabolic Programs Support Iron-Dependent Adaptation to RAS-MAPK Pathway Inhibition in Pancreatic Cancer. Cancer Discov 2022;12(9):2198–219 doi 10.1158/2159-8290.CD-22-0044. [PubMed: 35771494]

31. Seppala TT, Zimmerman JW, Suri R, Zlomke H, Ivey GD, Szabolcs A, et al. Precision Medicine in Pancreatic Cancer: Patient-Derived Organoid Pharmacotyping Is a Predictive Biomarker of Clinical Treatment Response. Clin Cancer Res 2022;28(15):3296–307 doi 10.1158/1078-0432.CCR-21-4165. [PubMed: 35363262]

32. Kang Y, Deng J, Ling J, Li X, Chiang YJ, Koay EJ, et al. 3D imaging analysis on an organoid-based platform guides personalized treatment in pancreatic ductal adenocarcinoma. J Clin Invest 2022;132(24) doi 10.1172/JCI151604.

33. Grossman JE, Muthuswamy L, Huang L, Akshinthala D, Perea S, Gonzalez RS, et al. Organoid Sensitivity Correlates with Therapeutic Response in Patients with Pancreatic Cancer. Clin Cancer Res 2022;28(4):708–18 doi 10.1158/1078-0432.CCR-20-4116. [PubMed: 34789479]

34. Montano R, Khan N, Hou H, Seigne J, Ernstoff MS, Lewis LD, et al. Cell cycle perturbation induced by gemcitabine in human tumor cells in cell culture, xenografts and bladder cancer patients: implications for clinical trial designs combining gemcitabine with a Chk1 inhibitor. Oncotarget 2017;8(40):67754–68 doi 10.18632/oncotarget.18834. [PubMed: 28978069]

35. Banerji U, Plummer ER, Moreno V, Ang JE, Quinton A, Drew Y, et al. A phase I/II first-in-human trial of oral SRA737 (a Chk1 inhibitor) given in combination with low-dose gemcitabine in subjects with advanced cancer. Journal of Clinical Oncology 2019;37(15_suppl):3095- doi 10.1200/JCO.2019.37.15_suppl.3095.

36. Plummer ER, Kristeleit RS, Cojocaru E, Haris NM, Carter L, Jones RH, et al. A first-in-human phase I/II trial of SRA737 (a Chk1 Inhibitor) in subjects with advanced cancer. Journal of Clinical Oncology 2019;37(15_suppl):3094- doi 10.1200/JCO.2019.37.15_suppl.3094.

37. Jones R, Plummer R, Moreno V, Carter L, Roda D, Garralda E, et al. A Phase I/II Trial of Oral SRA737 (a Chk1 Inhibitor) Given in Combination with Low-Dose Gemcitabine in Patients with Advanced Cancer. Clin Cancer Res 2023;29(2):331–40 doi 10.1158/1078-0432.CCR-22-2074. [PubMed: 36378548]

38. Miller WH, Chu QS, Bouganim N, Jonker DJ, Batist G, Buhlaiga NJ, et al. A phase Ib study of oral Chk1 inhibitor LY2880070 as monotherapy in patients with advanced or metastatic cancer. Journal of Clinical Oncology 2020;38(15_suppl):3579- doi 10.1200/JCO.2020.38.15_suppl.3579.

39. Chu QS, Jonker DJ, Provencher DM, Miller WH, Bouganim N, Shields AF, et al. A phase Ib study of oral Chk1 inhibitor LY2880070 in combination with gemcitabine in patients with advanced or metastatic cancer. Journal of Clinical Oncology 2020;38(15_suppl):3581- doi 10.1200/JCO.2020.38.15_suppl.3581. [PubMed: 32897826]

40. Tuveson D, Clevers H. Cancer modeling meets human organoid technology. Science 2019;364(6444):952–5 doi 10.1126/science.aaw6985. [PubMed: 31171691]

41. 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(2):228–47 doi 10.1016/j.ejca.2008.10.026. [PubMed: 19097774]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

42. Sholl LM, Do K, Shivdasani P, Cerami E, Dubuc AM, Kuo FC, et al. Institutional implementation of clinical tumor profiling on an unselected cancer population. JCI Insight 2016;1(19):e87062 doi 10.1172/jci.insight.87062. [PubMed: 27882345]

43. Di Veroli GY, Fornari C, Wang D, Mollard S, Bramhall JL, Richards FM, et al. Combenefit: an interactive platform for the analysis and visualization of drug combinations. Bioinformatics 2016;32(18):2866–8 doi 10.1093/bioinformatics/btw230. [PubMed: 27153664]

44. Do KT, Kochupurakkal B, Kelland S, de Jonge A, Hedglin J, Powers A, et al. Phase 1 Combination Study of the CHK1 Inhibitor Prexasertib and the PARP Inhibitor Olaparib in High-grade Serous Ovarian Cancer and Other Solid Tumors. Clin Cancer Res 2021;27(17):4710–6 doi 10.1158/1078-0432.CCR-21-1279. [PubMed: 34131002]

45. Lim KS, Li H, Roberts EA, Gaudiano EF, Clairmont C, Sambel LA, et al. USP1 Is Required for Replication Fork Protection in BRCA1-Deficient Tumors. Mol Cell 2018;72(6):925–41 e4 doi 10.1016/j.molcel.2018.10.045. [PubMed: 30576655]

46. Samaan JS, Abboud Y, Oh J, Jiang Y, Watson R, Park K, et al. Pancreatic Cancer Incidence Trends by Race, Ethnicity, Age and Sex in the United States: A Population-Based Study, 2000–2018. Cancers (Basel) 2023;15(3) doi 10.3390/cancers15030870.

47. Ilic M, Ilic I. Epidemiology of pancreatic cancer. World J Gastroenterol 2016;22(44):9694–705 doi 10.3748/wjg.v22.i44.9694. [PubMed: 27956793]

48. Cancer Genome Atlas Research Network. Electronic address aadhe, Cancer Genome Atlas Research N. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. Cancer Cell 2017;32(2):185–203 e13 doi 10.1016/j.ccell.2017.07.007. [PubMed: 28810144]

49. Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun 2015;6:6744 doi 10.1038/ncomms7744. [PubMed: 25855536]

50. Konstantinopoulos PA, da Costa A, Gulhan D, Lee EK, Cheng SC, Hendrickson AEW, et al. A Replication stress biomarker is associated with response to gemcitabine versus combined gemcitabine and ATR inhibitor therapy in ovarian cancer. Nat Commun 2021;12(1):5574 doi 10.1038/s41467-021-25904-w. [PubMed: 34552099]

51. Hill SJ, Decker B, Roberts EA, Horowitz NS, Muto MG, Worley MJ Jr., et al. Prediction of DNA Repair Inhibitor Response in Short-Term Patient-Derived Ovarian Cancer Organoids. Cancer Discov 2018;8(11):1404–21 doi 10.1158/2159-8290.CD-18-0474. [PubMed: 30213835]

52. Nieminuszczy J, Schwab RA, Niedzwiedz W. The DNA fibre technique - tracking helicases at work. Methods 2016;108:92–8 doi 10.1016/j.ymeth.2016.04.019. [PubMed: 27102626]

53. Abrams TA, Meyer G, Meyerhardt JA, Wolpin BM, Schrag D, Fuchs CS. Patterns of Chemotherapy Use in a U.S.-Based Cohort of Patients with Metastatic Pancreatic Cancer. Oncologist 2017;22(8):925–33 doi 10.1634/theoncologist.2016-0447. [PubMed: 28476943]

54. Wolpin BM, Rubinson DA, Wang X, Chan JA, Cleary JM, Enzinger PC, et al. Phase II and pharmacodynamic study of autophagy inhibition using hydroxychloroquine in patients with metastatic pancreatic adenocarcinoma. Oncologist 2014;19(6):637–8 doi 10.1634/theoncologist.2014-0086. [PubMed: 24821822]

55. O'Reilly EM, Oh DY, Dhani N, Renouf DJ, Lee MA, Sun W, et al. Durvalumab With or Without Tremelimumab for Patients With Metastatic Pancreatic Ductal Adenocarcinoma: A Phase 2 Randomized Clinical Trial. JAMA Oncol 2019;5(10):1431–8 doi 10.1001/jamaoncol.2019.1588. [PubMed: 31318392]

56. Strickler JH, Satake H, George TJ, Yaeger R, Hollebecque A, Garrido-Laguna I, et al. Sotorasib in KRAS p.G12C-Mutated Advanced Pancreatic Cancer. N Engl J Med 2023;388(1):33–43 doi 10.1056/NEJMoa2208470. [PubMed: 36546651]

57. Bekaii-Saab TS, Yaeger R, Spira AI, Pelster MS, Sabari JK, Hafez N, et al. Adagrasib in Advanced Solid Tumors Harboring a KRAS(G12C) Mutation. J Clin Oncol 2023:JCO2300434 doi 10.1200/JCO.23.00434.

58. Pishvaian MJ, Blais EM, Brody JR, Lyons E, DeArbeloa P, Hendifar A, et al. Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. The Lancet Oncology 2020;21(4):508–18 doi 10.1016/s1470-2045(20)30074-7. [PubMed: 32135080]

59. Misra S, Moro CF, Del Chiaro M, Pouso S, Sebestyen A, Lohr M, et al. Ex vivo organotypic culture system of precision-cut slices of human pancreatic ductal adenocarcinoma. Sci Rep 2019;9(1):2133 doi 10.1038/s41598-019-38603-w. [PubMed: 30765891]

60. Seppala TT, Zimmerman JW, Sereni E, Plenker D, Suri R, Rozich N, et al. Patient-derived Organoid Pharmacotyping is a Clinically Tractable Strategy for Precision Medicine in Pancreatic Cancer. Ann Surg 2020;272(3):427–35 doi 10.1097/SLA.0000000000004200. [PubMed: 32657929]

61. Hogenson TL, Xie H, Phillips WJ, Toruner MD, Li JJ, Horn IP, et al. Culture media composition influences patient-derived organoid ability to predict therapeutic responses in gastrointestinal cancers. JCI Insight 2022;7(22) doi 10.1172/jci.insight.158060.

62. Halbrook CJ, Lyssiotis CA, Pasca di Magliano M, Maitra A. Pancreatic cancer: Advances and challenges. Cell 2023;186(8):1729–54 doi 10.1016/j.cell.2023.02.014. [PubMed: 37059070]

63. Hansen RJ, Strouse B, Anderes K, Smith G, Hassig C. Abstract B181: The Chk1 inhibitor, SRA737, demonstrates chemical synthetic lethality with replication stress-inducing agents, including low-dose gemcitabine, in preclinical models of cancer. Molecular Cancer Therapeutics 2018;17(1_Supplement):B181-B doi 10.1158/1535-7163.Targ-17-b181.

TRANSLATIONAL RELEVANCE

Targeting therapeutic vulnerabilities of replication stress in pancreatic adenocarcinoma (PDAC) has been efficacious in preclinical models. We report a phase 1 expansion cohort of low-dose gemcitabine and the Chk1 inhibitor LY2880070 in patients with heavily pretreated metastatic PDAC. Although there were no clinical responses, two patient-derived organoid cultures developed from pretreatment biopsies of trial patients were sensitive to gemcitabine and LY2880070 *in vitro*. These organoids demonstrated increased expression of replication stress markers with drug treatment, as evidenced by immunoblotting and replication fork stability assays. The inconsistency between the gemcitabine/LY2880070 results in the clinic and *in vitro* testing suggests that organoid culture evaluation of the drug sensitivity may not predict clinical responses to replication stress-inducing strategies and underscores the need for ongoing efforts to define clinical biomarkers of response and resistance to these strategies.
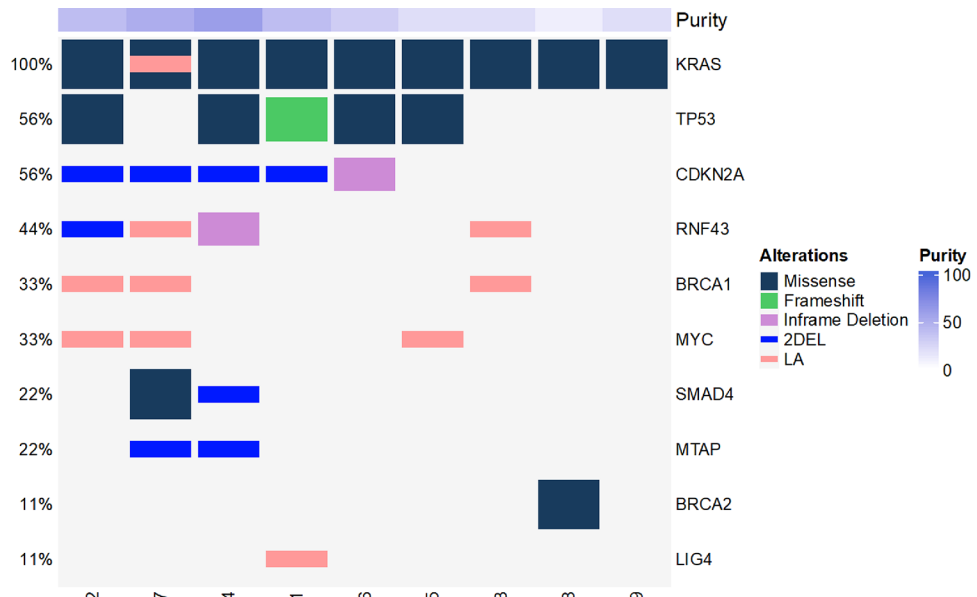
Figure 1. Genomic alterations in tumors from patients with PDAC enrolled in the trial.
OncoPanel next generation sequencing was performed on tumor samples for nine patients
enrolled in the trial. The co-mutation plot highlights the frequency of the most common
oncogenic genomic alterations. Columns represent individual patients with PDAC, and rows
indicate oncogenic genomic alterations. Abbreviations: 2DEL (2 copy deletion) and LA (low
level amplification [6 copies]).
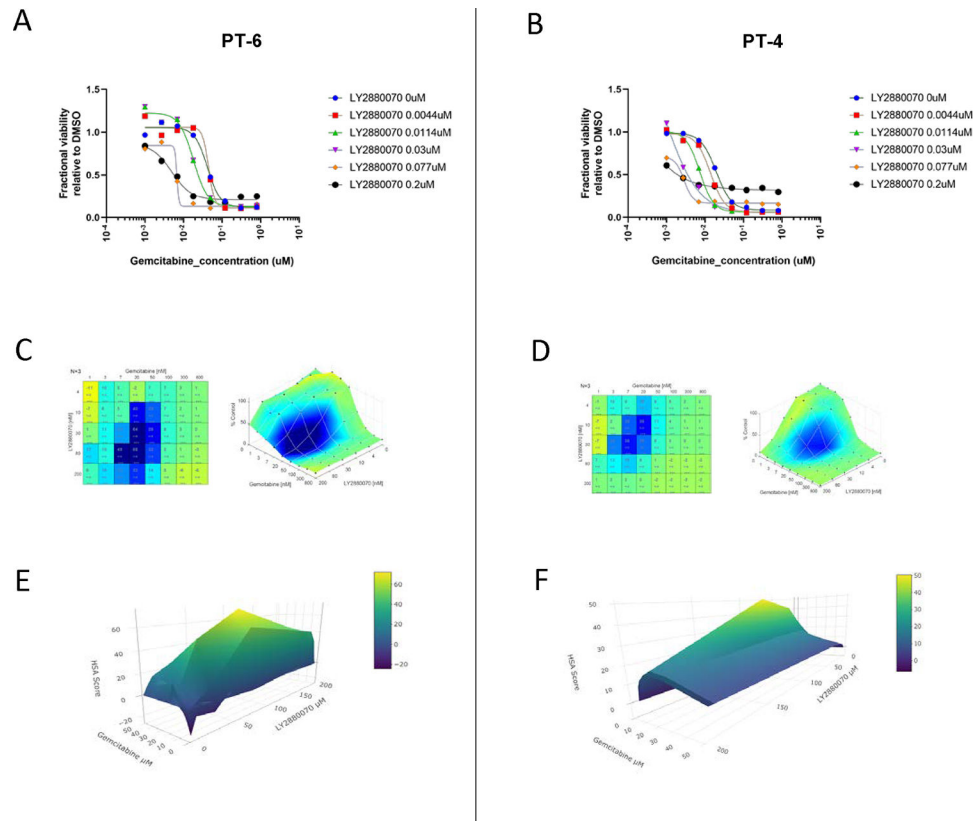
Figure 2. Combination treatment with LY2880070 and gemcitabine shows synergistic cell death in organoids.

Organoid cultures were grown in triplicate with graded concentrations of drugs. Synergy/antagonism between the drugs was determined with the Bliss model. **A-B** Survival plots of organoid cultures after 10 days' exposure to increasing concentrations of gemcitabine and LY2880070 for the organoids PT-6 (**A**) and PT-4 (**B**). **C-D** Bliss synergy/antagonism levels on the experimental combination dose–response surface. Bliss synergy/antagonism levels in a matrix format are shown. Bliss scores greater than zero (green/blue shading) indicate synergy between gemcitabine and LY2880070. (**C**) The Bliss synergy score indicated that the PT-6 organoid culture had maximal synergistic efficacy with 20 nM gemcitabine + 80 nM LY2880070. (**D**) The Bliss synergy score indicated that the PT-4 organoid culture had maximal synergistic efficacy with 20 nM gemcitabine + 30 nM LY2880070. **E-F** Highest Single Agent (HSA) synergy plot for the PT-6 organoid (**E**) and PT-4 organoid (**F**).
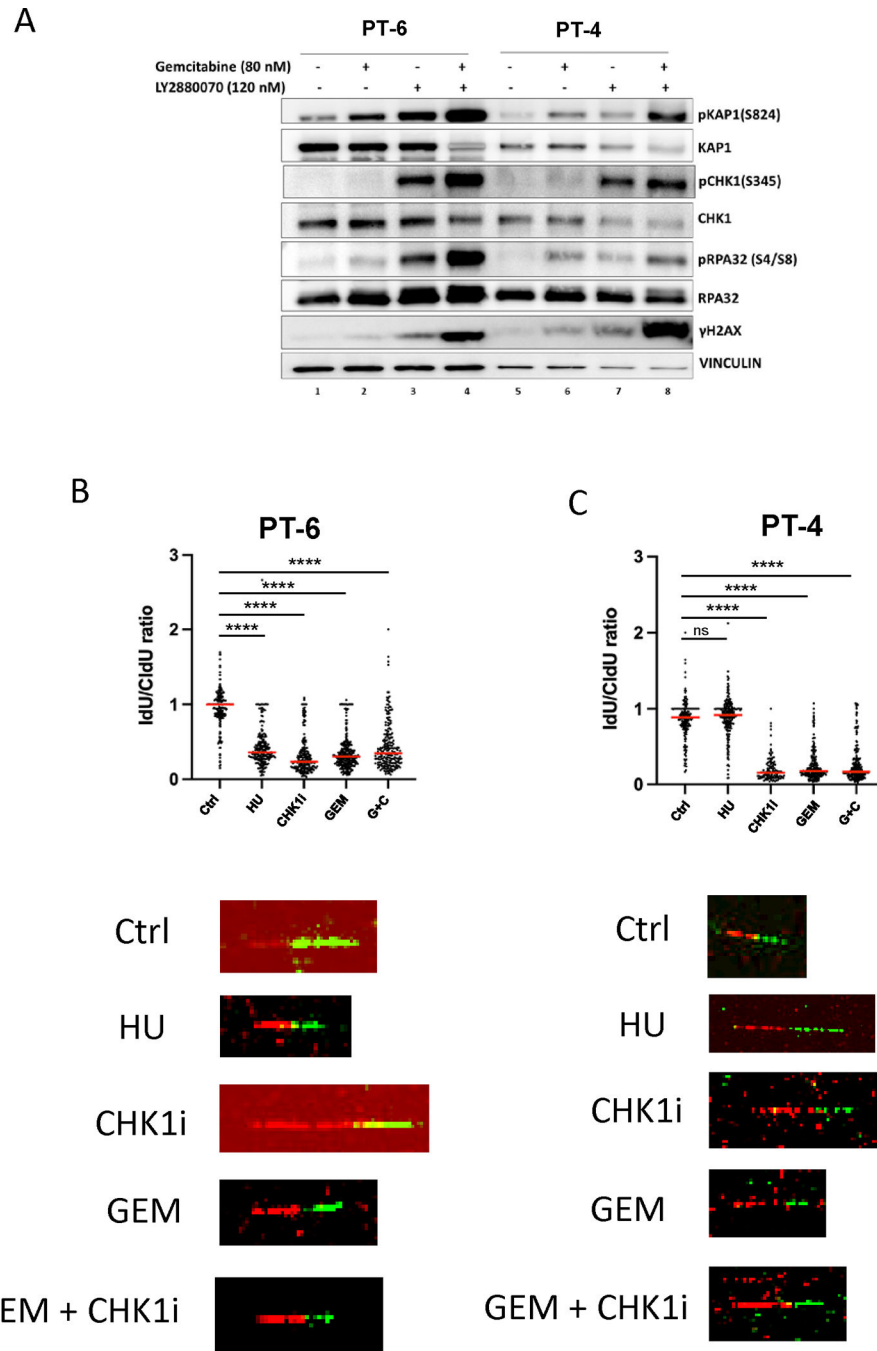
Figure 3. Combination treatment with LY2880070 and gemcitabine induces synergistic DNA damage and replication stress responses in organoids derived from pre-treatment biopsies. (**A**) Western blots of lysates from organoid cultures exposed to the indicated concentrations of LY2880070, gemcitabine, or combination treatment for 24 hours. (**B-C**) Replication fork stability assays for PT-6 (using gemcitabine 80 nM, LY2880070 50 nM) (**B**) and PT-4 (using gemcitabine 30 nM, LY2880070 15 nM) (**C**). Abbreviations: Ctrl: control, HU: hydroxyurea, CHK1i: CHK1 inhibitor, GEM: gemcitabine, G+C: gemcitabine and CHK1 inhibitor. **** signifies p value < 0.001; ns: not significant.

Tumor Biopsy
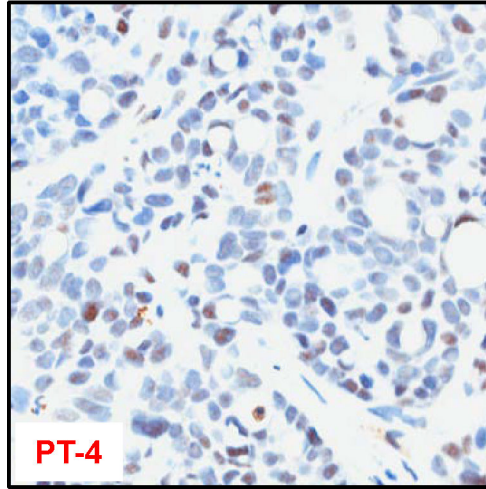
Organoid Culture
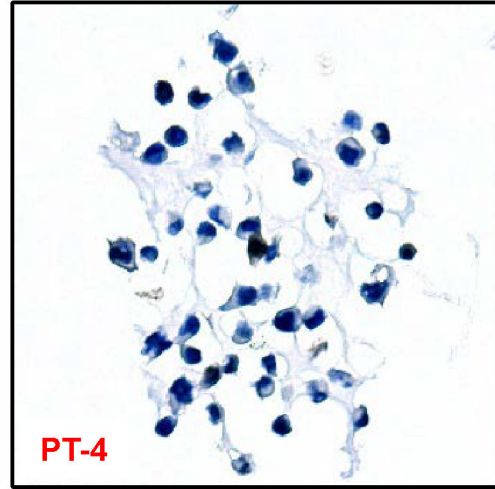
Figure 4. Heterogeneity of replication stress biomarker staining in a tumor sample and an organoid culture from the same biopsy.

Immunostaining for pRPA32 in the pretrial tumor biopsy sample from PT-4 (left) and the organoid culture (right) that was grown from the same biopsy.

Table 1:

Baseline Patient Characteristics

Characteristic	LY2880070/Gemcitabine (N=11)
Age	
Median (years)	65
Range (years)	43–70
Gender	
Female	3 (27.3%)
Male	8 (72.7%)
Race	
Other	1 (9.1%)
White	10 (90.9%)
Performance Status	
ECOG 0	5 (45.5%)
ECOG 1	6 (54.5%)
Baseline lymphocyte-to-monocyte ratio	
Median	1.74
Range	0.07–5.6
Baseline neutrophil-to-lymphocyte ratio	
Median	4.31
Range	1.08–123.7
Baseline Albumin	
Median (g/dL)	4.1
Range (g/dL)	3.5–4.6
Baseline CA19–9	
Median (g/dL)	2167
Range (g/dL)	27–15810
Prior Treatment	
1 prior line	0 (0%)
2 prior lines	6 (5.5%)
3 prior lines	5 (45.5%)
Prior Treatment Type	
5FU/nanoliposomal irinotecan	1 (9.1%)
Capecitabine	1 (9.1%)
FOLFIRINOX (metastatic)	7 (63.6%)
FOLFIRINOX (perioperative)	4 (36.4%)
Gemcitabine	1 (9.1%)
Gemcitabine/nab-paclitaxel	8 (72.7%)
Niraparib	1 (9.1%)
Other	2 (18.2%)

Characteristic	LY2880070/Gemcitabine (N=11)
Prior Treatment Type	
Radiation	5 (45.5%)
Pancreatic surgical resection	3 (27.3%)
Systemic therapy	11 (100%)
Sites of Metastases	
Bone	4 (36.4%)
Liver	11 (100%)
Lung	9 (81.8%)
Other	5 (45.5%)
Pancreas	7 (63.6%)
Peritoneum	4 (36.4%)

Table 2:

Any Treatment-Emergent Adverse Events

	Any Grade Treatment-Emergent Adverse Events	Grade 3 Treatment-Emergent Adverse Events
Abdominal pain	1 (9.1%)	1 (9.1%)
Acute Kidney Injury	1 (9.1%)	1 (9.1%)
Alanine Aminotransferase increased	5 (45.5%)	2 (18.2%)
Anemia	7 (63.6%)	3 (27.3%)
Aspartate Aminotransferase increased	4 (36.4%)	1 (9.1%)
Asthenia	1 (9.1%)	
Blood alkaline phosphatase increased	1 (9.1%)	1 (9.1%)
Chills	1 (9.1%)	
Constipation	2 (18.2%)	
Creatine phosphokinase increased	1 (9.1%)	1 (9.1%)
Decreased appetite	3 (27.3%)	
Dehydration	3 (27.3%)	
Depression	1 (9.1%)	1 (9.1%)
Diarrhea	2 (18.2%)	
Dyspnea	1 (9.1%)	
Fatigue	7 (63.6%)	1 (9.1%)
Gastric hemorrhage	1 (9.1%)	1 (9.1%)
Influenza like illness	1 (9.1%)	
Maculopapular rash	1 (9.1%)	
Myocardial infarction	1 (9.1%)	1 (9.1%)
Nausea	9 (81.8%)	
Neutropenia	5 (45.5%)	1 (9.1%)
Peripheral edema	1 (9.1%)	
Pneumonia cryptococcal	1 (9.1%)	
Sepsis	2 (18.2%)	2 (18.2%)
Streptococcal bacteremia	1 (9.1%)	1 (9.1%)
Thrombocytopenia	6 (54.5%)	
Upper Abdominal pain	1 (9.1%)	
Vomiting	3 (27.3%)	