8 SUPPLEMENTARY METHODS

8.1 Pharmacokinetics

All patients had peripheral blood drawn for PK studies during cycle 1 only at the following times: day -2, pre-treatment, and 25, 60, 90 min, 2, 3, 4, 6, and 8 h post-treatment; day 1, prior to gemcitabine infusion, 15, 25, 60, 90 min, 2, 3, 4, 6, 8, 24, and 48 h post start of infusion. Day 1 vacutainers were pretreated with 10 µL of 10 mg/mL THU for each mL of blood collected to prevent *ex vivo* deamination of gemcitabine. Plasma concentrations of veliparib (day -2 and 1) and gemcitabine and its deaminated metabolite dFdU (day 1) were quantitated using LC-MS(/MS) assays developed and validated at the UPCI Cancer Pharmacokinetics and Pharmacodynamics Facility (CPPF) [36,37]. Plasma pharmacokinetic parameters were derived from the data by non-compartmental methods with PK Solutions 2.0™ (Summit Research Services, Montrose, CO, USA).

8.2 Pharmacodynamics

Pharmacodynamic (PD) analysis of PAR levels was performed on PBMCs collected on cycle 1, day -2 prior to and at 1 and 6 h, respectively, after the first veliparib dose and on cycle 1, day 1 prior to and at 1, 6 and 24 h after the start of the gemcitabine infusion. PAR levels were analyzed by the PAR immunoassay developed by the National Cancer Institute Division of Cancer Treatment and Diagnosis or using a commercially available kit (Trevigen HT Chemiluminescent PAR Sandwich ELISA Cat# 4418-096-K; currently sold as "PARP in vivo pharmacodynamic assay II kit Cat#: 4520-096-K). In addition, the level of DNA damage response in PBMCs, as assessed by activation of ataxia telangiectasia mutated (ATM) and histone 2A (H2AX), was determined using a semi-quantitative multiplexed immunoblot assay previously developed by the CPPF [32].

8.3 Patient with ascites

A 69-year-old man with metastatic adenocarcinoma of the pancreas was enrolled on this phase I trial. He had been initially diagnosed with adenocarcinoma of the pancreas and pulmonary metastases and had undergone first-line chemotherapy with gemcitabine. He progressed with peritoneal carcinomatosis and cytologically documented malignant ascites. The rapid re-accumulation of ascites, despite repeated paracenteses, necessitated ultrasound-guided placement of an indwelling catheter into the peritoneal cavity. The catheter was attached to a drainage bag, and the patient or his caregiver emptied the bag daily. The patient was started on DL 1 of the 28-day cycle. During day 1, serial blood and ascites samples were collected for PK analysis of veliparib, gemcitabine and dFdU and for measurement of PAR on PBMCs and ascites cells.

Ascitic fluid samples (5 mL) were collected in tubes containing THU. They were stored in an ice bath until centrifuged at 3,800 x g for 15 min at 4°C. The resulting supernatant was stored at -70 °C until analysis. The cell pellet was counted and stored for analysis of PAR. Ascitic cells were collected on day 1, before, and at 25 min, 1 h, and 6 h after administration of the study drugs.

8.4 Protein binding of veliparib

For the evaluation of veliparib protein binding in plasma and ascites, veliparib was added to blank plasma or ascites to attain a final concentration of approximately 50, 125, and 1250 ng/mL. In a commercially available rapid equilibrium dialysis device (Pierce Biotechnology, ThermoFischer Scientific, Rockford, IL) [38], plasma or ascitic fluid samples (500 μL) were dialyzed against 750 μL of phosphate-buffered saline (PBS) at 37 °C for 4 h (the time point at which equilibrium had been reached) and the concentration of veliparib was quantitated in plasma or ascitic fluid and PBS. Because of decreased sensitivity of the method in PBS samples, PBS was mixed with plasma at a ratio of 2:1, which resulted in adequate sensitivity of the LC-MS assay for the quantitation of veliparib. The unbound fraction (Fu) of veliparib was calculated from the ratio of veliparib concentration in PBS to the concentration in plasma or ascitic fluid.

8.5 Cytidine deaminase activity

Cytidine deaminase (CDA) enzymatic activity was determined in plasma at pre-treatment and on day 1 1.5 h post-veliparib as previously described [39]. Briefly, CDA-mediated generation of ammonium was monitored spectrophotometrically after overnight incubation at 37°C. Protein concentration was determined with the BCA Protein Assay kit (PierceTM, Thermo scientific, Rockford, IL 61105). Each sample was assayed in duplicate and results averaged. The potential relationship of gemcitabine clearance and the metabolic ratio of dFdU/gemcitabine-ratio *versus* pre-treatment CDA activity were explored. Pre-treatment and day 1 1.5 h post-veliparib samples were compared by paired non-parametric Wilcoxon to assess any effect of veliparib on CDA activity.