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A phase I/II study of ivaltinostat combined with gemcitabine and erlotinib in patients with untreated locally advanced or metastatic pancreatic adenocarcinoma

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Abstract

This phase I/II study evaluated the safety and efficacy of a new histone deacetylase (HDAC) inhibitor, ivaltinostat, in combination with gemcitabine and erlotinib for advanced pancreatic ductal adenocarcinoma (PDAC). Patients diagnosed with unresectable, histologically confirmed PDAC who had not undergone previous therapy were eligible. Phase I had a 3 + 3 dose escalation design to determine the maximum tolerable dose (MTD) of ivaltinostat (intravenously on days 1, 8 and 15) with gemcitabine (1000 mg/m^2 intravenously on days 1, 8 and 15) and erlotinib (100 mg/day, orally) for a 28-day cycle. In phase II, patients received a six-cycle treatment with the MTD of ivaltinostat determined in phase I. The primary endpoint was the objective response rate (ORR). Secondary endpoints included overall survival (OS), disease control rate (DCR) and progression-free survival (PFS). The MTD of ivaltinostat for the phase II trial was determined to be 250 mg/m^2. In phase II, 24 patients were enrolled. The median OS and PFS were 8.6 (95% confidence interval [CI]: 5.3-11.2) and 5.3 months (95% CI: 3.7-5.8). Of the 16 patients evaluated for response, ORR and DCR were 25.0% and 93.8% with a median OS/PFS of 10.8 (95% CI: 8.3-16.7)/5.8 (95% CI: 4.6-6.7) months. Correlative studies showed that mutation burden detected by cfDNA and specific blood markers such as TIMP1, pro-MMP10, PECAM1, proMMP-2 and IGFBP1 were associated with clinical outcomes. Although the result of a small study, a combination of ivaltinostat, gemcitabine and erlotinib appeared to be a potential treatment option for advanced PDAC.

KEYWORDS
chemotherapy, erlotinib, gemcitabine, ivaltinostat, pancreatic cancer

Abbreviations: CR, complete response; DCR, disease control rate; DLT, dose-limiting toxicity; ECOG, Eastern Cooperative Oncology Group; EGFR, epithelial growth factor receptor; FAS, full analysis set; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; MTD, maximum tolerable dose; ORR, objective response rate; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; PP, per protocol; PR, partial response.

Our study was previously presented at a poster session at the ESMO 22nd World Congress on Gastrointestinal Cancer, 2020.

What's new?

Histone deacetylase inhibitors (HDACi) are approved for use as anticancer drugs for a range of tumors. While no clinical trials have evaluated HDACi efficacy in pancreatic ductal adenocarcinoma (PDAC), the novel intravenous HDACi ivaltinostat appears to exert a synergistic anticancer effect in PDAC cells with gemcitabine/erlotinib combinations in vitro and in vivo. This phase I/II prospective single-arm study suggests that ivaltinostat combined with gemcitabine/erlotinib may represent a potential treatment option with an acceptable safety profile for advanced pancreatic ductal adenocarcinoma. Moreover, the results point to potential blood markers that could help predict responses to ivaltinostat treatment.

1 | INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer mortality with poor overall survival (OS).[1] The 5-year survival rate for advanced PDAC has remained at approximately 3% even with recent improvements in combination therapies of FOLFIRINOX and gemcitabine/nab-paclitaxel.[2,3] These cytotoxic chemotherapies improve treatment outcomes but also increase toxicities. Currently, there have been only one approved targeted agent for advanced PDAC, erlotinib, an epithelial growth factor receptor (EGFR) inhibitor, combined with gemcitabine.[4] However, this regimen only marginally improves patient outcomes.

Histone acetylation is a posttranslational modification associated with cancer initiation and progression. Nucleic histone deacetylase (HDAC) removes the acetyl group from the N-terminal tail of the histone and stabilizes the DNA-histone complexes, thus inducing chromatin compaction.[5,6] This process can reduce the expression of genes associated with tumor suppression and differentiation and can lead to tumorigenesis. HDAC inhibitors (HDACis) are currently used as anticancer drugs for hematologic malignancies. In 2006, vorinostat, a hydroxamate-based HDACi, was the first HDACi to earn United States Food and Drug Administration (USFDA) approval for the treatment of cutaneous T-cell lymphoma.[7] For the next decade, other HDACis, such as romidepsin, belinostat and panobinostat, proved their efficacy in clinical trials and were approved by USFDA for various hematological malignancies.[8-11] HDACis were not successful in clinical trials as monotherapies for solid tumors.[5] When combined with other chemotherapeutic agents, HDACis were reported to be effective in ovarian cancers and renal cell carcinoma.[12,13] There have been no clinical trials evaluating the efficacy of HDACis against PDAC, although a phase I study reported that an HDACi was well tolerated by patients with pretreated biliary tract or pancreatic cancer.[14] Additionally, through in vitro experiments using cell lines, recent studies have proposed the use of HDACis as therapeutic agents for pancreatic cancer.[15-18]

Ivaltinostat, previously known as CG200745, is a recently developed intravenous hydroxamate-based pan-HDACi, similar to other HDACis such as vorinostat and belinostat approved by the USFDA.[19-22] Anticancer effects of ivaltinostat were reported in various solid tumors[22] like prostate cancer,[19] nonsmall cell lung cancer,[21] cholangiocarcinoma,[23] and pancreatic cancer.[24] Ivaltinostat induced cell death by modulating acetylation of p53, a tumor suppressor,[20] and induced antitumor effects via miRNAs targeting the Hippo pathway in cancer cells.[23] A first-in-human study of ivaltinostat reported it could be safely administered at effective dose levels that inhibited HDAC in tumor tissue in 28 patients with refractory solid malignancies.[22] Regarding pancreatic cancer, we previously reported ivaltinostat inhibited pancreatic cancer cell growth and improved chemosensitivity to gemcitabine.[24] Ivaltinostat induced the expression of apoptotic proteins and increased acetylated histone H3 levels in pancreatic cancer cell lines and enhanced sensitivity of gemcitabine-resistant pancreatic cancer cells to gemcitabine with decreased expression of ATP-binding cassette-transporter genes. In particular, ivaltinostat exerted a synergistic anticancer effect in PDAC cells with gemcitabine/erlotinib combinations in vitro and in vivo.

Based on the above considerations, we conducted a phase I/II prospective single-arm study to evaluate the safety and efficacy of the combination therapy of ivaltinostat, a novel HDACi, combined with gemcitabine and erlotinib in patients with untreated locally advanced or metastatic PDAC. In a correlative study, we evaluated potential biomarkers showing differences in expression in the patient group according to the treatment response.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients diagnosed with histologically confirmed unresectable PDAC without previous history of anticancer chemotherapy, radiation or biologics were enrolled. Eligibility criteria included age 20 to 75 at enrollment; Eastern Cooperative Oncology Group performance status (ECOG PS) 0 to 2; estimated life expectancy at the time of enrollment more than 3 months; and adequate hematologic (absolute neutrophil count 1500/mm^3, hemoglobin 9.0 g/dL, platelets ≥100 000/mm^3); hepatic (serum bilirubin <2× upper limit normal [ULN], aspartate aminotransferase [AST]/alanine aminotransferase [ALT] <2.5× ULN, alkaline phosphatase <5× ULN with liver metastasis, AST/ALT <5× ULN, prothrombin time [PT] or partial thromboplastin time 1.5× ULN); and renal function (serum creatinine or creatinine clearance rate 60 mL/min [using the Cockcroft-Gault equation] and normal serum electrolyte values [calcium: 8.3-10.5 mg/dL, magnesium: 1.58-3.0 mg/dL,

phosphorous: 2.4-4.5 mg/dL and potassium: 3.3-5.5 mmol/L]). Exclusion criteria included patients who had experienced a major surgery within 2 weeks prior to the screening visit with evidence of uncontrolled brain metastasis (except patients with radiologically and neurologically stable brain metastasis without corticosteroid therapy for at least 2 weeks); subjects ineligible for oral drugs, or having difficulty in absorbing the study drugs due to a history of major gastrointestinal surgery or pathological findings; patients treated with antibiotics within the last 7 days due to an active bacterial infection prior to the enrollment (topical antibiotic therapies were excluded); patients diagnosed with malignancies within past 5 years (except for basal cell skin cancer, in situ cervical cancer or papillary thyroid tumor); pregnancy or lactating patients; fertile patients who did not consent to the effective contraception during the study period and up to 3 months after the completion of the study; patients ineligible for anticancer chemotherapy due to a systemic disease (ie, chronic renal failure); patients treated with any other investigational drug within 4 weeks prior to the screening visit; patients with a history of hypersensitivity to the study drugs; and patients that were human immunodeficiency virus-positive. The first subject enrolled on 7 April 2016, and the last subject completed the study on 14 February 2020.

2.2 | Study design and treatment

This single-arm phase I/II clinical study evaluated the efficacy and safety of ivaltinostat in combination with gemcitabine and erlotinib for advanced pancreatic cancer. Phase I had a 3 + 3 dose escalation design to determine the maximum tolerable dose (MTD) and the dose-limiting toxicity (DLT) of ivaltinostat in combination with gemcitabine and erlotinib. The DLT was assessed according to the National Cancer Institute Common Toxicity Criteria version 4.0. A DLT was defined as: (a) grade 4 neutropenia lasting six or more days or grade 4 or grade 3 thrombocytopenia with bleeding or grade 3 neutropenia associated with fever >38.5°C; (b) any drug-related nonhematologic grade 3 or 4 toxicity with the exception of mucositis, nausea, vomiting, anorexia, dermatitis and fatigue; (c) drug-related nonhematologic grade 4 toxicity, including mucositis, nausea, vomiting, anorexia, dermatitis and fatigue if supportive interventions were unsuccessful; (d) alopecia was an exception for DLT regardless of grades; (e) treatment-related toxicities that caused discontinuation of treatment more than twice or occurring during three consecutive weeks or total delay of treatment for more than 3 weeks. The MTD was defined as the dose at which one or zero patients developed a DLT among six-patient cohort. Based on the 3 + 3 dose escalation design, each cohort consists of three or six subjects. If one in three subjects had experienced DLT in a specific dose, the dose was administered to an additional three subjects and a total of six patients were checked for the occurrence of DLT. If two or more subjects in any cohort had experienced a DLT, the previous dose was considered MTD, and the dose escalation ended. The initial dose of ivaltinostat was 187.5 mg/m^2 and extended to 250 or 312.5 mg/m^2 based on the results of the cohort of three subjects per dose level. Gemcitabine and erlotinib were administered as fixed doses; gemcitabine (1000 mg/m^2 intravenously), and erlotinib (100 mg/day orally), whereas ivaltinostat was administered according to the dose level for each cohort. Each cycle consisted of 28 days. Both ivaltinostat and gemcitabine were administered on days 1, 8 and 15 and erlotinib was administrated daily for each 28-day cycle.

Overall, 24 patients were enrolled in phase II, which utilized Simon's two stage design,[25] and received ivaltinostat (250 mg/m^2 intravenously, determined based on the results of phase I study), gemcitabine (1000 mg/m^2 intravenously) and erlotinib (100 mg/day orally). Both ivaltinostat and gemcitabine were administered via the same protocol as that in the phase I study, until development of unacceptable toxicity or disease progression. The entire treatment period was six cycles and could be extended to 12 cycles if the patients wanted to continue the regimen and the principal investigator agreed. Tumor assessment was evaluated at the end of every two cycles. Dose reduction or delay could be applied for intolerant patients according to the study protocol (Appendix S1).

2.3 | Assessments

The primary endpoint was the ORR, which was defined as the rate (%) of patients who had best tumor assessment with complete response (CR) or partial response (PR) during the treatment period. Secondary endpoints included disease control rate (DCR), OS and progression-free survival (PFS). DCR was defined as the rate (%) of patients who had best tumor assessment with CR, PR or stable disease (SD) during the treatment period. OS was defined as the time from the date of the first enrollment to the date of death from any cause. Patients who were still alive or withdrew at the date of cut off were censored. PFS was defined as the time from the date of the first enrollment to the date of tumor progression or to the date of death from any cause. Patients were followed up for survival until death, withdrawal or study closure.

Tumor evaluation was performed at baseline, at the end of every two cycles and/or at the end of the trial. For patients with measurable/nonmeasurable lesions, the Response Evaluation Criteria in Solid Tumors version 1.1 was used to evaluate target/nontarget lesions. Serial measurements of carbohydrate antigen 19-9 (CA19-9) levels were performed at baseline and at the beginning of each treatment cycle. Patients were followed up for survival until death or study closure. Safety evaluation included vital signs, adverse events (AEs), adverse drug reactions, serious AEs, ECG and clinical laboratory tests, which were performed on days 1, 8 and 15 each cycle. AEs were graded according to the Common Terminology Criteria for Adverse Events, version 4.0, by the National Cancer Institute.

Full analysis set (FAS), per protocol (PP) and safety set analysis were defined according to the study protocol. FAS was defined as patients who had been evaluated for tumor response at least once after baseline. The PP analysis set included patients who received at least 70% and more of the intended study drug throughout the treatment period and without any significant violation of the protocol (Appendix S1). The safety cohort included all patients who had received the study drug at least once.

2.4 | Correlative studies

2.4.1 | Patient's blood sample collection

Peripheral blood samples for correlative studies including cfDNA analysis, protein array and ELISA analyses were collected predose on days 1 and 8 of the first treatment cycle, on days 1, 3 and 5 of the treatment cycle and on the day the treatment ended. The blood sample was transferred to a plain tube and an EDTA-containing tube immediately after collection, centrifuged within 30 minutes and stored below −70°C.

2.4.2 | cfDNA analysis

The cfDNA samples were extracted from plasma samples. The concentration of DNA sample was assayed using the Qubit Fluorometer and the size distribution was measured using TapeStation. DNA barcodes were attached to samples during barcoding PCR to distinguish each sample in sequencing process. The PCR product for each sample was pooled and subsequently purified. The sample sets were subjected to the enrichment process for 96 mutations. The enriched outputs were used for library preparation, and the final library was sequenced on the Illumina MiSeq. Sequencing data was analyzed in a fully automated fashion using BWA analysis scripts for alignment to a custom reference library composed of sequences within the 96-gene mutation panel, and using SAM Tools for further data manipulation following alignment. Mutation quantification was performed using in-house scripts. ONCOCHASER (Theragen Bio Inc., Gyeonggi-do, Korea) test reports on the absence or presence of each 96 mutations with over two mutant DNA copies per plasma samples. The input DNA mass was the total amount of cfDNA from the plasma sample used in the assay. Since the assay detects internal positive controls to measure all copies of the genome, the input DNA mass may differ from the Qubit mass. Mutant DNA abundance (%) was also reported relative to input DNA mass, with reference to limit of detection. The sequencing coverage and quality statistics for samples are summarized in Table S1. ONCOCHASER targeted mutation list are described in Table S2.

2.4.3 | Protein array

Protein was extracted from plasma using a protein extraction buffer (Fullmoon Biosystems, Sunnyvale, California) and protein expression was analyzed using antibody microarray analysis (Fullmoon Biosystems) according to the manufacturer's protocol. Briefly, 50 μg of protein sample was labeled and incubated with coupling mixture on the antibody microarray slide (Fullmoon Biosystems) and detected with Cy3-streptavidin (GE Healthcare, Chalfont St. Giles, UK). The slide was rinsed scanned using the GenePix 4100A scanner (Axon Instrument, USA) at 10 μm resolution, optimal laser power and PMT. Following image scanning, they were gridded and quantified with GenePix 7.0 Software (Axon Instrument, USA). The protein information was annotated using UniProt DB. Comparing good and poor responders to treatment, markers that clearly showed a difference in expression before and after treatment were selected as subjects for further verification by ELISA.

2.4.4 | ELISA analysis

Serological marker measurement was performed using ELISA kits according to the manufacturer's protocols: IL4 (D4050), TIMP1 (DTM100), IL3 (D3000), IL1A (DLA50), PDGFB (DBB00), TEK (or TIE2, DTE200), PECAM1 (DCD310), MMP2 (MMP200), pro-MMP10 (DM1000), KLK3/PSA (DKK300), free IGFBP1 (DGB100), CX3CL1 (DCX310), IL19 (D1900) (all R&D SYSTEMS); and YAP (LifeSpan BioSciences, Inc., LS-F49700). A calibration curve was generated using known concentrations of analyte. Samples were further diluted and reassayed if readings were above the linear range of the calibration curve. Data are reported as mean ± SD. ELISA kits details used for serological marker measurement are listed in Table S3. The degree of correlation between blood markers and patient PFS were evaluated to choose potential biomarkers for predicting responses to treatment or disease prognosis.

2.5 | Statistical analysis

To identify MTD and DLT, the 3 + 3 dose escalation design was utilized and based on the method of determining MTD described above. In total, 10 patients were enrolled in the phase I study. The estimated sample size for the phase II study after the determination of MTD was calculated based on Simon's two stage design[25] with a reference of published data of 8.6% ORR from a phase III randomized controlled trial of gemcitabine and erlotinib combination therapy.[4] Assuming a difference in response probability of study drug = 20%, $\alpha = .05$ and statistical power = 90%, the estimated minimally required sample size for phase II study based on binomial distribution probability would be 9 for initial stage and increase to a total of 24 patients if more than one patient had responded to the study drug during the initial stage. The safety analysis included all patients who had received a single dose of the study drug. The main efficacy analysis was based on the FAS population, defined as a group of patients who had received at least single dose of study drug and had at least one tumor response evaluation after the baseline assessment.

The final data analysis was performed using IBM SPSS 748 Statistics for Windows, v25.0 (IBM Corp, Armonk, New York) with a data cutoff date of 20 October 2020. Data from patients who were alive at that time were censored for survival analysis. Statistical analysis of correlated studies used Pearson's correlation and Mann-Whitney *U* test to evaluate the correlation of ELISA results with patient

outcomes. Data analysis were performed using GraphPrism v5.0 (GraphPad Software, California).

3 | RESULTS

3.1 | Patients characteristics

The baseline demographic and disease characteristics of patients for the phase I/II study are summarized in Table 1. Ten patients (six male and four female) with a median age of 59 years were enrolled in the phase I study. One subject presented ECOG PS2 (10%) and others presented ECOG PS1 (90%). Baseline CA19-9 was 599 U/mL (median) and primary tumor sites were four in the head (40%), five in the body or tail (50%) of the pancreas and one in an overlapped area (10%). One patient had locally advanced disease (10%) and nine patients had metastatic disease (90%).

Figure 1 presents a schematic summary of the phase I study. Ten patients received three different dose levels of ivaltinostat with gemcitabine and erlotinib. One DLT, a febrile neutropenia of grade 3, was observed among the four patients who received the 312.5 mg/m^2 dose of ivaltinostat. According to the protocol, two more patients were supposed to be enrolled in the same dose cohort. However, due to the increased frequency and severity of AEs at the dose level of 312.5 mg/m^2 compared to the cohorts of lower dose levels, the Data and Safety Monitoring Board recommended not continuing at that dose level of 312.5 mg/m^2. Thus, MTD and the recommended dose for phase II for ivaltinostat was determined to be 250 mg/m^2.

3.2 | Pharmacokinetic analyses

Data for the pharmacokinetics analysis are presented in Table S4. The half-life ($t_{1/2}$) of ivaltinostat at the dose level of 187.5, 250 and 312.5 mg/m^2 was 7.3, 5.7 and 6.6 hours, respectively. $AUC_{0\text{-}24h}$ of ivaltinostat at the dose level of 187.5, 250 and 312.5 mg/m^2 were 28 264, 35 143 and 65 431 h·ng/mL, while C_{max} was 9553, 8062 and 12 782 ng/mL, respectively (Table S4 and Figure S1).

3.3 | Efficacy

Twenty-four patients (13 male and 11 female) with a median age of 64 years (range 47.3-74.7) were enrolled in phase II study. The location of the primary tumor site was observed mainly in the body/tail (62.5%) and the head (33.3%) of the pancreas (Table 1). One patient presented ECOG PS0 (4.2%) and the other patients presented ECOG PS1 (95.8%). The baseline CA19-9 was 456 U/mL (median), seven patients had locally advanced disease (29.2%) and 17 patients had metastatic disease (70.8%).

Figure 1 presents the trial flow of the phase II study. Eight of 24 patients were dropped early from the study without any response evaluation after the baseline; thus, 16 patients were included in the FAS analysis according to the study protocol. The reasons for the eight early drop-outs were: patient's choice (four cases, 50%), general

TABLE 1 Patient demographics and disease characteristics

Characteristics	Part 1	Part 2	Total
Number of subjects	10	24	34
Age, years (mean ± SD)	59.8 ± 11.7	62.7 ± 7.7	61.9 ± 9.0
Median	59.3	64.3	63.4
Sex, no. (%)			
Female	4 (40.0)	11 (45.8)	15 (44.2)
Male	6 (60.0)	13 (54.2)	19 (55.9)
ECOG PS, no. (%)			
0	0 (0.0)	1 (4.2)	1 (2.9)
1	9 (90.0)	23 (95.8)	32 (94.1)
2	1 (10.0)	0 (0.0)	1 (2.9)
Baseline CA 19-9, U/mL (median, range)[a]	599 (2-20 000)	456 (1-20 000)	549 (1-20 000)
Primary tumor site, no. (%)			
Head	4 (40.0)	8 (33.3)	12 (35.3)
Body or tail	5 (50.0)	15 (62.5)	20 (58.8)
Overlap	1 (10.0)	1 (4.2)	2 (5.9)
Extent of disease, no. (%)			
Locally advanced	1 (10.0)	7 (29.2)	8 (23.5)
Metastatic	9 (90.0)	17 (70.8)	26 (76.5)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.
[a]Measurement upper limit of the CA 19-9 was 20 000 U/mL.

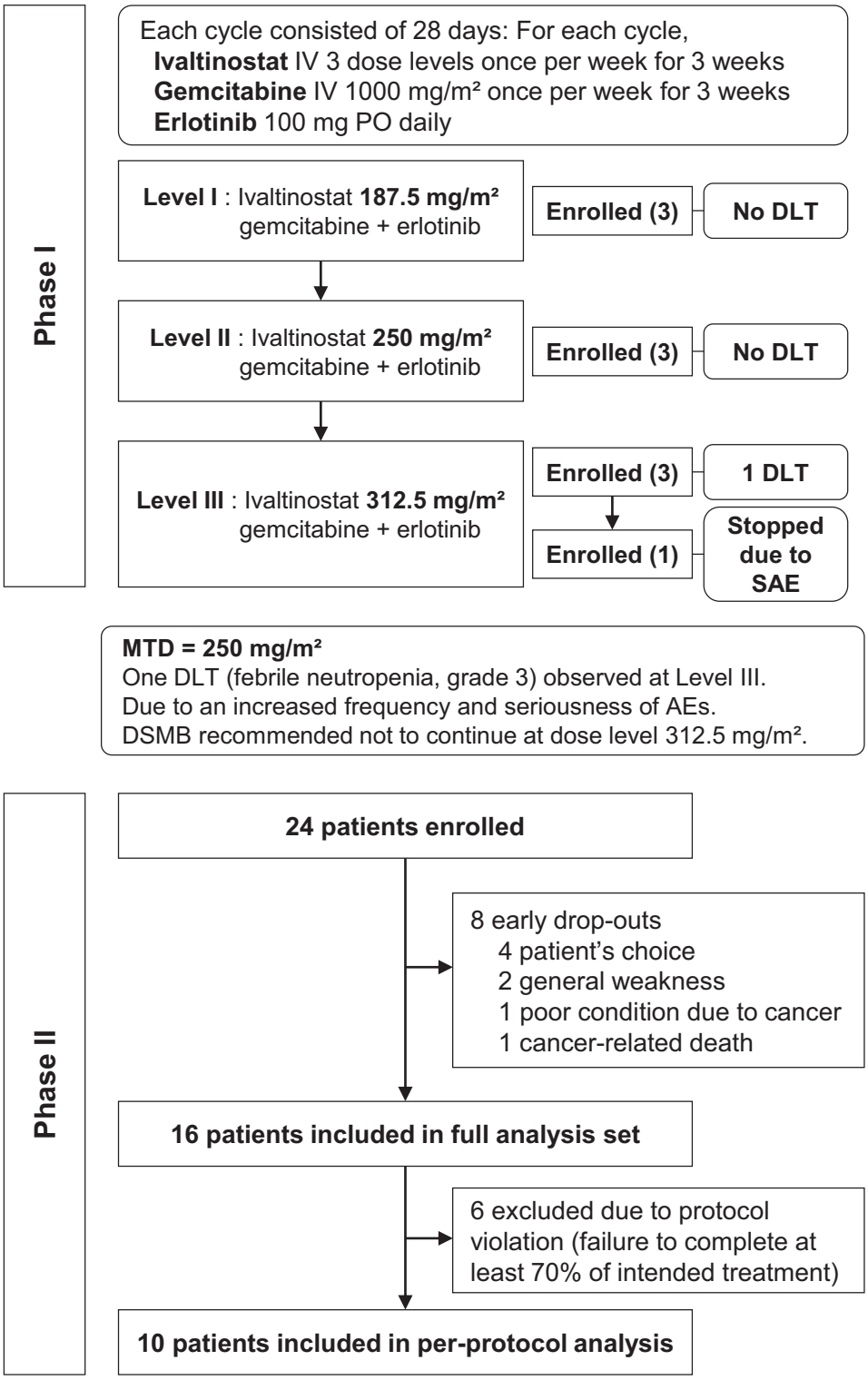

FIGURE 1 Trial flow of the phase I and II study

weakness (two cases, 25%), poor conditions due to cancer (one case, 12.5%) and cancer-related death (one case, 12.5%). Ten patients were included in the PP analysis after six patients were excluded due to failure to complete >70% of the intended treatment of six cycles. All patients, except one patient who withdrew consent (n = 23), were followed every 2 months for survival analysis. Figure 2 depicts each patient's tumor response and OS in swimmer plot.

At the time of the final follow-up for OS, 15 patients had died, 7 patients were lost to follow-up and one patient was still alive. All causes of death were related to cancer. Median OS and PFS based for all patients (n = 23) were 8.6 months (95% CI: 5.3-11.2) and 5.3 months (95% CI: 3.7-5.8), respectively. In the FAS analysis (n = 16), ORR was 25.0% and DCR was 93.8% with 4 PRs (25%) and 11 SDs (68.8%). Median OS was 10.8 months (95% CI: 8.3-16.7) and

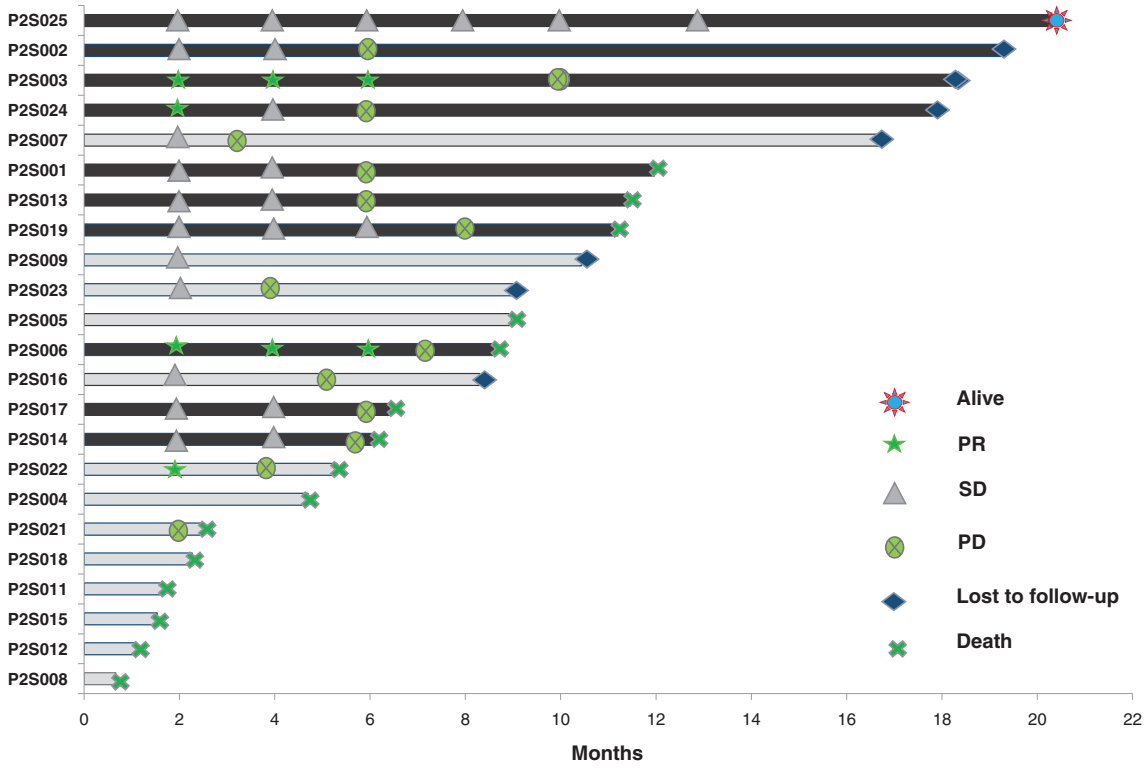

FIGURE 2 Swimmer plot graph of patients in the phase II study. Color codes for the bars: Dark brown solid line represents subjects who had completed six cycles or more; Gray solid line represents subjects who received fewer than six cycles of treatment. Loss-to-follow-up cases are presented at the middle point between the dates known to be alive and lost to follow-up [Color figure can be viewed at wileyonlinelibrary.com]

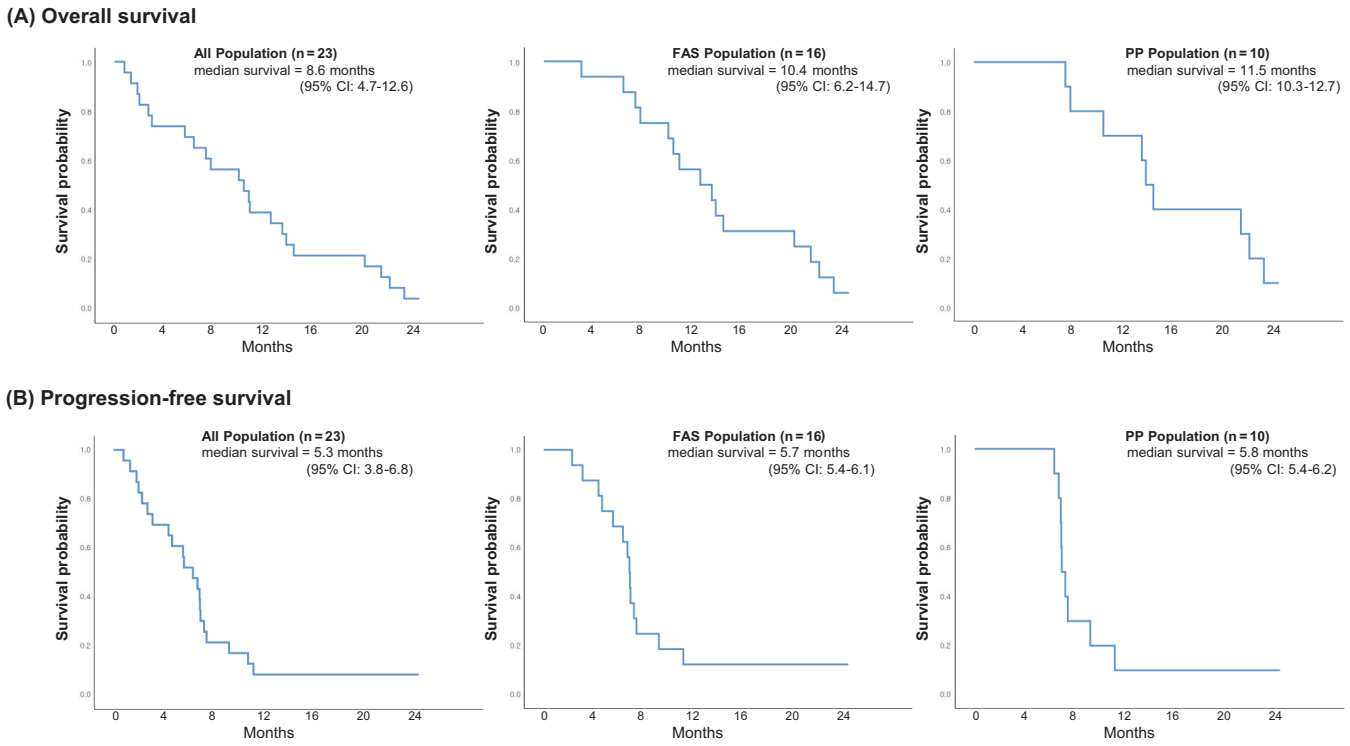

FIGURE 3 Kaplan-Meier curves for overall survival and progression-free survival for the phase II patients. (A) Overall survival according to study populations. (B) Progression-free survival according to study populations [Color figure can be viewed at wileyonlinelibrary.com]

median PFS was 5.8 months (95% CI: 4.6-6.7). In the PP analysis (n = 10), ORR was 30% and DCR was 100% with 3 PRs (30%) and 7 SDs (70%). Median OS was 11.7 months (95% CI: 8.6-18.4) and median PFS was 5.9 months (95% CI: 5.7-8.5). Figure 3 presents Kaplan-Meier curves for OS and PFS.

3.4 | Safety and adverse events

Twenty-four patients received at least one dose of ivaltinostat and were included in the safety analysis. The patients received a median of 3.8 cycles (mean 3.6 cycles) with a range of 0.33 to six cycles of treatment, with 10 patients completing six cycles of treatment. Of these, nine patients stayed on their starting dose of the study drug for the entire duration of treatment. Doses of ivaltinostat and gemcitabine were reduced in 3 of 24 patients (12.5%, respectively) due to occurrence of AEs and no patient received a reduced dose of erlotinib.

Of the 218 AEs reported, 114 adverse drug reactions (ADRs) occurred among all 24 subjects. The most frequently reported ADRs were decreased platelet count (15 events), decreased appetite (11 events), rash (11 events), nausea (10 events) and vomiting (10 events). A total of 26 ADRs with grade 3 or 4 severity were reported in 15 subjects (62.5%) (Table 2). There were no grade 5 (death) events.

The most frequently reported treatment-related grade 3 or 4 AEs were neutropenia (eight cases, 33.3%), thrombocytopenia (seven

TABLE 2 NCI-CTCAE grade 3 and 4 treatment-related adverse events in phase II study

		Number of patients (%)		
System organ class	Adverse events	Grade 3 (n = 24)	Grade 4 (n = 24)	Total (n = 24)
Hematologic	Neutropenia	3 (12.5)	5 (20.8)	8 (33.3)
	Febrile neutropenia	2 (8.3)	0	2 (8.3)
	Thrombocytopenia	4 (16.7)	3 (12.5)	7 (29.2)
	Anemia	4 (16.7)	0	4 (16.7)
Nonhematologic	Pneumonia	0	1 (4.2)	1 (4.2)
	Fatigue	2 (8.3)	0	2 (8.3)
	Anorexia	2 (8.3)	0	2 (8.3)

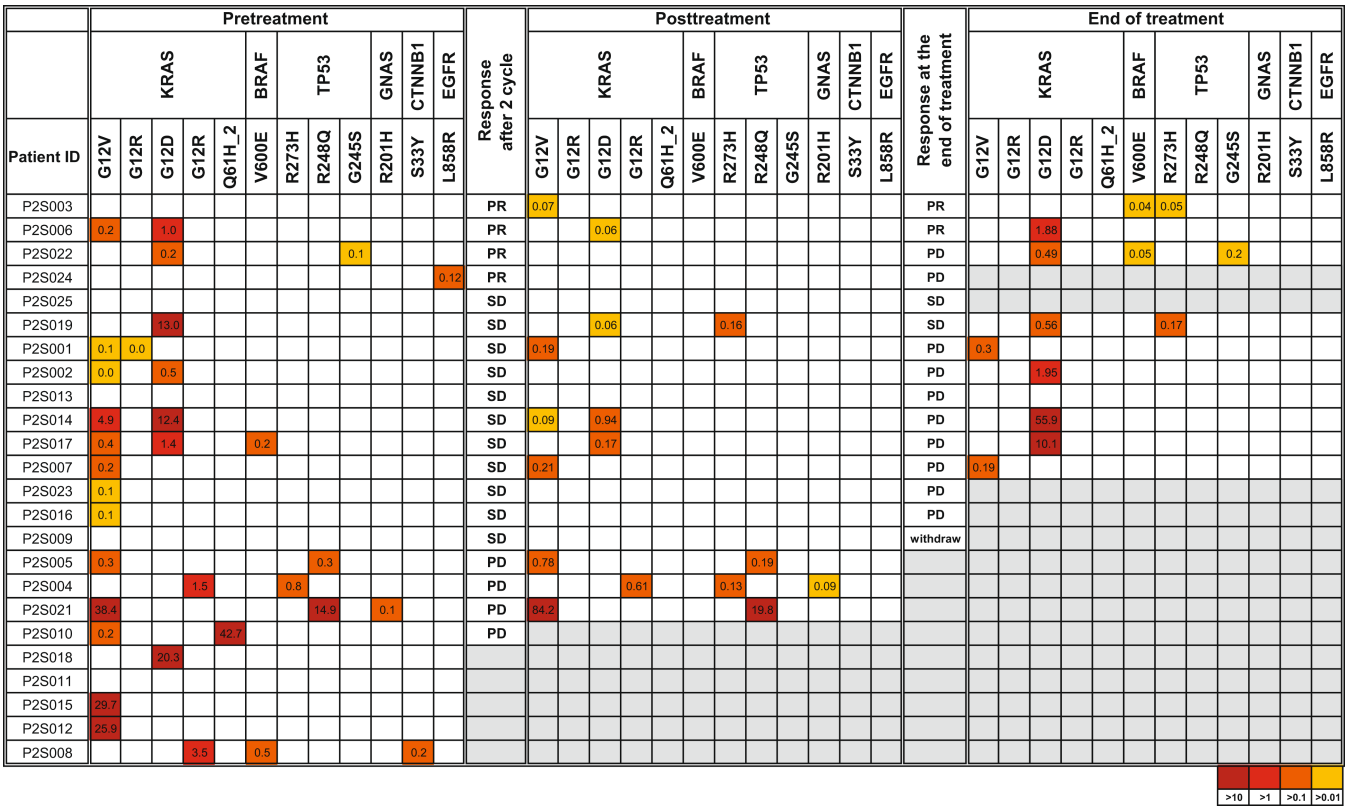

FIGURE 4 Change in tumor mutation burden at the beginning of the study and after treatment over time. Mutant DNA abundance (%) was presented by yellow (low) to red (high) color [Color figure can be viewed at wileyonlinelibrary.com]

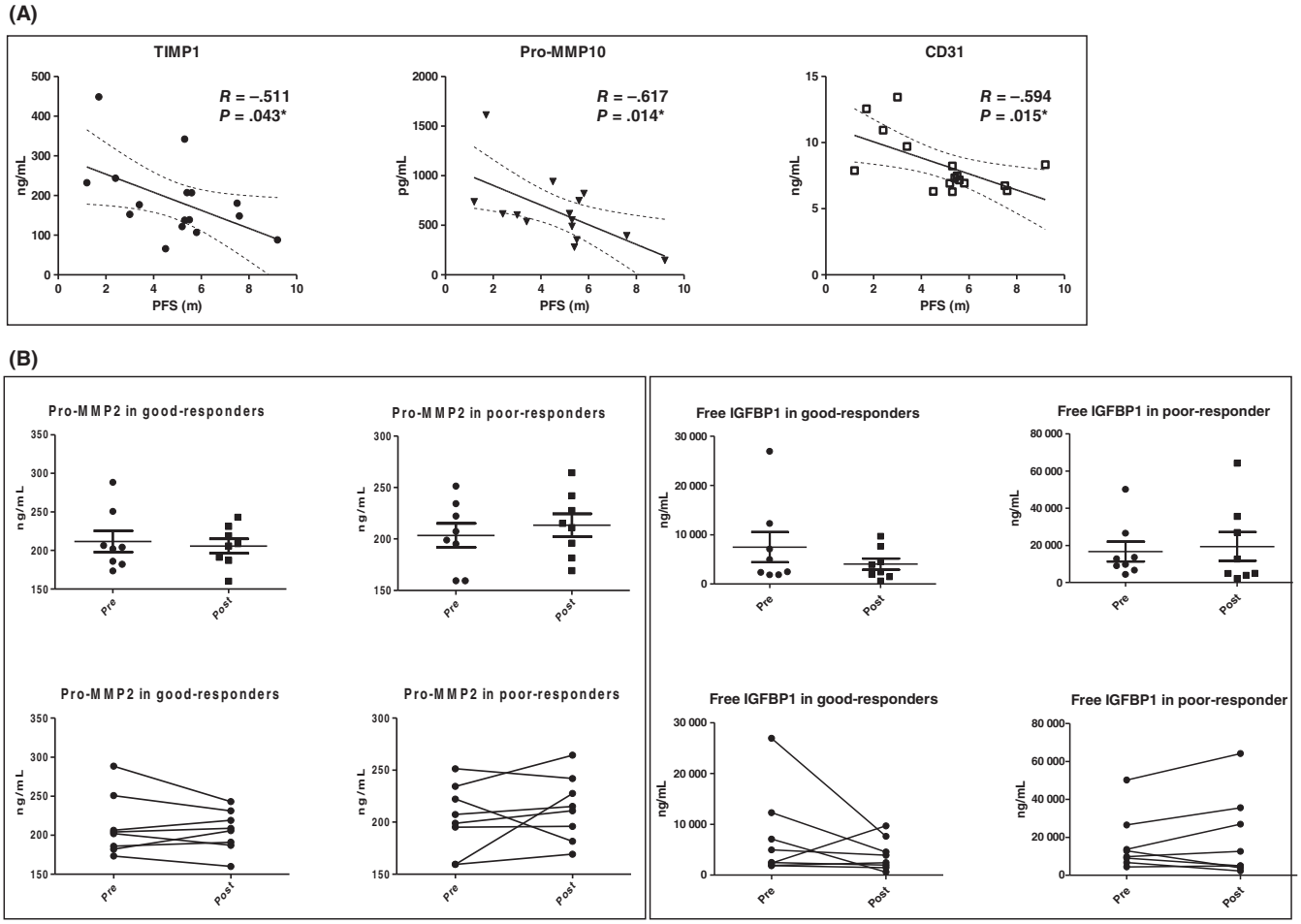

FIGURE 5 Expression of blood markers evaluated by ELISA. (A) The correlation between blood markers and progression-free survival (PFS) of patients is illustrated as a scatter plot where each dot represents a single blood sample. Pearson's correlation values (*R*) and the *P*-values are indicated. (B) Different modulation of blood markers in good and poor responders according to PFS

cases, 29.2%) and anemia (four cases, 16.7%). All AEs were well controlled with supportive treatment, there were no cases that had dropped out of the study due to intolerable AEs.

3.5 | Correlative studies

According to previous reports, KRAS (~90%) and TP53 mutations (50%-60%) were observed with high frequency, while GNAS, BRAF, CTNNB1 and EGFR mutations were observed at low frequency.[26,27] Figure 4 shows representative genetic mutations of PDAC among the patients in our study, listed in the order of response to therapy. The initial mutation rate of KRAS was 75% (18/24 cases) and TP53 was 16.7% (4/24 cases). BRAF, GNAS, CTNNB1 and EGFR were mutated in one or two of 24 cases. Patients who achieved a PR after 2 cycles of therapy presented lower mutation burden in expression level and diversity, compared to patients who achieved SD or PD. In general, patients who obtained PD after 2 cycles or could not be evaluated due to poor general condition or death showed higher mutation burden. Additionally, the overall mutation burden of patients with PR or SD showed a decreasing trend, while those of patients with PD showed an increasing trend. These findings may suggest that the mutation burden detected by cfDNA reflects the tumor response to ivaltinostat therapy and can be used to monitor the early response of each patient after treatment.

To determine potential markers of use in predicting chemotherapy responses to ivaltinostat or disease prognosis, patient samples were analyzed at the time of pretreatment, on day 8 after treatment, and at the end of the trial using antibody microarray analysis. Three good and three poor responders were selected to compare differences in marker expression stratified by patient treatment response (Figure S2). Accordingly, protein expression had clearly changed according to the patient's prognosis. Comparing individual patients before and after treatment, specific protein markers could clearly distinguish patients according to prognosis (Figure S2A). Differently expressed markers in the poor responders were compared to the good responders at pretreatment, and differently expressed markers on day 8 and at end of the trial compared to pretreatment were selected for validation by ELISA in all available patient samples (Figure S2B,C).

Serial blood samples at pretreatment and posttreatment (day 8), were available for 16 patients for ELISA analysis. Using Pearson's correlation coefficient, we examined the degree of correlation between blood markers and PFS of patients (Figure 5A). A negative correlation trend with PFS was observed with expression levels of TIMP1 ($R = -.511$, $P = .043$), pro-MMP10 ($R = -.617$, $P = .014$) and CD 31 (=PECAM1) ($R = -.594$, $P = .015$) between the markers tested. These three markers were considered potential biomarkers to predict the chemotherapy response to ivaltinostat before treatment. To determine early response markers, 16 patients were divided into good responders (n = 8) and poor responders (n = 8) according to PFS. Figure 5B shows the down-regulation of pro-MMP2 and free IGFBP1 in responders, whereas these markers were up-regulated in nonresponders. Thus, blood markers may have a potential role as response predictors of ivaltinostat in patients with PDAC.

4 | DISCUSSION

To our best knowledge, this is the first complete report on a phase II clinical trial of HDACi-based chemotherapy for PDAC. Ivaltinostat with the gemcitabine/erlotinib regimen presented the results of ORR 25% and DCR 93.8% (PR 25%, SD 68.8%) among 16 patients who had evaluated tumor response at least once after enrollment (baseline) and the estimated median OS and PFS of these patients were 10.4 and 5.7 months. As the primary endpoint, the ORR of 25% was much higher than the ORR of 8.6% reported in a previous phase III study with a gemcitabine and erlotinib regimen.[4] The secondary end points of DCR, OS and PFS were also superior to those of the previous study (DCR 57.5%, OS 6.24 months and PFS 3.75 months).[4] Due to small number of patients in our study, it is difficult for use to compare ivaltinostat with the current standard therapies; however, ivaltinostat does appear to show promise in terms of efficacy as a new treatment option for PDAC.

The toxicity profile of ivaltinostat with the gemcitabine/erlotinib combination appeared to be acceptable. In the phase I study, the MTD of ivaltinostat was explored under a combination regimen with regular doses and a schedule of administration of gemcitabine (1000 mg/m^2 IV, days 1, 8 and 15) and erlotinib (100 mg/day orally). A DLT, febrile neutropenia grade 3, was reported in the high-dose cohort at 312.5 mg/m^2, and subsequently, ivaltinostat at 250 mg/m^2 was determined to be MTD. During the phase II study period, the most frequently reported grade 3 or 4 AEs were hematologic disorders, such as neutropenia (33.3%), thrombocytopenia (29.2%) and anemia (16.7%). Most AEs were well controlled by supportive care and did not require treatment interruption. Overall toxicity was considered acceptable, with levels similar to those of the gemcitabine/erlotinib regimen[4] or current first-line therapies for PDAC.[2,3]

Overexpression of the HDAC family genes has been reported in PDAC and the HDAC family was suggested to be an important regulator of PDAC.[28,29] Preclinical studies revealed that inhibition of HDAC could suppress tumor growth in pancreatic cancer cell lines.[29,30] Nevertheless, HDACis have shown limited success in clinical trials for solid tumors, including PDAC.[31] A phase II study of tacedinaline, and an oral HDACi, combined with gemcitabine reported no advantage over gemcitabine alone in patients with advanced PDAC.[32] Another phase II study of panovinostat with bortezomib in patients with PDAC after gemcitabine-based therapy was reportedly suspended due to lack of response and toxicity after only seven patients were enrolled.[33] In the phase I/II study of mocetinostat for advanced PDAC, mocetinostat with gemcitabine combination was associated with significant toxicities and showed only limited clinical activity.[34] A possible cause of the ineffectiveness of HDACis in solid tumors is considered to be their short half-life and low C_{max}, which interfered with maintaining suitable treatment concentrations after the drug is delivered to the target organ. However, it is theorized that ivaltinostat compensates for this weaknesses in pharmacokinetic properties due to the extended accumulation of acetylated histone in tumor tissues observed in previous animal and human studies.[22,35] Moreover, our previous study revealed that combined therapy of ivaltinostat with gemcitabine and erlotinib had synergistic effects in pancreatic cancer cell lines and gemcitabine-resistant cell lines.[24] Based on these findings, we conducted this phase I/II clinical trial of ivaltinostat with gemcitabine/erlotinib combination for patients with PDAC and the results obtained, suggested a promising response rate and possible survival gain.

Correlative studies were conducted to find possible markers for predicting responsiveness to the ivaltinostat with gemcitabine/erlotinib combination therapy, and as a result, several candidates including cfDNA and protein biomarkers were elucidated. cfDNA is considered a potential valuable tool in diagnosis, monitoring therapy response and postoperative recurrence in PDAC.[36-38] In our study, cfDNA analysis was based on targeted NGS strategy. Representative PDAC driver mutation, KRAS^{mut}, was detected in 75% of patients and was comparable to previously reported KRAS^{mut} detection rates (40%-80%).[37,39,40] The cfDNA analysis found that the anticancer effect was high in a group with less initial mutation burden and poor responders presented an increasing tendency of mutation burden over time. These results suggested that the mutation burden detected by cfDNA is an indicator of the tumor response to ivaltinostat therapy and can be used to monitor the early response of a patient to treatment. The clinical significance of these findings is limited as there was no control arm in the study and it is unclear whether these results were from ivaltinostat treatment or from other chemotherapy agents.

Correlative studies have suggested serological protein markers for the prediction of the response to the ivaltinostat treatment. The pretreatment blood level of TIMP1, pro-MMP10 and CD 31 were negatively correlated with PFS of the patients. We also found a difference before/after treatment in the blood levels of pro-MMP2 and free IGFBP1 between good and poor responders. These markers are known to be cell markers related to tumor microenvironments, including fibrosis and vascular functions. The activity of matrix metalloproteinases (MMPs) is critical for cancer cells to invade through extracellular matrices. PDAC is characterized by a strong tumor microenvironment and contains many proteases consisting of MMPs.[41] Experimental studies frequently use MMPs, including

MMP2 and MMP10, as indicators for the diagnosis and progression of PDAC.[42,43] TIMP1 is known as a natural inhibitor of MMP and plays a role in the diagnosis and progression of PDAC.[43,44] CD31 is a well-known pan-endothelial cell marker and has been reported as a venous invasion marker for pancreatobiliary cancer.[45] Tissue expression of CD31 is also known to be a poor prognostic factor associated with desmoplastic stroma in PDAC.[46] One report indicated that circulating levels of CD31 decreased during treatment of metastatic breast cancer; however, the relationship between circulating CD31 and PDAC is not well known. IGFBP-1 is a component of the insulin growth factor axis and is important in cancer cell migration and metabolism.[47] The clinical role of IGFBP-1 in PDAC development remains uncertain,[48] although several studies have reported that elevated blood IGFBP-1 is associated with poor prognosis in PDAC.[49] In a blood biomarker study of results from the CALGB80303 trial, a phase III study comparing gemcitabine/bevacizumab vs gemcitabine/placebo, researchers reported that plasma IGFBP-1 elevations were related with poor survival in PDAC regardless of treatment group.[49] The relationship between these markers and HDAC has been reported in several cancers,[50-52] but the relationship in pancreatic cancer needs to be investigated. Antifibrotic and antiangiogenetic effects are one of the presumed action mechanisms of HDACis, including ivaltinostat,[53-56] thus further evaluation is needed in PDAC. Since our study was a single-arm study, there are limitations in determining the clinical significance between markers and ivaltinostat therapy. These markers may also be prognostic markers of PDAC regardless of treatment. A large-scale phase III clinical trial is currently in preparation, and we expect to provide more convincing results in a correlative study.

There are limitations to this phase I/II study. First, this was a nonrandomized single-arm study and patient selection could have influenced outcomes. Further, the response rate and survival outcomes could not be directly compared to conventional therapy. The relatively high dropout rate (8 of 24 cases) was also a weak point of our study. Thus, the results may not be generalizable. Secondly, another gemcitabine-based regimen, a combination with nab-paclitaxel, has proven its efficacy and has become a standard therapy. However, this trial was not performed with gemcitabine/nab-paclitaxel combination because of our positive result of previous in vitro experiments of gemcitabine/erlotinib combination and regulatory difficulties in nab-paclitaxel use at the time of initiation of the current clinical trial. Although our study was designed based on a preclinical study that reported a synergistic effect of ivaltinostat with the gemcitabine plus erlotinib regimen,[24] more studies are needed to confirm whether combinations with current standard therapies can lead to better results. To overcome this limitation, we plan to evaluate the effect of ivaltinostat in combination with current standard chemotherapy, FOLFORINOX or a gemcitabine/nab-paclitaxel regimen in a large-scale randomized controlled phase III clinical trial. Furthermore, considering the antifibrotic effect of HDACis, combination with immune checkpoint inhibitors might produce new opportunities for treatment for PDAC featuring an immunosuppressive tumor microenvironment.

In conclusion, results from the present phase I/II clinical trial indicate that combination therapy of ivaltinostat, gemcitabine and erlotinib can be a potential treatment option with an acceptable safety profile for patients with locally advanced or metastatic PDAC.

AUTHOR CONTRIBUTIONS

Conceptualization: Si Young Song. *Methodology*: Jung Hyun Jo, Dawoon E. Jung, Sangsook Cho and Si Young Song. *Software*: Jung Hyun Jo, Dawoon E. Jung and Sangsook Cho. *Validation*: Hee Seung Lee, Soo Been Park, Moon Jae Chung, Jeong Youp Park, Seungmin Bang, Seung Woo Park and Si Young Song. *Formal analysis*: Jung Hyun Jo, Dawoon E. Jung and Sangsook Cho. *Investigation*: Jung Hyun Jo, Dawoon E. Jung, Sangsook Cho and Si Young Song. *Resources*: Si Young Song. *Data curation*: Jung Hyun Jo, Dawoon E. Jung and Sangsook Cho. *Writing—original draft preparation*: Jung Hyun Jo, Dawoon E. Jung and Sangsook Cho. *Writing—review and editing*: Hee Seung Lee, Soo Been Park, Moon Jae Chung, Jeong Youp Park, Seungmin Bang, Seung Woo Park and Si Young Song. *Visualization*: Jung Hyun Jo, Dawoon E. Jung and Sangsook Cho. *Supervision*: Si Young Song. *Project administration*: Si Young Song. *Funding acquisition*: Si Young Song. All authors have read and agreed to the published version of the article. The work reported in the article has been performed by the authors, unless clearly specified in the text.

CONFLICT OF INTEREST

Sangsook Cho is an employee of CG Pharmaceuticals, Inc. The other authors do not have a conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The raw protein microarray data generated in our study is available in the GEO database under accession number GSE189724. Other data that support the findings of our study are available from the corresponding author upon request.

ETHICS STATEMENT

This phase I/II study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. Informed consent was obtained from all subjects. (ClinicalTrials.gov Identifier: NCT02737228.)

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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