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PHASE I STUDIES

Phase Ib trial combining capecitabine, erlotinib and bevacizumab in pancreatic adenocarcinoma - REBECA trial

Christian Dittrich[1,2] · Robert Königsberg[1,2] · Martina Mittlböck[3] · Klaus Geissler[4] · Azra Sahmanovic-Hrgovcic[5] · Johannes Pleiner-Duxneuner[6] · Martin Czejka[5] · Philipp Buchner[5]

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Summary

Background Purpose of this phase Ib trial was to establish the maximum tolerable dose (MTD) of capecitabine and to escalate the dosages of erlotinib and bevacizumab to determine the recommended phase II dose (RP2D) in patients with advanced/metastatic pancreatic adenocarcinoma not pretreated for metastatic disease. *Methods* Starting doses were capecitabine 500 mg/m^2 bid orally continuously, erlotinib 100 mg orally daily, and bevacizumab 5 mg/kg intravenously q 2 weeks. Dose escalation was performed according to a 3 + 3 design for capecitabine until MTD, for erlotinib and bevacizumab until the maximum doses registered by applying a substance-related, toxicity-based scheme accompanied by pharmacokinetic analysis. Circulating tumor cells (CTCs) were determined pretherapeutically by immunohistochemical identification after enrichment with immunomagnetic separation. *Results* Thirty patients were evaluable at six dose levels. 900 mg/m^2 bid were determined as MTD for capecitabine based on dose-limiting toxicities: cutaneous in two patients and vascular in another. The most severe (Grade (G)3/4) drug-related treatment-emergent adverse events (toxicities) belonged to the categories gastrointestinal, vascular, cutaneous, cardiovascular, metabolic/nutritional or hematological. G3 toxicities occurred in 14 (47%), G3 + G4 in a single (3%) patient. 2 out of 28 patients (7%) exerted partial response, 17 (61%) stable disease. Pharmacokinetic evaluation revealed lack of drug-drug interaction between capecitabine and erlotinib and their metabolites. Presence of CTCs was associated with shorter progression-free survival ($p = 0.009$). *Conclusions* The study met the primary objective. RP2D was capecitabine 800 mg/m^2 bid continuously, erlotinib 150 mg daily, and bevacizumab 10 mg/kg q 2 weeks. The regimen could be applied safely, but demonstrated limited efficacy.

Keywords Locally advanced / metastatic pancreatic adenocarcinoma · Phase Ib · Capecitabine · Erlotinib · Bevacizumab · Pharmacokinetics

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✉ Christian Dittrich
ch.dittrich@chello.at

[1] Applied Cancer Research — Institution for Translational Research Vienna (ACR-ITR VIEnna), Bernardgasse 24/2, 1070 Vienna, Austria

[2] Ludwig Boltzmann-Institute for Applied Cancer Research, Centre for Oncology and Haematology, Kaiser Franz Josef-Spital, Kundratstrasse 3, 1100 Vienna, Austria

[3] Section for Clinical Biometrics, Centre for Medical Statistics, Informatics, and Intelligent Systems (CeMSIIS), Medical University of Vienna, Spitalgasse 23, 1090 Vienna, Austria

[4] 5th Medical Department with Oncology and Palliative Medicine, Hietzing Hospital, Wolkersbergenstrasse 1, 1130 Vienna, Austria

[5] Division of Clinical Pharmacy, Department of Pharmaceutical Chemistry, Centre of Pharmacy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

[6] Medical and Regulatory Affairs, Roche Austria, Engelhorngasse 3, 1211 Vienna, Austria

Introduction

Improvement of systemic therapy of pancreatic adenocarcinoma (PAC) continues to be an unmet medical need. An almost complete lack of a personalized therapeutic approach in PAC becomes better understandable due to the fact that there are no drugs available that target driver mutations in a higher percentage of PACs [1]. Until recently, single-agent gemcitabine (GEM) has been considered the cornerstone of chemotherapy for patients with advanced PAC [2]. Compared with GEM, FOLFIRINOX resulted in significantly improved median overall survival (OS), median progression-free survival (PFS), rates of objective response (ORR), and time to definitive deterioration in quality of life [3]. Due to significant toxicity, this regimen remains restricted to patients with excellent performance status (PS). In the pivotal phase III MPACT trial, the combination of the stroma-targeted nab-paclitaxel and GEM demonstrated a significant survival benefit along with good tolerability in PAC, now representing a standard for the patient category with average PS [4].

At the time of conception of this phase Ib trial, combining GEM with a second cytotoxic drug in this palliative setting was and is again questioned due to the unsatisfactory effectiveness and increased toxicity [5, 6]. 5-fluorouracil (5-FU) continuous infusion was found to be more effective than bolus 5-FU, a reason why to restrain from using GEM, but to test the orally administered prodrug of 5-FU, capecitabine (C), substituting 5-FU continuous infusion [7]. The rationale for testing a continuous application of C was based on a phase I study by Budman et al. who determined 1331 mg/m^2/day continuously divided into two portions as recommended dose for phase II (RP2D) [8]. A prospective randomized phase II trial, which aimed at determining the anti-tumor activity of three different arms, one with continuous C, one with intermittent C and one with C combined with leucovorin, met the chosen statistical requirements [9]. In our trial, the continuous application was selected in order to establish a periodically repetitive drug administration for C and erlotinib (E) as long as a potential pharmacokinetic drug interaction was not excluded. The starting dose with 1000 mg/m^2/day was chosen to be below the dose recommended in the phase I by Budman et al. and tested in the phase II by Van Cutsem et al. [8, 9].

The relatively high incidence and immunostaining intensity of epidermal growth factor (EGF) and its receptor (EGFR) expression in PAC was the rationale to investigate E in this disease setting [10]. In a phase III trial, E yielded a statistically significant, albeit clinically questionable survival benefit in patients with advanced disease in combination with GEM compared with GEM alone [11].

The interest in exploring the angiogenesis inhibitor bevacizumab (B) was based on preliminary preclinical data and the clinical exploited involvement of vascular endothelial growth factor A (VEGF-A) in PAC [12, 13]. There was consistent biologic rationale for combining anti-EGFR and anti-angiogenesis therapies in cancer treatment [14]. In preclinical non-pancreatic tumor models, the additive or synergistic effect of anti-EGFR and anti-VEGF therapies when used in combination was well documented [15, 16].

At the time of the initiation of this trial, there existed no clinical data on the combination for C with E and B. As only little or no overlap between the toxicity profiles of the agents had been observed, the combination was expected to have an acceptable safety and tolerability profile and to provide greater benefit than either agent alone. Evidence had emerged that the detection of circulating tumor cells (CTCs) provided important prognostic information, especially in breast, colon and prostate cancer [17]. Only scarce and contradictory data regarding pancreatic cancer were available and provided very preliminary evidence that CTCs were or were not associated with the prognosis [18, 19].

The primary objective of the REBECA trial was to develop stepwise a safe and tolerable regimen as well as to identify the RP2D for its further development as a new first-line therapy in locally advanced or metastatic PAC that is not refractory to previous potentially received adjuvant therapy with GEM. The secondary objectives were to assess pharmacokinetics (PK) of C and E, any preliminary evidence of anti-tumor activity, and the prognostic value of the pretherapeutic assessment of CTCs.

Patients and methods

Patient population

The eligibility criteria included patients age 18 or older with histologically or cytologically confirmed locally advanced, unresectable and/or metastatic PAC according to the 6th Edition of the American Joint Commission on Cancer (AJCC) Staging System for Pancreatic Adenocarcinoma [20]. Patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 2, adequate bone marrow, liver and kidney function as well as an activated partial thromboplastin time $\leq 1.5 \times$ ULN within 7 days prior to enrolment. The urine dipstick for proteinuria had to be <2+. Main exclusion criteria were resectable pancreatic cancer, previous therapy for metastatic or locally advanced disease (first-line treatment) and/or pre-exposition to one of the three drugs to be tested. Prior adjuvant radiochemotherapy was allowed.

The trial (NCT 00925769; EudraCT 2008-004444-36) was conducted in accordance with the ethical standards of the Ethics Committee of the City of Vienna (EK 08-159-0908) and with the Helsinki Declaration. All patients provided written informed consent before trial entry.

Study design

This phase Ib, open-label, dose-escalation study of the combination of C, E, and B was undertaken at two centers in Austria between December 2008 and May 2014. The primary endpoint was to determine the maximum tolerable dose (MTD) of this triple drug regimen based on dose-limiting toxicities (DLTs) in ≥33% of patients whereby the DLTs had to belong to the same quality of toxicity categorized into gastrointestinal, vascular, cutaneous, eyed, cardiovascular, respiratory, constitutional, metabolic/nutritional, hematological, and infectious. The toxicity quality categories used in our trial were defined based on the toxicities to be expected from the single agent use of the three drugs tested. Secondary endpoints consisted in the determination of the complete PK parameters of C and E and their metabolites in all study patients, of the ORR, of the disease control rate (DCR = ORR + stable disease (SD)), % freedom from progression at 6 months, PFS, and the enumeration of CTCs.

The dose escalation was performed according to a 3 + 3 design using a drug-related toxicity-based dose escalation scheme (Table 1). C was not administered in a 2-week on followed by 1-week off mode as registered, but continuously daily. For C, a dose of 500 mg/m^2 bid orally continuously considered to be minimal potentially effective was used as starting dose whereas for E 100 mg orally daily and for B 5 mg/kg intravenously q 2 weeks, the lower doses of E and B registered in any disease entity were applied as starting doses. Dose escalation started with C, followed by E and was completed with B. Initially, three patients were to be treated at the first dose level (DL1) for 3 cycles. Two weeks of treatment were defined as one cycle, three cycles (6 weeks of treatment) as the evaluation period. If no DLTs were recorded during the first evaluation period, treatment was continued and three further patients were treated at the subsequent higher DL (DL2). If one patient (out of three) developed a DLT, the number of patients treated at that DL was expanded to a maximum of 6. Dose was escalated at maximum until ≥33% of the patients of the expanded cohort had experienced DLTs of the same quality category during the evaluation period, namely until the MTD of C was reached. The immediate next lower DL was therewith the RP2D of C. As a next step, the dose of E was increased to 150 mg and three patients were entered at this DL. Even if no DLT or MTD was reached for E, the recommended dose of E was defined by the investigators and the sponsor not to go beyond the highest dosage the drug was registered for. Similarly, at the following DL (DL6), the dose of B was increased to 10 mg/kg to determine the tolerability for B, but not higher than the registered dose. Patients excluded from the study for reasons other than toxicity in the DLT evaluation period had to be replaced by new patients.

Safety and efficacy assessment

On-study evaluation included weekly physical examination, assessment of vital signs and toxicity as well as measurements of hematological, renal and hepatic function as well as blood coagulation.

Treatment-emergent adverse events (TEAEs) and drug-related TEAEs (toxicities) were graded according to the CTCAE version 3.0. DLT was defined as any grade (G)3 or G4 toxicity as well as stopping of C and/or E intake ≥7 days and/or cancellation of one or more B infusions due to AEs. During the drug evaluation phase, no dose modification was foreseen. If a DLT occurred, the treatment could be delayed for a maximum of 2 weeks and was continued, provided that

Table 1 Dose escalation – dose limiting toxicities (DLTs)

Dose level	C mg/m^2 bid po cont	E mg total daily po	B mg/kg q 2 weeks iv	Patient #	Patients evaluable (N = 30)	Patients with DLT (N = 6)	Grade 3/4 Toxicities[a] (N = 7)	DLTs (Qualities)
1	500	100	5	1–7	6	1	2	Diarrhea, myocardial infarction (gastrointestinal, cardiovascular)
2	650	100	5	8–13	6	1	1	Erythema, pustules (cutaneous)
3	800	100	5	14–21	6	1	1	Diarrhea (gastrointestinal)
4	900	100	5	22–29	6	3	3	eczema herpeticum (exanthema), hand-foot syndrome, acute rectal bleeding (cutaneous, vascular)
5	800[b]	150[b]	5	30–32	3			
6	800	150	10[b]	33–35	3			

C capecitabine, *po* orally, *cont* continuously, *E* erlotinib, *B* bevacizumab, *iv* intravenously, # consecutive patient identification number, *N* number of patients

[a] Graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 3.0

[b] Recommended doses for phase II (RP2D)

toxicities returned to baseline or were ≤ G1. All patients who received the study drugs in the entire evaluation period or who stopped the study for treatment-related AEs and were therefore not to be replaced, were to be included into the safety evaluation.

Tumor response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) every 6 weeks [21]. In brief, complete response (CR) was defined as the disappearance of all tumor lesions, partial response (PR) as 30% decrease of the sums of the longest diameters of the target lesions, both confirmed at ≥4 weeks. SD was diagnosed if neither PR nor progressive disease (PD) criteria were met. Disease control was defined as CR plus PR plus SD. PD was a 20% increase in the sums of the longest diameters of the target lesions without prior CR, PR, or SD.

Patients continued to receive therapy until occurrence of unacceptable toxicity and/or progression of the disease.

Pharmacokinetic assessments and PK calculation method

As to C, 5′-deoxy-5-fluorocytidine (DFCR) and 5′-deoxy-5-fluorouridine (DFUR), blood samples were drawn pre-dose and 30, 60, 90, 120, 150, 180, 240, 300 and 360 min after ingestion of C on day 1. C and metabolites were isolated from blood matrix compounds by solid phase extraction and quantitated by two separate high-performance liquid chromatography (HPLC) methods [22, 23]. Blood samples were drawn pre-dose and 1, 2, 3, 4, 6, 8 and 24 h after ingestion of E on day 1. E and OSI-420 were isolated from blood matrix compounds by solid phase extraction and quantitated by use of a selective and validated reversed-phase HPLC assay as described by Lepper et al. [24].

The determination of the PK of B was relinquished since previous investigations did not detect a significant PK interaction neither between C and B nor between E and B [25, 26].

For curve fitting and PK analysis, the Phoenix WinNonlin version 6.2.1 software was used (Pharsight Corporation, a Certara™ company, Princeton, New Jersey, USA). For both drugs, non-compartment PK was calculated using the model 302 (linear trapezoid rule; linear up-log down) of the WinNonlin library for oral administration. Statistical evaluation of the obtained PK parameters was performed by use of the scientific software GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, California, USA). To detect potential outliers, a ROUT Test (Q = 1%) was carried out. Moreover, student's t-test or ANOVA analysis, both one-sided with a significance level of 5%, was used.

Determination of CTCs

Before start of therapy, blood samples were collected for assessment of CTCs by a two-step procedure as previously described [27]. In brief, an immuno-magnetic procedure was used for enrichment of CTCs, namely by positive selection of anti-epithelial cell adhesion molecule (EpCAM)-expressing cells. For direct immuno-magnetic labelling of EpCAM-positive cells, MACS HEA 125-microbeads® were added to the cell suspension enriched with nucleated cells. Then, cells were subjected to magnetic separation using MACS® separation columns (Miltenyi Biotec Inc., Bergisch Gladbach, Germany). In a second step, the identification of CTCs was based on the reactivity of the murine monoclonal antibody A45-B/B3 with the CTCs' cytoskeleton. The immunochemical staining of CTCs was performed according to the EPIMET® (Micromet, Munich, Germany) cell detection kit manufacturer's instructions with the alkaline phosphatase-conjugated A45-B/B3 Fab antibody fragment. Cells were classified as CTCs when staining was positive for cytokeratin and morphological criteria were fulfilled according to Borgen et al. [28].

Statistical methods

The results of the statistical analysis presented were based on the data documented in the clinical study database at the time-point of database-lock (April 2014). Presentation of study data is primarily descriptive. Data were summarized in total and by DL group using absolute and relative frequencies for categorical variables and descriptive statistics of mean, standard deviation (SD), minimum, median and maximum for continuous variables. Time to event data were graphically displayed by Kaplan-Meier curves, and group differences were tested by log-rank test. Group differences were also quantified by hazard ratios (HR) and corresponding 95% confidence intervals (95% CI) by the Cox regression model. PFS was defined as the number of days between the first administration of study treatment and the first on-study assessment of PD or the last date of on-study tumor assessment at which the patient was considered to be progression-free. All tests were based on a two-sided significance level of 5%. Statistical calculations were performed with the software SAS© (SAS Institute Inc., Cary, North Carolina, USA).

Results

Patient demographics and disease characteristics

Thirty-five patients were screened. Three out of them were screening failures (due to pulmonary embolism ($N = 1$); impossibility to swallow tablets due to permanent nausea and emesis ($N = 1$); clinical PD before start of therapy ($N = 1$)). Additional two patients were not evaluable due to early PD resulting in 30 patients that constituted the per-protocol population.

Patient demographics and characteristics are detailed in Table 2. Overall, 30 patients received 264 cycles of therapy; in median 6 cycles per person (minimum 2, maximum 32 cycles).

Dose escalation, dose-limiting toxicities (DLTs), maximum tolerable doses (MTDs), and recommended dose for phase II (RP2D)

Details of the dose escalation are presented in Table 1. Due to the fact that the 2 DLTs, diarrhea G3 and myocardial infarction G4, occurred in a single patient out of 3 patients the criterion of MTD was not fulfilled. Three more evaluable patients were entered at DL1. Dose escalation of C was continued to 650 mg/m^2 bid continuously (DL2) and 800 mg/m^2 bid continuously (DL3) with a single patient experiencing a DLT at each of these DLs. Three out of the 6 evaluable patients of DL4 developed G3 toxicity representing DLTs; acute rectal bleeding in 1 patient, eczema herpeticum and a hand-foot-syndrome, each in a separate further patient. Since the latter two toxicities belonged to the same toxicity category, i.e. cutaneous toxicity, MTD was therewith reached. Therefore, 800 mg/m^2, the DL closest to, but below the MTD was determined as the dose recommended for consecutive dose escalation of E and B, respectively. The maximum planned doses of E (150 mg daily) and B (10 mg/kg) did not result in DLTs and proved to be safe.

Safety and efficacy

TEAEs were reported in all 30 (100%) patients, toxicities in 29 (96.7%) patients (Supplementary Table S1). The most common toxicities irrespective of their grade were rash/acne/exanthema/erythema in 22 (73%), fatigue in 16 (53%), hand-foot-syndrome and nausea, each in 14 (47%), vomiting in 13 (43%), diarrhea in 12 (40%), hypertension in 9 (30%), lack of appetite in 8 (27%) patients, dry skin, dysgeusia or hoarseness in 7 (23%) each, stomatitis and anemia in 6 (20%) each, and conjunctivitis, dry nasal mucosa, alopecia or paronychia in 5 (17%) patients each. In Table 3, the onset of the maximum (G3/4) toxicities and their allocation to the individual patients are detailed according to the respective treatment cycle. G3 toxicity occurred in 14 (47%) patients, G3 + G4 in a single (3%) patient. The only G4 toxicity was a myocardial infarction that occurred in a patient experiencing already G3 diarrhea. Only one (3%) patient had no toxicity, whereas the maximum toxicity per patient was G1 in 2 (7%), G2 in 12 (40%), G3 in 14 (47%) and G4 in 1 (3%).

Since two (7%) patients went off the study due to early toxicity before having reached the time interval foreseen for restaging, only 28 out of 30 patients evaluable for safety could be also assessed for response according to RECIST. Two (7%) patients reached an objective response in form of PR lasting 267 and 252 days, respectively. 17 (61%) patients fulfilled the criteria of SD resulting in disease control in 19 (68%) patients and in freedom from progression at 6 months in 8 out of 28 (29%) patients. PD was observed in 21 out of 28 patients. Based on Kaplan-Meier, PFS was in median 77 days (95% CI: 42–274 days; range 35–455 days).

Table 2 Demographics and patient characteristics ($N = 30$)

Age, years (mean ± S.D.)	63.9 ± 8.4
Gender	
Male	15 (50.0%)
Female	15 (50.0%)
ECOG performance status	
0	23 (76.7%)
1	7 (23.3%)
Disease	
Locally advanced	1 (3.3%)
Metastatic	29 (96.7%)
Measurable	29 (96.7%)
Non-measurable	1 (3.3%)
Prior adjuvant treatment	
Yes	1 (3.3%)
No	29 (96.7%)

N number of evaluable patients, *S.D.* standard deviation, *ECOG* Eastern Cooperative Oncology Group

Pharmacokinetic assessments

Since C is an orally administered drug, the PK parameter AUC_{last} (area under concentration-time curve from time = 0 h to the last collected blood sample) was chosen for depiction. It best represents the bioavailability of the drug in the central compartment. In Fig. 1a, the data of C and its metabolites 5′-DFCR and 5′-DFUR are shown as geometric mean and 95% CI and therefore resulted in a higher variability compared to mean values including SD. The AUC of C continuously increased with higher doses, whereas 5′-DFCR and 5′-DFUR AUCs were independent from the C dose and exceeded the concentration of the parent compound, giving evidence for the desired sufficiently high plasma concentrations of the 5-FU precursor 5′-DFUR. Figure 1b shows the AUC_{last} values of E and its metabolite OSI-420 as geometric mean and 95% CI. Both AUCs are independent from the DL except for DL 6; at this DL, 150 mg E was applied instead of 100 mg. In general, the AUC of OSI-420 is about a tenth of the AUC of E. Extensive PK data of C and E, including the data of their metabolites, are summarized in Supplementary Tables S2 and S3.

Six patients each were evaluable for DL1-4, and 3 patients each for DL5 and 6.

Table 3 Onset of Grade 3 or 4 drug-related treatment-emergent adverse events (TEAEs) (toxicities) allocated to the individual patient according to the respective treatment cycle

Toxicities	Patient identification number														
	2	7	9	11	13	14	15	18	20	23	24	27	28	29	30
Gastrointestinal															
Diarrhea	2[a]	.	.	.	.	2[a]	.	.	.	23	.	.	.	.	.
Dysphagia	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.
Stomatitis	.	.	.	.	.	.	5	.	.	.	.	.	.	.	.
Vascular															
Acute rectal bleeding	.	.	.	.	.	.	.	.	.	.	.	2[a]	.	.	.
Hemorrhage	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8
Thrombosis	.	.	.	.	.	.	.	1[b]	.	.	.	.	.	.	.
Cutaneous															
Blistering	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4
Cheilitis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6
Hand-foot syndrome	.	.	.	12	.	.	.	.	4	.	7	.	3[a]	.	8
Paronychia	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.
Rash / acne / exanthema / erythema	.	.	1[a]	.	.	.	4	.	.	3[a]	.	.	.	5	.
Cardiovascular															
Myocardial infarction	2[a]	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Metabolic / nutritional															
Hyperbilirubinemia	.	10	16	.	4	.	.	.	.	.	.	.	.	.	.
Hypokalemia	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.
Hematological															
Anemia	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.

Entries are numbers of respective treatment cycles

[a] Toxicities representing dose-limiting toxicities (DLTs)

[b] Thrombosis of central venous access not defined as DLT

Inter-individual variability was high for all parameters in the investigated compounds and their metabolites (Supplementary Tables S2 and S3). Dose escalation of C from DL1-4 yielded a likewise increase of its AUC_{last} and peak plasma concentration (C_{max}), indicating dose linearity (Supplementary Table S2). After ingestion of C, C_{max} occurred rapidly within 30 to 90 min. Apart from DL3, $C_{AUClast}$ and C_{cmax} increased incrementally within each DL (C → 5′-DFCR→5′-DFUR) reaching their peak levels in 5′-DFUR, the direct precursor of 5-FU. Formation of almost equal amounts of 5′-DFUR was observed in DL1-4, regularly exceeding the plasma concentrations of C. DL5 and 6 are to be considered separately since the dose of E (DL5) and additionally of B (DL6) changed, respectively. Compared to DL3 – all three DLs using a dosage of C of 800 mg/m^2 bid – there was a substantial elevation of C_{cmax} and $C_{AUClast}$ in DL5 and 6 that did not result in a rise of 5′-DFUR as expected.

Apart from OSI-420 in DL4, almost equal C_{max} and AUC_{last} amounts of E and its metabolite OSI-420 (roughly 10% of E) were observed after administration of 100 mg of E (DL1-4) ($p = 0.2840$) (Supplementary Table S3). Apart from DL5, C_{max} was reached after 2–3 h. According to expectations, $E_{AUClast}$ and E_{cmax} increased in DL6, but surprisingly not in DL5 in which the $E_{AUClast}$ equaled that in DL1-4. E_{cmax}, however, was even lower than in DL1-4.

CTC analysis

CTCs could be assessed in 22 (73%) out of the 28 patients evaluable for response. In 10 (36%) patients, at least one CTC was detected and a statistically highly significant shorter PFS (median 42 days, 95% CI: 35–148 days, range 35–148 days) was observed compared to those 12 patients without CTCs (median 274 days, 95% CI: 43–414 days, range 42–455 days; HR = 4.3 (95% CI 1.6–14.8); $p = 0.009$) (Fig. 2).

Discussion

All objectives of this phase Ib trial were met; for this triple-drug combination, the RP2D was defined to be C 800 mg/m^2 bid orally continuously, E 150 mg daily orally and B 10 mg/kg

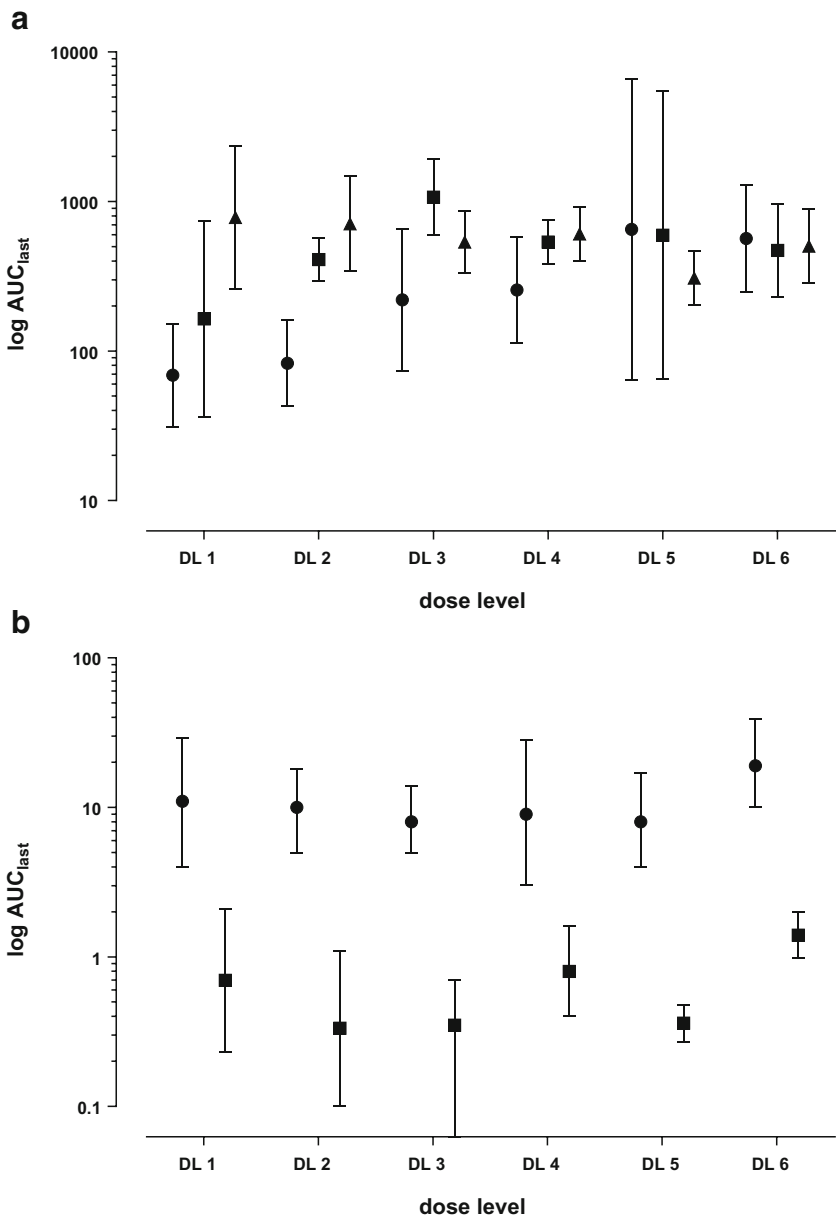

Fig. 1 **a**, **b** Area under the concentration-time curve $(AUC)_{last}$ values are depicted as geometric mean and 95% CI for capecitabine (C) and erlotinib (E) as well as for their metabolites for each dose level. Footnote: 1a: C (circles), 5′-DFCR (squares), 5′-DFUR (triangles); 1b: E (circles), OSI-420 (squares). AUC_{last} concentration-time curve from time = 0 h to the last collected blood sample

iv q 2 weeks, respectively. PK were found to be linear from DL1 to DL6 without demonstrating drug-drug interaction between C and E; and last but not least we were able to confirm the hypothesized worse prognosis of patients with CTCs. Overall, the regimen turned out to be safe, but of limited activity.

Since the superiority of FOLFIRINOX over GEM the development of first-line regimens based on a fluorinated pyrimidine instead of GEM was reconsidered [29]. Our choice of C as cytotoxic backbone of the regimen found indirectly support by Tempero et al. who defended the use of first-line regimen based on a fluorinated pyrimidine without GEM including regimen with selected targeted drugs in this therapeutic situation [29]. The success of the GEST study with S1, an oral fluoropyrimidine derivative, in Asian patients was a further argument in this direction [30]. Very recently, Manji et al. concluded that there is no compelling evidence to envision a chemotherapy-free regimen for PAC in the near future [31].

When we conceived our trial, E was the only targeted drug registered for PAC [11]. A second-line study combining C 1000 mg/m^2 bid given in the way it was registered, namely for 2 weeks orally, followed by 1–week rest, with E 150 mg daily orally was found to be active (10% PR) and safe although about half of the patients (11 out of 23) required dose reduction of E to 100 mg due to toxicity, mainly diarrhea [32]. Overall, these data are in line with ours although not perfectly comparable since we have chosen continuous daily ingestion of C and ended up with 800 mg/m^2 bid in combination with E

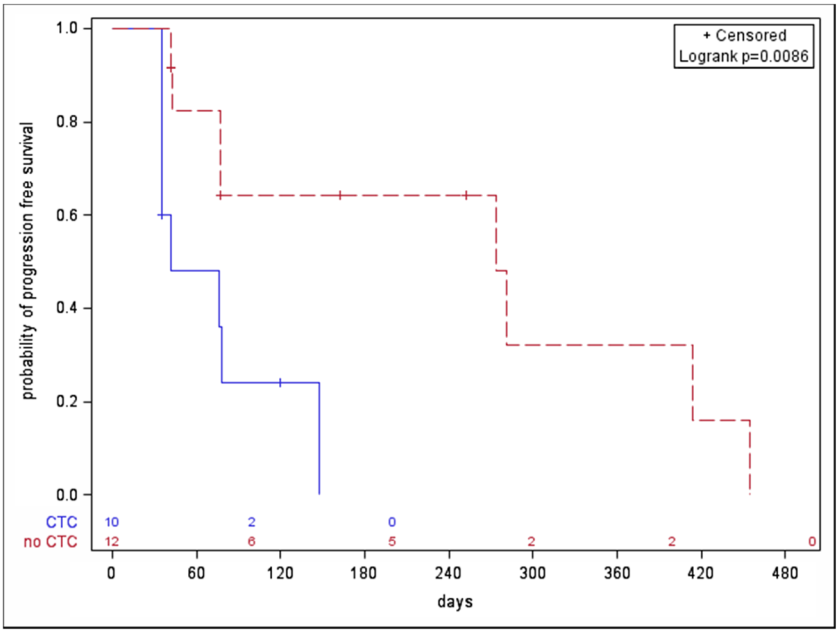

Fig. 2 Kaplan-Meier plot of progression-free survival (PFS) according to absence or presence of circulating tumor cells (CTCs) in patients with pancreatic adenocarcinoma. Footnote: PFS was significantly longer in terms of median 274 days (95% CI, 43–414 days; range 42–455 days) in patients without CTCs ($N = 12$; - - - 0 CTC) in comparison to median 42 days (95% CI, 35–148 days; range 35–148 days) in patients with CTCs ($N = 10$; —≥1 CTC). (HR 4.3; 95% CI 1.6–14.8; log-rank $p = 0.009$)

150 mg daily orally as RP2D. The anti-tumor activity with 7% observed in our study was lower considering that our trial was performed in a first-line setting whereas that of Kulke et al. in GEM refractory patients [32]. Toxicity was quite comparable with diarrhea G3 in 17%, rash/desquamation G3 in 13%, and hand-foot skin reaction G3 in 13% in the trial of Kulke et al. and in 10%, 20%, and 17%, respectively, in our trial (Table 3) [32]. Whether the myocardial infarction occurring in our trial in that patient who had already experienced a G3 diarrhea was the consequence of dehydration and consecutive increased blood viscosity or a C dependent toxicity or the consequence of both is a matter of speculation.

The combination of E and B has been tested in several tumor entities. Ko et al. studied the combination of the two compounds in GEM refractory metastatic PAC and found it safe but relatively ineffective [33]. As consequence of a first-line study with cetuximab and B he dissuaded from a "targeted only" approach with dual EGFR/VEGF inhibition [34]. When the dual anti-targeted approach including E and B was combined with GEM and tested against GEM/E the primary endpoint was not met, only PFS, but not OS was significantly prolonged [35]. Remission maintenance with sunitinib after chemotherapy in patients with at least SD was encouraging to further pursue the anti-angiogenic approach [36]. Starling and co-workers were combining GEM plus C with E and B [37]. With all restrictions necessary when comparing efficacy phase Ib data of separate studies, there is the obvious impression of better outcome with the quadruple regimen by Starling et al. with a PR rate of 50%, DCR of 85%, and a median PFS and OS of 9.0 and 12.5 months, respectively [37]. In contrast, we observed PR only in 7%, a DCR of 68%, and a median PFS of 77 days (2.5 months). To what degree this is due to a higher anti-tumor activity of the quadruple regimen and how much the differences in patient characteristics between the two small cohorts contributed thereto is a matter of speculation. The short intervals between the scheduled response assessments of only every 6 weeks in our trial in comparison to every 8 weeks in that of Starling et al. and in those of several others might be an additional reason for the extremely short median PFS in our trial [11, 35, 37–39].

Interestingly, Chadha et al. performed a phase Ib trial in unresectable pancreatic cancer combining radiotherapy concurrently with C, E and B [39]. Their RP2D were C 825 mg/m^2 bid, E 150 mg daily and B 5 mg/kg q 2 weeks and thus almost identical to ours. On the base of lacking G4 toxicity as well as the occurrence of several pathological responses, the authors considered the regimen to be safe, tolerable, and worth to be further tested. In general, the anti-angiogenic approach seems to be not successful enough in PAC [38, 40, 41]. This may be the consequence of complex stromal interaction with preponderant fibrosis and relative hypovascularity of PAC. Patient selection on the base of a negative prediction such as the SNP rs7993418 in VEGF receptor 1 may justify a reconsideration of anti-angiogenic therapy in PAC [42].

As the metabolic pathways of the two small molecules C and E and the monoclonal antibody B differ from each other, PK interactions seemed unlikely. However, the potential had to be evaluated and excluded for safety purposes.

Since B is not only structurally but also concerning its protein-like PK characteristics completely distinct from the other two compounds, we anticipated no impact of C and E on the PK of B. Our hypothesis was supported by findings from Han et al., who recently stated that the PK of B rather

depends on patient's physiological conditions independent of administration of additional chemotherapeutic agents [43]. An investigation of Herbst et al. demonstrated a lack of PK interaction between B and E, and own investigations on combining C and B could not identify a significant influence of B on the metabolic activation of C [26, 44]. Another two publications also supported the thesis of a lack of PK interactions between C and B [25, 45]. Therefore, we restrained from a further PK investigation of the monoclonal antibody B.

Dose escalation of C in DL1-4 was associated with a likewise proportional increase of C_{cmax} and $C_{AUClast}$, as to be expected when linear PK are prevailing that was also reported by Budman et al. being in line with our findings [8]. Pronk et al. confirmed a substantial extent of inter-subject variability of the PK of C as also observed in our trial [46]. The concomitant administration of C with E did not affect its metabolic activation nor its subsequent biotransformation. Observed PK parameters in our DL4 representing the highest DL reached in our study were of the order of those described by Farkouh et al., although their C_{max} and AUCs were clearly higher due to higher administered doses of C [44]. Moreover, our DL4 data were also in line with those reported by Louie et al. taking into account that their dose of C was 1000 mg/m^2 bid [47]. However, the reason for similar plasma levels of 5′-DFUR across our DL1-4 cohorts despite different doses of C remained unclear.

We found no indications of a drug-drug interaction between C and E concerning the metabolic activation of C that is consistent with the results published by Twelves et al. [48]. Our C_{cmax} and $C_{AUClast}$ were of the magnitude of those respective parameters described by Twelves et al. albeit ours were partly higher in DL3, the dose of C representing RP2D [48]. Our 5′-DFUR C_{max} in DL3 were only slightly above those of Ma et al. and almost equal to those of Van Cutsem et al. [49, 50]. Correlation with Twelves et al. was not possible since these authors presented PK data of C only [48]. The PK data of E, especially exposure in DL1-4, were not altered by dose escalation of C. This gave evidence of C to have no impact on the metabolic pathway of E. In DL5, where E was administered at a dose of 150 mg, E_{cmax} and $E_{AUClast}$ surprisingly were found not to be increased in comparison to those in DL1-4, whereas DL6 yielded significantly higher values. Not only our results, but also those published in the literature remained contradictory. Whereas Twelves et al. and Van Cutsem et al. reported 3-fold higher amounts of E_{cmax} and $E_{AUClast}$ compared to our 100 mg data, our DL1-4 data were in line with Ma et al. [48–50]. Inter-patient variability was high and might have contributed together with the small sample size to our partly inconsistent results. Prediction of the PK of E is considered to be difficult and highly variable [51]. Whereas no interaction between C and E was reported by Twelves et al., a trend for reduced C in the presence of E was described by Van Cutsem et al. [48, 50]. Thus, our data are in line with Twelves et al. not supporting the existence of an interaction between these two compounds [48].

The detection rate of CTCs in 55% patients of our cohort was in line with that of 50% reported by Bidard et al. for four small cohorts [52]. Overall, detection rates of CTCs described in various patient cohorts and reached with different methodologies ranged from 5 to 100% [53, 54]. There are also contradictory results as to the prognostic significance of CTCs reported in different studies [52]. Our data of a significant shorter PFS in patients in whom CTCs could be identified pretherapeutically are in line with results of de Albuquerque et al. and those of the two meta-analyses by Han et al. or Ma et al. [55–57]. Due to a lack of standardization of the detection methodologies and technologies as well as the heterogeneity concerning the patient populations studied CTCs have not reached the status of a surrogate marker for PFS or OS in PAC yet. Since post-treatment CTCs were not measured interpretation of our data is restricted to predict the natural history and/or burden of disease, but does not allow to estimate any benefit from treatment.

Even now, after having learned from many clinical trials performed after the time when our trial had started, many questions raised during that time are still unanswered or have been raised again from a different perspective. A more detailed tumor characterization allowing the identification of more relevant targets with consecutive better patient selection as well as the development of more suitable drugs interfering with the extremely complex tumor microenvironment of PAC are the most pertinent features for a more successful treatment of PAC in the immediate next future.

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Authors' contribution Conception and design: CD, RK, JP-D, MC, PB.

Acquisition of data: CD, RK, MM, KG, AS-H, MC, PB.

Analysis and interpretation of data: MM, CD, PB, RK, MC.

Writing (CD, MM, PB), review and/or revision of the manuscript: all authors.

Approval of the final version: all authors.

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influence the production of the manuscript nor the data analysis nor the interpretation of the data presented.

Compliance with ethical standards

Conflict of interest CD's non-profit research institutes (Ludwig Boltzmann-Institute for Applied Cancer Research (LBI-ACR VIEnna) and Applied Cancer Research – Institution for Translational Research Vienna, both forming the Central European Anticancer Drug Development Platform (CEADDP)) were funded by the Austrian Research Promotion Agency (FFG) – SELP (Strategisches Exzellenz Leitprojekt) Structural Programme, and the LBI-ACR VIEnna additionally by Roche Austria. CD received compensation as a member of a scientific advisory board of Roche Austria. He also consulted for Roche Austria and received compensation.

MM has received compensation for statistical analyses of the study from Roche Austria.

KG has received compensation as a member of scientific advisory boards and for presentations from Roche Austria.

JP-D is Roche Austria employee.

RK, AS-H, MC, and PB declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. The study protocol was approved by the Ethics Committee of the City of Vienna (reference number EK 08-159-0908).

Informed consent Informed consent was obtained from all individual participants included in the study.

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