

Phase I/II Study of Refametinib (BAY 86-9766) in Combination with Gemcitabine in Advanced Pancreatic cancer

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Abstract

Background Activating *KRAS* mutations are reported in up to 90% of pancreatic cancers. Refametinib potently inhibits MEK1/2, part of the MAPK signaling pathway. This phase

I/II study evaluated the safety and efficacy of refametinib plus gemcitabine in patients with advanced pancreatic cancer. **Methods** Phase I comprised dose escalation, followed by phase II expansion. Refametinib and gemcitabine plasma

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levels were analyzed for pharmacokinetics. *KRAS* mutational status was determined from circulating tumor DNA.

Results Ninety patients overall received treatment. The maximum tolerated dose was refametinib 50 mg twice daily plus standard gemcitabine (1000 mg/m² weekly). The combination was well tolerated, with no pharmacokinetic interaction. Treatment-emergent toxicities included thrombocytopenia, fatigue, anemia, and edema. The objective response rate was 23% and the disease control rate was 73%. Overall response rate, disease control rate, progression-free survival, and overall survival were higher in patients without detectable *KRAS* mutations (48% vs. 28%, 81% vs. 69%, 8.8 vs. 5.3 months, and 18.2 vs. 6.6 months, respectively).

Conclusion Refametinib plus gemcitabine was well tolerated, with a promising objective response rate, and had an acceptable safety profile and no pharmacokinetic interaction. There was a trend towards improved outcomes in patients without detectable *KRAS* mutations that deserves future investigation.

Key Points

Ascertaining tumor mutation status using circulating tumor DNA may be a promising diagnostic tool for optimization of treatment

KRAS mutation status may be both prognostic for disease progression and predictive of benefit from the refametinib plus gemcitabine combination for patients with advanced pancreatic cancer

1 Introduction

Pancreatic cancer is among the leading causes of cancer-related mortality worldwide [1], and activating *KRAS* mutations are reported in up to 90% of pancreatic cancers [2, 3]. These mutations cause constitutive activation of the mitogen-activated protein kinase signaling pathway (RAS-RAF-MEK-ERK) which, in turn, persistently activates downstream effectors leading to dysregulated cellular proliferation, survival, and metastasis [4–6]. Therefore, mitogen-activated protein kinase signaling is an important target for therapeutic inhibition [7]. However, only a subset of patients may benefit from single-agent treatment approaches as multiple pathways are commonly dysregulated.

Gemcitabine monotherapy has long been the standard of care for advanced pancreatic cancer and still represents an option (along with the oxaliplatin, irinotecan, fluorouracil, and leucovorin regimen, and gemcitabine plus albumin-bound paclitaxel) for first-line therapy in metastatic or locally advanced, unresectable disease [8–10]. However, the survival improvement with gemcitabine monotherapy is modest [11].

Previous phase II and III trials of gemcitabine combined with other cytotoxic agents have shown acceptable safety but inconsistent survival improvement versus monotherapy [10, 12–16]. The promising activity of cytotoxic combinations has also been associated with high toxicity [9, 10].

Refametinib (BAY 86-9766; Bayer Pharma AG, Berlin, Germany) is an orally available, potent, selective, allosteric (non-adenosine triphosphate competitive) inhibitor of MEK1/2 [17]. Refametinib has demonstrated both single-agent activity [18] and synergistic activity in combination with gemcitabine [19] in preclinical models of pancreatic cancer.

A single-arm, open-label, phase I/II study (NCT01251640) evaluated the safety and efficacy of refametinib plus gemcitabine in patients with advanced pancreatic cancer eligible for first-line gemcitabine. Phase I investigated the safety, tolerability, and pharmacokinetics of the combination; phase II evaluated the efficacy, safety, and biomarker analysis of the recommended phase II dose.

2 Methods

2.1 Ethical Approval and Informed Consent

The study protocol and all protocol amendments were reviewed and approved by independent ethics committees and institutional review boards for each study site before the start of the study and before implementation of the amendments. 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 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

2.2 Study Design

This open-label, non-randomized, multicenter study (ClinicalTrials.gov number NCT01251640 (<http://clinicaltrials.gov/show/NCT01251640>)) comprised two phases: phase I evaluated three dose levels to determine the maximum tolerated dose and recommended phase II dose of refametinib plus gemcitabine; phase II evaluated the efficacy, safety, and biomarker analysis of the recommended phase II dose. The primary outcome measure in phase II was objective response rate (confirmed complete response and confirmed partial response) per blinded independent radiological review. Secondary outcome measures included disease control rate (complete response, partial response, and stable disease), progression-free survival, overall survival, toxicity, and determination of *KRAS* mutational status (wild type or mutant). Additional secondary measures were pre-planned correlation of

KRAS mutational status with response and survival, and analysis of biomarkers relevant to RAS pathway activation or to pathways known to influence activity of RAS-RAF-dependent signal transduction.

Patients received standard gemcitabine intravenously at 1000 mg/m² weekly on day 1; continuous oral treatment with refametinib twice daily began on day 2. Patients received refametinib plus gemcitabine for 7 out of 8 weeks (cycle 1), then 3 out of 4 weeks in subsequent cycles. Enrollment of up to 18 patients in phase I was planned. Dose escalation followed a 3 + 3 design. If no dose-limiting toxicity was seen within the first three patients at the starting dose level (refametinib 30 mg twice daily plus standard gemcitabine) and within the first 4 weeks of treatment, the next highest dose level (refametinib 50 mg twice daily plus standard gemcitabine) was to be initiated immediately. All three patients enrolled at the starting dose level were to continue treatment until they had received a full 8 weeks of therapy. If a dose-limiting toxicity occurred within the first three patients at the starting dose level and after the first 4 weeks of treatment, further recruitment to the higher dose cohort was to be paused and three additional patients were to be enrolled to the starting dose cohort. If no dose-limiting toxicities were observed in these additional three patients within 4 weeks of treatment, then enrollment to the higher dose level continued. If two or more patients out of a maximum of six showed dose-limiting toxicities at the starting dose level within cycle 1, the next lowest dose level would be investigated. The maximum tolerated dose was the highest dose level at which no more than one patient out of six experienced a dose-limiting toxicity. Following identification of the maximum tolerated dose, the data monitoring committee was to be involved in the definition of the recommended phase II dose.

Protocol-defined dose-limiting toxicities, as defined by the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0, included grade 4 anemia; grade 4 neutropenia lasting more than 10 days; grade 3 or 4 neutropenia with fever greater than 38 °C; thrombocytopenia or grade 3 or 4 thrombocytopenia associated with serious bleeding; signs of serious bleeding; grade 3 or higher non-hematological toxicity; grade 3 or higher diarrhea if refractory to maximal anti-diarrheal therapy; grade 3 skin toxicity for more than 2 weeks with maximum supportive treatment; grade 4 skin toxicity (with subsequent removal from the study); missing more than 14 days of consecutive treatment; and increase in aspartate aminotransferase or alanine aminotransferase from grade 1 to grades 2–4 or from grade 2 (in patients with liver metastases) to grade 3 or 4 in the case of a second occurrence after a first recovery to baseline level taking more than 14 days, or a third occurrence.

In phase II, treatment with refametinib at the recommended phase II dose, plus standard gemcitabine 1000 mg/m² weekly, began on cycle 1, day 1. Treatment continued until

progressive disease, unacceptable toxicity, or other discontinuation criteria were met, as follows: initiation of a new anti-cancer regimen; development of a second malignancy; deterioration of Eastern Cooperative Oncology Group performance status to 4 or more; increased aspartate aminotransferase or alanine transaminase from grade 1 to grades 2–4 or from grade 2 (in patients with liver metastases) to grade 3 or 4 in the case of a second occurrence after a first recovery to baseline level taking more than 14 days, or a third occurrence; at the patient's request; if continuation would be harmful to the patient's health (as determined by the investigator); substantial non-compliance with study requirements; development of any intercurrent illness that may affect clinical status assessment or study endpoints; positive serum pregnancy test; use of illicit drugs or other substances that may contribute to toxicity; interruption in study drug administration because of drug-related toxicities for more than 22 days and/or delay of more than 22 days for gemcitabine; or if more dose reductions were required than allowed according to protocol.

2.3 Eligibility

Patients were eligible if aged 18 years or older and with histologically or cytologically confirmed, locally advanced or metastatic pancreatic adenocarcinoma not amenable to curative surgery or radiotherapy. Other eligibility criteria included life expectancy of 12 weeks or more; at least one unidimensional lesion measurable by computed tomography or magnetic resonance imaging (Response Evaluation Criteria in Solid Tumors version 1.1); resolution of all acute toxic effects of any prior local treatment to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 grade 1 or 0; Eastern Cooperative Oncology Group performance status of 2 or under; adequate laboratory criteria (platelet count $\geq 100 \times 10^9/L$; hemoglobin ≥ 10 g/dL; absolute neutrophil count $\geq 1.5 \times 10^9/L$; total bilirubin ≤ 1.5 mg/dL; alanine aminotransferase and aspartate aminotransferase each $\leq 2.5 \times$ upper limit of normal [ULN; or $\leq 5 \times$ ULN in case of liver metastases]; amylase and lipase $\leq 1.5 \times$ ULN; serum creatinine $\leq 1.5 \times$ ULN; prothrombin time or international normalized ratio and partial thromboplastin time $\leq 1.5 \times$ ULN); and normal cardiac function as estimated by echocardiography.

2.4 Assessments

Screening included demographics and baseline characteristics, echocardiography, ophthalmic examination, plasma and tumor biopsy for genotyping and biomarker analysis, and tumor evaluation (blinded) by computed tomography or magnetic resonance imaging (Response Evaluation Criteria in Solid Tumors version 1.1). In phase I, serial blood samples for pharmacokinetic analysis of gemcitabine and refametinib

and the respective inactive metabolites difluorodeoxyuridine and M-17 were collected up to 24 h post-infusion at cycle 1, days 1 and 22, and up to 8 hours post-dose at cycle 1, days 21 and 22. Blinded tumor assessments were performed independently every 8 weeks during treatment until progressive disease or the end of treatment. Confirmatory scans were performed 4 or more weeks after an objective tumor response (complete response or partial response) was documented. Investigator assessment of tumor scans was performed for all patients who received treatment. Safety (changes in laboratory values, vital signs, electrocardiogram, and physical examination) was assessed at screening, at cycle 1, day 1, and weekly thereafter. Adverse events and concomitant medications were assessed continuously from screening onwards. Following the end of treatment, patients entered a 30-day safety follow-up including adverse-event documentation. Survival follow-up was performed monthly up to 8 months after the first treatment of the last patient.

2.5 Pharmacokinetic Assessments

Plasma concentrations of all analytes were measured using fully validated high-pressure liquid chromatography with mass spectrometric detection, and pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin® (Version 4.1; Pharsight Corporation, Princeton, NJ, USA).

2.6 Biomarker Studies

Circulating tumor DNA in plasma was analyzed for *KRAS* mutational status by beads, emulsions, amplification, and magnetics (BEAMing) technology (Covance Inc., Princeton, NJ, USA), with an assay cutoff of 0.02% mutant allele for positivity. Circulating microRNA from plasma was analyzed by quantitative polymerase chain reaction using a human microRNA panel (Exiqon, Woburn, MA, USA). Tumor biopsies were collected where available, as freshly frozen or formalin-fixed and paraffin-embedded. Histological analysis comprised hematoxylin and eosin staining and Ki67 immunolabeling. Targeted archival tumor gene next-generation sequencing was performed using the FoundationOne® panel (Foundation Medicine, Cambridge, MA, USA). Gene expression was analyzed using RNA isolated from tumor biopsy samples using the Ovation® formalin-fixed and paraffin-embedded circulating DNA synthesis kit (NuGen, San Carlos, CA, USA), and RNA sequencing was performed using an Ion Proton™ system (Life Technologies, Grand Island, NY, USA). Reads were mapped to hg19 using TopHat2 [20] with Bowtie2 [21]. Gene-level read counts and reads per kilobase of transcript per million values were calculated with Expressionist® Refiner Genome (Genedata, Lexington, MA, USA).

2.7 Statistical Analysis

Phase I data were analyzed descriptively. The primary efficacy endpoint in phase II tested the null hypothesis that the overall response rate would be 7% or less on the α level of 12.5% using a one-sided binomial test; assuming a true overall response rate of 17% under study treatment, exactly 60 patients treated at the recommended phase II dose were required to be analyzed for efficacy for a power of 90% (primary analysis set). The null hypothesis was to be rejected if seven or more patients in the primary analysis set experienced confirmed complete response or confirmed partial response. Other secondary efficacy endpoints in phase II were analyzed descriptively; corresponding *p*-values are not confirmatory. Descriptive statistics and frequency tables were used for safety analysis for all patients who received at least one dose of study treatment (safety analysis set).

3 Results

3.1 Baseline Demographics and Disease Characteristics

Of the 24 patients enrolled in phase I, 20 were treated and evaluable for safety assessment (Fig. 1a). Ten patients were assigned to dose level 1 (refametinib 30 mg twice daily) and 10 to dose level 2 (refametinib 50 mg twice daily). In phase II, 107 patients were enrolled and screened; 80 were treated and evaluable for safety assessment (Fig. 1b), of whom 10 were originally enrolled at dose level 2 in phase I and are therefore accounted for twice. Overall, 55.6% of patients were male and the median age was 63 years (Table 1). Most patients (85.6%) had metastatic disease.

In phase II, of the 60 patients centrally evaluated for response (primary analysis set; Fig. 1b), 39 (65%) had *KRAS* mutations, as determined from circulating tumor DNA. Frequently observed *KRAS* mutations included G12D, G12V, and G12R; mutations in codon 38 or 436 were not observed. Molecular tumor characterization was performed in 23 out of 30 archival samples (77%) with sufficient tumor content. Tumor exome sequencing revealed *KRAS* mutations (G12D, G12R, G12V, Q61H, Q61R, A59G) in 15 out of 16 patient samples containing sufficient DNA (Fig. 2). Frequent co-occurring somatic mutations or amplifications in patients with *KRAS* mutations included *TP53* (14 out of 15 [93%]), *CDKN2A* (5 out of 15 [33%]), *C-MYC* (4 out of 15 [27%]), and *KAT6A* (2 out of 15 [13%]). One patient with stable disease and low Ki67 H-score had two co-existing *KRAS* mutations (A59G, Q61R). Discordance was observed in *KRAS* mutational status, as determined by BEAMing technology, in three samples.

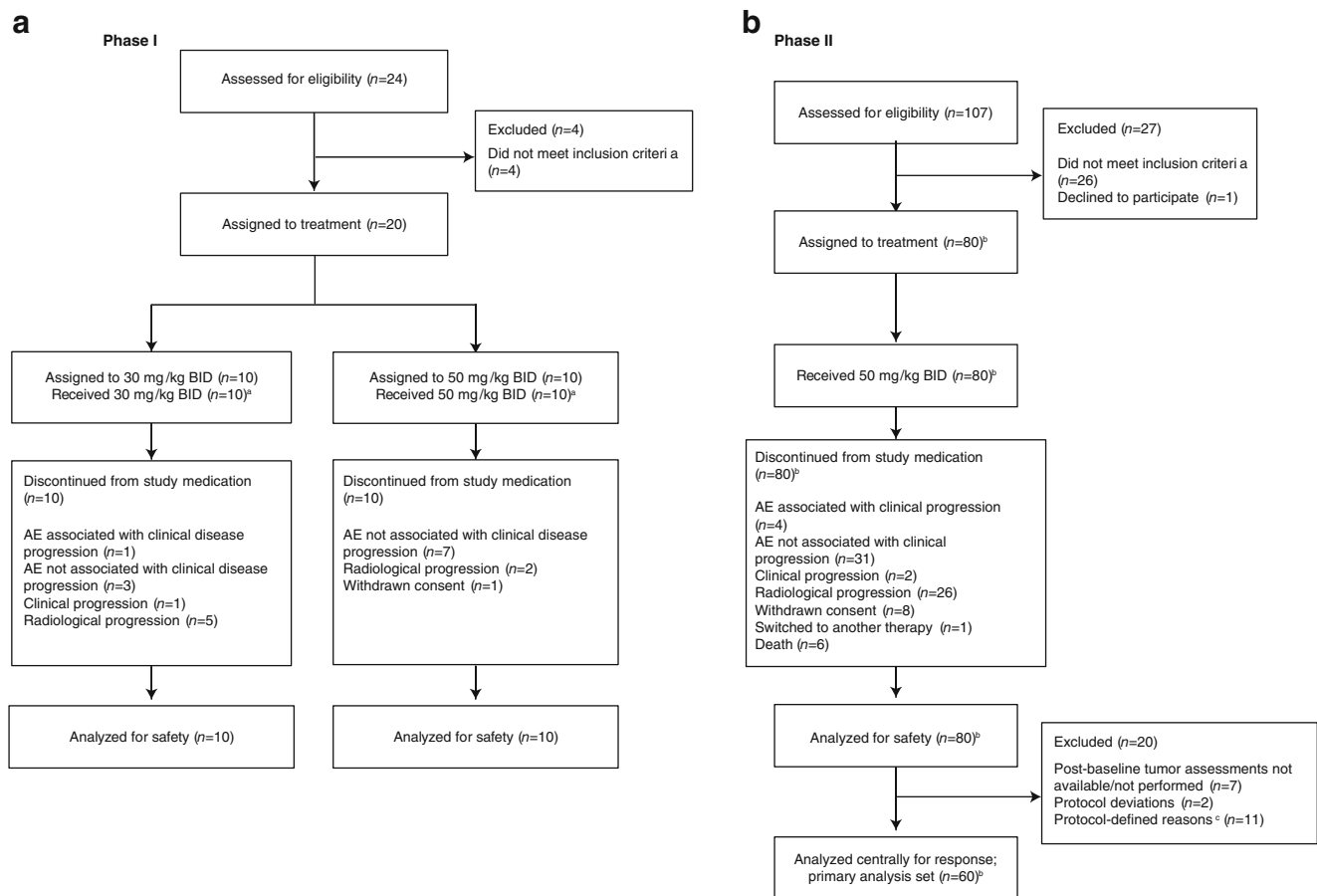


Fig. 1 Patient disposition and flow in phase I (a) and phase II (b). ^aFour patients were replaced because of non-dose-limiting toxicity events before the end of the first cycle. ^bIncludes 10 patients from the 50 mg/kg twice daily dose level in phase I. ^cProtocol-defined reasons refer to the

statistical requirement of exactly 60 treated patients to be centrally evaluated for the primary efficacy endpoint; patients excluded from the primary efficacy analysis were evaluated for response by investigator assessment. *AE* adverse event, *BID* twice daily other $n=2$

Nineteen samples with sufficient tumor content had sufficient RNA for analysis of gene expression. Messenger RNA expression data for all genes and for genes with published *KRAS* pathway signatures [22] were tested for correlation with response to treatment; visual inspection of principal component analysis and hierarchical clustering results showed no obvious correlation (data not shown; no statistical analysis was performed because of the small sample number). Correlation between copy number alteration and messenger RNA expression level was investigated for genes with copy number alteration in more than one patient, and expression of *C-MYC* and *KAT6A* correlated with gene amplification (Online Resource 1).

MicroRNA expression data were generated from baseline plasma samples. No individual difference in microRNA was observed for *KRAS* mutational status, response to treatment, or treatment (data not shown). An association between expression level and *KRAS* mutational status was observed for miR-96-5p, miR-214-3p, and miR-877 (Online Resource 2). The false discovery rate for each analysis was 0.33.

3.2 Exposure and Safety

In phase I, the mean daily dose of refametinib was 56.8 mg in the 30 mg cohort (range 40.7–59.8) and 91 mg in the 50 mg cohort (range 66.8–100). Mean refametinib treatment duration was 23.5 weeks (range 4.6–54) in the 30-mg cohort and 11.3 weeks (range: 2–32.3) in the 50-mg cohort. During phase I, treatment was tolerated at dose level 2 (refametinib 50 mg twice daily plus standard gemcitabine), which was declared the maximum tolerated dose and recommended phase II dose. During phase I, one patient in the 30-mg cohort experienced grade 3 deterioration of general status (dose-limiting toxicity), which led to dose interruption, remained unresolved, and was considered unrelated to treatment. This patient subsequently experienced grade 5 steatohepatitis which was deemed treatment-related (gemcitabine); a relationship to refametinib could not be excluded. One patient in the 50-mg cohort experienced grade 3 pneumonitis (dose-limiting toxicity), considered treatment-related, leading to dose interruption and study withdrawal. In phase I, four patients from each dose level were not evaluable for dose-limiting toxicities because they had not

Table 1 Baseline demographics and disease characteristics

	Phase I Refametinib 30 mg BID (<i>n</i> = 10)	Phase II Refametinib 50 mg BID (<i>n</i> = 80)	Total (<i>N</i> = 90) ^a
Males, <i>n</i> (%)	6 (60.0)	44 (55.0)	50 (55.6)
Median age, years	64.5	63.0	63.0
Race, <i>n</i> (%)			
White	10 (100)	77 (96.3)	87 (96.7)
Black	0	2 (2.5)	2 (2.2)
Asian	0	1 (1.3)	1 (1.1)
Ethnicity, <i>n</i> (%)			
Not Hispanic or Latino	10 (100)	80 (100)	90 (100)
Median BMI, kg/m ²	26.3	24.1	24.5
ECOG PS ^b , <i>n</i> (%)			
0	6 (60.0)	30 (37.5)	36 (40.0)
1	4 (40.0)	49 (61.3)	53 (58.9)
Tumor histology, <i>n</i> (%)			
Adenosquamous carcinoma	0	2 (2.5)	2 (2.2)
Ductal adenocarcinoma	6 (60.0)	64 (80.0)	70 (77.8)
Mucinous cystadenocarcinoma	0	1 (1.3)	1 (1.1)
Mucinous non-cystic carcinoma	0	1 (1.3)	1 (1.1)
Osteoclast-like giant-cell tumor	0	1 (1.3)	1 (1.1)
Serous cystadenocarcinoma	0	1 (1.3)	1 (1.1)
Undifferentiated carcinoma ^c	1 (10.0)	1 (1.3)	2 (2.2)
Other ^d	3 (30.0)	9 (11.3)	12 (13.3)
Status of primary tumor, <i>n</i> (%)			
Resected	1 (10.0)	5 (6.3)	6 (6.7)
Resected, with presence of residual/ recurrent tumor	0	7 (8.8)	7 (7.8)
Unresected	9 (90.0)	68 (85.0)	77 (85.6)
TNM classification at study entry, <i>n</i> (%)			
IIA	0	1 (1.3)	1 (1.1)
IIB	1 (10.0)	1 (1.3)	2 (2.2)
III	1 (10.0)	10 (12.5)	11 (12.2)
IV	8 (80.0)	68 (85.0)	76 (84.4)
Median (range) time from initial diagnosis to start of study treatment, days	26 (8–674)	30 (3–776)	30 (3–776)
Prior systemic anticancer therapy ^e , <i>n</i> (%)	1 (10.0)	6 (7.5)	7 (7.8)
Prior anticancer radiotherapy ^e , <i>n</i> (%)	0	2 (2.5)	2 (2.2)
Number of target lesions, <i>n</i> (%)			
1	4 (40.0)	25 (31.3)	29 (32.2)
2	1 (10.0)	22 (27.5)	23 (25.6)
3	3 (30.0)	19 (23.8)	22 (24.4)
4	2 (20.0)	9 (11.3)	11 (12.2)
5	0	5 (6.3)	5 (5.6)
Number of non-target lesions, <i>n</i> (%)			
0	4 (40.0)	18 (22.5)	22 (24.4)
1	2 (20.0)	26 (32.5)	28 (31.1)
2	2 (20.0)	15 (18.8)	17 (18.9)
3	1 (10.0)	13 (16.3)	14 (15.6)
4	1 (10.0)	7 (8.8)	8 (8.9)
5	0	1 (1.3)	1 (1.1)

Table 1 (continued)

	Phase I Refametinib 30 mg BID (n = 10)	Phase II Refametinib 50 mg BID (n = 80)	Total (N = 90) ^a
Sites of disease ^f , n (%)			
Pancreas	9 (90.0)	69 (86.3)	78 (86.7)
Liver	5 (50.0)	46 (57.5)	51 (56.7)
Lymph node	3 (30.0)	43 (53.8)	46 (51.1)
Lung	3 (30.0)	24 (30.0)	27 (30.0)
Abdominal cavity	1 (10.0)	8 (10.0)	9 (10.0)
Peritoneum	0	6 (7.5)	6 (6.7)
Others ^g	2 (20.0)	25 (31.3)	27 (30.0)
Median (range) time from most recent progression to start of study treatment, days	27 (6–54)	12 (2–77)	12 (2–77)

BID twice daily, BMI body mass index, ECOG PS Eastern Cooperative Oncology Group performance status, TNM tumor node metastasis

^a Includes 10 patients from the 50 mg/kg dose level in phase I

^b Data missing for one patient for whom screening was coincidentally performed at cycle 1, day 1, and for whom ECOG PS was 0

^c Includes spindle, giant, and small cell types

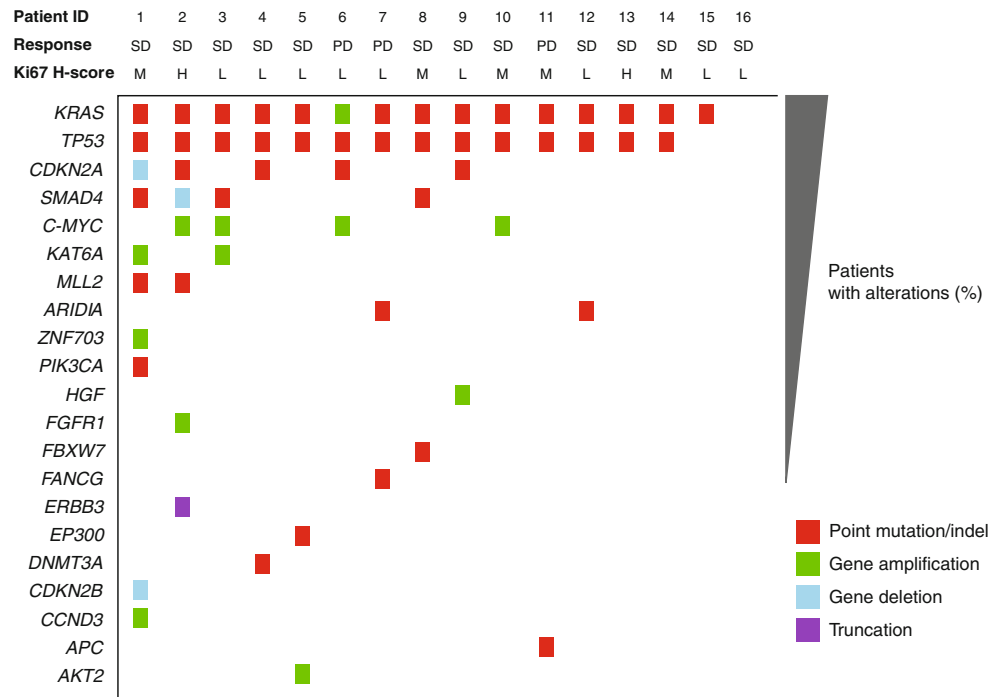
^d Histology was consistent with adenocarcinoma but cytology was not available

^e Patients were treated with adjuvant intent

^f Includes potential multiple sites per patient

^g Includes adrenal gland (phase II, n = 4), hilar lymph node and spleen (phase II, both n = 3), bone, celiac lymph node, and thorax (phase II, all n = 2), pleural cavity and regional lymph node (phase I, both n = 1; phase II, both n = 1), and cervical lymph node, iliac lymph node, kidney, mediastinal lymph node, mediastinum, ovary, and pelvis (phase II, all n = 1)

Fig. 2 Tumor exome sequencing (primary analysis set). H high, Indel insertion or deletion, L low, M medium, PD progressive disease, SD stable disease



reached the end of one cycle of treatment or had received too low a dose of treatment.

In phase II, the mean daily dose of refametinib was 88 mg overall (range 52.7–100; relative dose intensity 88%); 66% of patients (53 out of 80) received an average dose of 81–100 mg daily. Mean refametinib treatment duration, excluding interruptions, was 14.7 weeks (range 0.9–51.3). The mean weekly gemcitabine dose was 895.6 mg/m² (range 500–1000; relative dose intensity 90%); 95% of patients (76 out of 80) received 751–1000 mg/m² per week. The mean gemcitabine treatment duration, excluding interruptions, was 11.6 weeks (range 1–37).

The main reasons for study discontinuation in phase II were adverse events not associated with progressive disease (39%) or radiological progression (33%) (Online Resource 3). All patients experienced at least one treatment-emergent adverse event; most experienced at least one grade 3 (49%) or grade 4 (23%) treatment-emergent adverse event. The most common grade 3 or 4 treatment-emergent adverse event was neutropenia (39%; 14% grade 4). Overall, 66% of patients experienced at least one serious adverse event, considered refametinib-related in 24% of patients and gemcitabine-related in 26% of patients. No grade 5 adverse events were considered refametinib-related, although one patient (1.3%) had a grade 5 adverse event considered gemcitabine-related. Frequent treatment-emergent adverse events, occurring in 20% or more of patients, are shown in Table 2. In phase II, five patients had

pneumonitis (two each at grades 2 and 3, respectively, and one at grade 4), in addition to two patients in phase I (one at grade 2 and one at grade 3 [dose-limiting toxicity]).

3.3 Pharmacokinetics

In phase I, following multiple-dose oral administration, refametinib was well absorbed at both dose levels (30 mg twice daily and 50 mg twice daily), with comparable exposure without (cycle 1, day 21) and with (cycle 1, day 22) gemcitabine (Online Resource 4A). Refametinib and metabolite M-17 pharmacokinetic parameters were generally comparable with historical data in patients with other cancer types [23] (Online Resource 5). Gemcitabine exposure was comparable when administered without (cycle 1, day 1) and with (cycle 1, day 22) refametinib (Online Resource 4B). The pharmacokinetic parameters of gemcitabine and metabolite difluorodeoxyuridine are shown in Online Resource 6.

3.4 Efficacy

Of the 60 patients evaluated for response by independent radiological review, none had confirmed complete responses and 14 (23%) had confirmed partial responses, giving an objective response rate of 23%; the disease control rate was 73% (Table 3). The null hypothesis of objective response rate of 7% or less could

Table 2 Incidence of treatment-emergent adverse events occurring in at least 20% of patients, by body system and worst grade according to Common Terminology Criteria for Adverse Events v4.0 (all patients evaluable for safety assessment in phase II)

<i>n</i> (%)	Grade 1 (<i>n</i> = 80)	Grade 2 (<i>n</i> = 80)	Grade 3 (<i>n</i> = 80)	Grade 4 (<i>n</i> = 80)	Grade 5 (<i>n</i> = 80)	Total (<i>N</i> = 80)
Thrombocytopenia	11 (13.8)	20 (25.0)	15 (18.8)	2 (2.5)	0	48 (60.0)
Fatigue	16 (20.0)	19 (23.8)	9 (11.3)	0	0	44 (55.0)
Anemia	3 (3.8)	29 (36.3)	10 (12.5)	1 (1.3)	0	43 (53.8)
Limb edema	23 (28.8)	19 (23.8)	1 (1.3)	0	0	43 (53.8)
Nausea	22 (27.5)	13 (16.3)	4 (5.0)	0	0	39 (48.8)
Neutropenia	0	6 (7.5)	20 (25.0)	11 (13.8)	0	37 (46.3)
Diarrhea	17 (21.3)	16 (20.0)	4 (5.0)	0	0	37 (46.3)
Fever	20 (25.0)	16 (20.0)	0	0	0	36 (45.0)
Vomiting	22 (27.5)	11 (13.8)	2 (2.5)	0	0	35 (43.8)
Acneiform rash	11 (13.8)	16 (20.0)	6 (7.5)	0	0	33 (41.3)
Abdominal pain	13 (16.3)	15 (18.8)	2 (2.5)	0	0	30 (37.5)
Mucositis oral	12 (15.0)	10 (12.5)	6 (7.5)	0	0	28 (35.0)
Increased alanine aminotransferase	7 (8.8)	9 (11.3)	11 (13.8)	0	0	27 (33.8)
Anorexia	17 (21.3)	7 (8.8)	2 (2.5)	0	0	26 (32.5)
Increased aspartate aminotransferase	7 (8.8)	5 (6.3)	11 (13.8)	1 (1.3)	0	24 (30.0)
Dyspnea	10 (12.5)	11 (13.8)	1 (1.3)	1 (1.3)	1 (1.3)	24 (30.0)
Thromboembolic event	2 (2.5)	10 (12.5)	10 (12.5)	1 (1.3)	0	23 (28.8)
Constipation ^a	14 (17.5)	3 (3.8)	0	0	0	18 (22.5)
Increased creatinine phosphokinase	6 (7.5)	6 (7.5)	4 (5.0)	1 (1.3)	0	17 (21.3)
Hypokalemia	11 (13.8)	0	5 (6.3)	0	0	16 (20.0)
Rash maculopapular	7 (8.8)	9 (11.3)	0	0	0	16 (20.0)

^a Data missing for one patient

Table 3 Efficacy of refametinib plus gemcitabine (primary analysis set)

Response n (%) [75% CI]	Total (N = 60)
Best overall response (RECIST)^a	
Partial response	14 (23.3) [16.89–30.99]
Unconfirmed partial response	7 (11.7) [7.00–18.15]
Stable disease	23 (38.3) [30.63–46.57]
Progressive disease	6 (10.0) [5.69–16.23]
Objective response rate (confirmed complete response and partial response only)	14 (23.3) [16.89–30.99]
Disease control rate (partial response ^b and stable disease)	44 (73.3) [65.47–80.13]

CI confidence interval, RECIST Response Evaluation Criteria in Solid Tumors

^a Data missing for 10 patients

^b Includes unconfirmed partial response

thus be rejected. Seven patients had unconfirmed partial responses (12%) and no patients had unconfirmed complete responses.

Median progression-free survival was 6.3 months and median overall survival was 8.9 months (Fig. 3).

There were 10 (13%) confirmed partial responses and no confirmed complete responses among all 80 investigator-

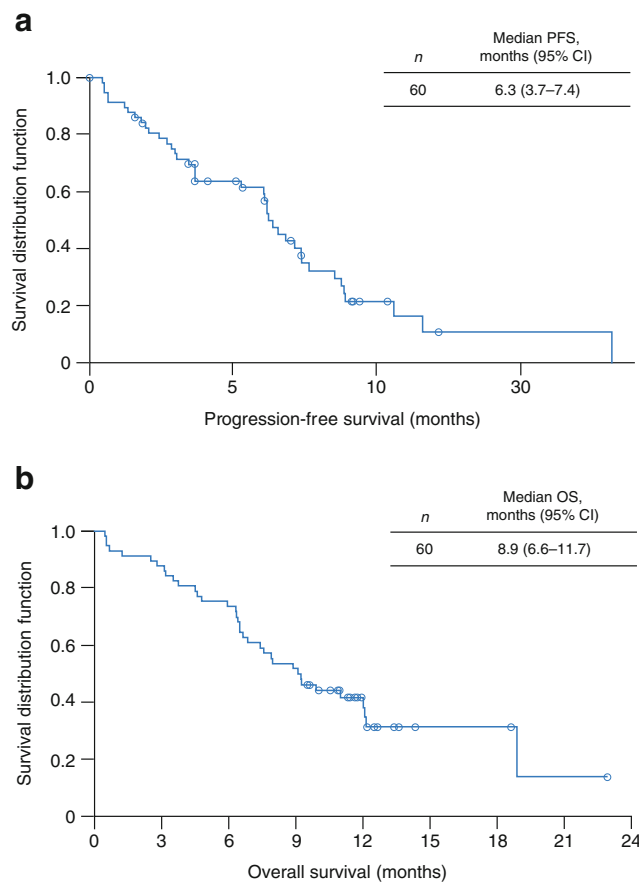


Fig. 3 Median PFS (a) and median OS (b) (primary analysis set). CI confidence interval, OS overall survival, PFS progression-free survival

assessed patients, giving an objective response rate of 13%. Another 10 patients (13%) had unconfirmed partial responses and 35 patients (44%) had stable disease; the disease control rate was 69%. No patients had an unconfirmed complete response. For the 80 investigator-assessed patients, median progression-free survival was 5.4 months (range 0–18.2; 95% confidence interval 4.0–7.1) and median overall survival was 8.4 months (range 0.5–18.2; 95% confidence interval 6.4–11.6).

3.5 Response by KRAS Mutational Status

Of the 60 patients evaluated for response by independent radiological review and for KRAS mutational status in circulating tumor DNA, KRAS mutations were detected in 39 patients (65%). Of these patients, 11 (28%) had partial responses (including unconfirmed partial responses) and 16 (41%) had stable disease; the disease control rate was 69% (27 out of 39). For patients without detectable KRAS mutations, 10 (48%) had partial responses (including unconfirmed partial responses) and seven (33%) had stable disease; the disease control rate was 81% (17 out of 21). KRAS wild-type allele frequency tended to correlate with better tumor response (Fig. 4).

A greater proportion of patients without detectable KRAS mutations (11 out of 20 [55%]) showed best change in target lesion size of 30% or more compared with patients with detectable KRAS mutations (13 out of 31 [43%]; blinded assessment) (Fig. 5a).

Median progression-free survival was 5.3 months and 8.8 months (Fig. 5b), and median overall survival was 6.6 months and 18.2 months (Fig. 5c), for patients with and without detectable KRAS mutations, respectively.

Of the 54 patients in the primary analysis set evaluable for change in serum level of carbohydrate antigen 19-9 from

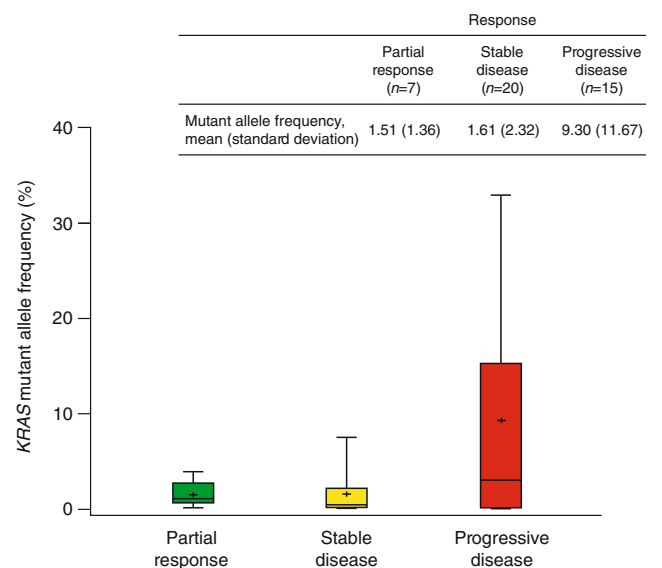
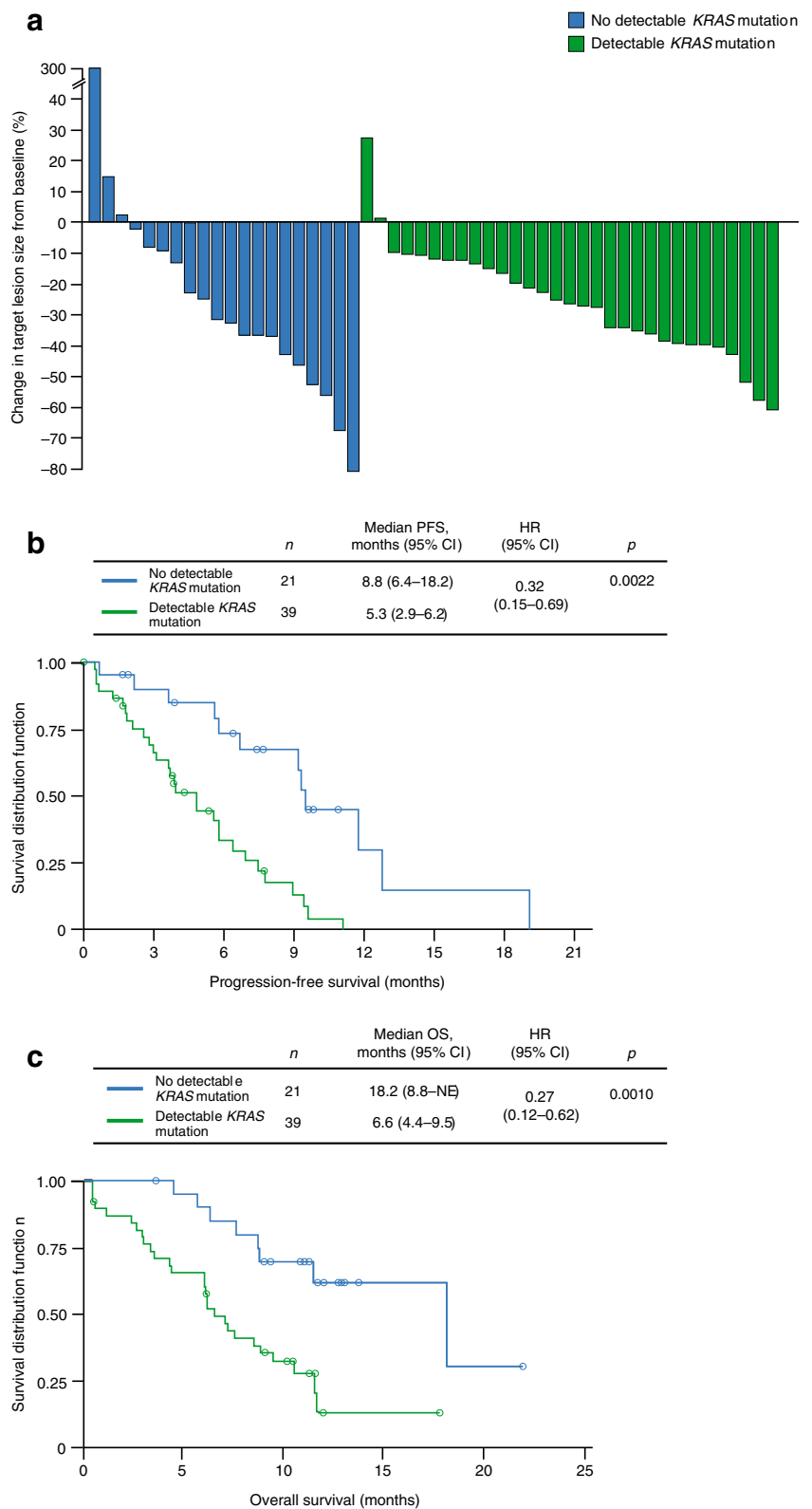


Fig. 4 Tumor response and mutant allele frequency (primary analysis set)

Fig. 5 Change from baseline in target lesion size by *KRAS* mutational status (a), median PFS in *KRAS* subgroups (b), and median OS in *KRAS* subgroups (c) (primary analysis set). Nine out of the 60 patients in the primary analysis set were not evaluated for change in target lesion size, of whom two experienced protocol deviations. Six patients were not evaluable for change in carbohydrate antigen 19-9 from baseline. *CI* confidence interval, *HR* hazard ratio, *NE* not evaluable, *OS* overall survival, *PFS* progression-free survival



baseline, 29 showed a 50% or higher decrease (Online Resource 7), which did not appear to be associated with *KRAS* status. However, wild-type *KRAS* was associated with

lower serum carbohydrate antigen 19-9 at baseline ($p = 0.0236$) and at cycle 1, day 29 ($p = 0.0154$) (Online Resource 8).

4 Discussion

This phase I/II study determined the maximum tolerated dose of refametinib plus gemcitabine and evaluated the efficacy and tolerability of the combination in patients with unresectable, locally advanced or metastatic pancreatic cancer, for whom gemcitabine-based therapy is indicated as first-line treatment.

The maximum tolerated dose in phase I was identified to be refametinib 50 mg twice daily plus standard gemcitabine, consistent with historical refametinib monotherapy data [23]. The combination appeared generally feasible, with the most frequent adverse events being grade 1 or 2. However, the incidence of grade 3 or 4 neutropenia in phase II (39%) was higher than in previous reports of gemcitabine in this patient population [9, 12, 14, 16]. In total, seven patients developed pneumonitis, a known toxicity of gemcitabine [24], although it remains possible that adding a MEK inhibitor may increase the incidence of pneumonitis, as seen in the phase II study of trametinib and gemcitabine (7 out of 80 cases vs. 2 out of 80 cases in the gemcitabine group) [25].

The primary efficacy endpoint in phase II was reached, with an objective response rate of 23% for the refametinib plus gemcitabine combination, which is more than twice as high as historical reports of gemcitabine monotherapy (range 5.4–10.5%) [11, 13, 26]. The overall disease control rate was consistent with historical reports of gemcitabine monotherapy (73% vs. 29.8–47.2%) [11, 13, 26]. Baseline demographics and disease characteristics were broadly similar to those seen in previous trials [13, 26]. The inclusion of patients with locally advanced, unresectable disease as well as those with metastatic disease remains representative of patients with advanced pancreatic cancer, and the evaluation of response rate in these patients was based on patients having at least one measurable lesion, consistent with recent trials [27–29].

Response, progression-free survival, and overall survival were similar to those reported for albumin-bound paclitaxel plus gemcitabine [10]. Partial response and overall survival were slightly lower than reported for the oxaliplatin, irinotecan, fluorouracil, and leucovorin regimen, although progression-free survival was similar [9]. Objective response rate and overall survival were also similar to those recently reported for the combination of trametinib and gemcitabine (objective response rate 22%; overall survival 8.4 months), along with the proportion of patients with detectable *KRAS* mutations (72%) [25]. In the trametinib and gemcitabine study, overall survival was greater with trametinib and gemcitabine than with gemcitabine and placebo in patients with mutant *KRAS* ($n = 103$; 8.3 vs. 6.7 months, respectively) and those with wild-type *KRAS* ($n = 40$; 8.6 vs. 5.9 months, respectively). In our study, median overall survival was also greater in patients without detectable *KRAS* mutations (18 vs. 6.6 months, respectively), as were median progression-free

survival and objective response rate (8.8 vs. 5.3 months and 48% vs. 28%, respectively).

The proportion of patients with detectable *KRAS* mutations as determined from circulating tumor DNA was similar to that in a previous study in pancreatic cancer (62.6%) [30]. In the latter study, overall survival was greater in patients with wild-type versus mutant *KRAS* (413 vs. 276 days, respectively), suggesting a negative prognostic role for *KRAS* mutations detected in circulating tumor DNA.

Nevertheless, the predictive or prognostic role of *KRAS* following first-line gemcitabine-based therapy in pancreatic cancer remains unclear. Retrospective analysis of first-line gemcitabine-based therapy revealed a lower objective response rate in patients with mutant *KRAS* compared with wild-type *KRAS* (11% vs. 26%, respectively) [31]. Subgroup analysis revealed longer overall survival with gemcitabine and erlotinib in patients with wild-type *KRAS* (9.7 vs. 5.2 months), with no overall survival difference between *KRAS* mutational subgroups treated with other gemcitabine-based regimens (7.0 vs. 7.0 months) [31]. Conversely, subgroup analysis of a phase III study [14] reported similar overall survival in patients treated with gemcitabine and erlotinib irrespective of *KRAS* mutational status (6.1 vs. 6.0 months in wild type and mutant, respectively), while the mutant *KRAS* subgroup appeared to have greater benefit from gemcitabine monotherapy compared with the wild-type subgroup (7.4 vs. 4.5 months, respectively) [32].

Results from serum carbohydrate antigen 19-9 levels in both patient subsets were ambiguous and do not allow for firm conclusions. A negative impact of *KRAS* mutations and high serum carbohydrate antigen 19-9 levels on overall survival has been reported [33].

C-MYC amplification was prevalent in mutant *KRAS* tumors, consistent with previous observations, suggesting *C-MYC* pathway activation in these patients [34]. These data suggest that targeting *C-MYC* pathways may provide an alternative therapeutic strategy in the treatment of pancreatic adenocarcinoma [35].

KRAS mutational status also tended to correlate with miR-96-5 and miR-214-3 expression, roles for which have been described as a tumor suppressor in pancreatic adenocarcinoma [36] and in the regulation of growth and invasion of stem-like cells in a hepatocellular carcinoma model [37], respectively. However, the significance level must be interpreted with caution because of the sample size analyzed (800 microRNA species), the high false discovery rate, and the lack of corrections for multiple comparisons. Although preliminary, these data may support a role for circulating microRNAs as biomarkers of disease aggressiveness, warranting further investigation.

Although concordance between the mutational status in tumor specimens and circulating tumor DNA is generally very high, discordance between the mutational status in tumor and

circulating tumor DNA from fresh plasma may occur and deserves further investigation [25, 38, 39]. Discordance was observed here in three samples between *KRAS* mutational status as determined by exome sequencing of tumor biopsies and BEAMing technology of fresh plasma. Although BEAMing technology is highly sensitive [40], sensitivity was not formally tested and false negatives could not be conclusively excluded in this small sample.

Refametinib is metabolized by liver enzymes CYP3A4 and CYP2C19, and is a substrate for glucuronidation by UGT2B7. Gemcitabine is metabolized by cytidine deaminase and is primarily eliminated in urine along with its metabolite difluorodeoxyuridine. As expected from these distinct metabolic and elimination pathways, no pharmacokinetic interactions were observed; refametinib and gemcitabine exposures were comparable when administered alone or in combination.

Overall, refametinib combined with gemcitabine is well tolerated in 8-weekly cycles up to the maximum tolerated dose, with no pharmacokinetic interaction. The primary endpoint of phase II was met: the combination showed a relatively high objective response rate in patients with advanced pancreatic cancer, with an acceptable safety profile. There was a trend towards improved survival in patients without detectable *KRAS* mutations compared with those with detectable *KRAS* mutations in circulating tumor DNA. This study also suggests that biomarker status in patients with *KRAS* mutations may provide predictive or prognostic information with regard to clinical benefit from refametinib plus gemcitabine.

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Compliance with Ethical Standards

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Conflict of Interest HR has performed a consulting or advisory role for Boehringer Ingelheim, Bayer, GlaxoSmithKline, Bristol-Myers Squibb, Roche, and Sanofi-Aventis. JJ has performed a consulting or advisory role for Eli Lilly, Pfizer, AstraZeneca, and Celgene; participated in a speaker bureau for Roche; received research funding from GlaxoSmithKline, Roche, and Novartis; and received travel, accommodation, and other expenses from Boehringer Ingelheim. MH has received honoraria from Celgene, research funding from Roche and Boehringer Ingelheim, and travel, accommodation, and other expenses from Celgene and Boehringer Ingelheim. MP has received honoraria and research funding from Bayer. PR has received honoraria from Roche, Sirtex, Bayer, and Celgene; performed a consulting or advisory role for Roche, Sirtex, Sanofi-Aventis, Celgene, Baxter, Merck Serono, and Daiichi Sankyo; received research funding from Sanofi-Aventis, Bayer, Roche, and Merrimack; and received travel, accommodation, and other expenses from Roche, Merck Serono, Celgene, and Sanofi-Aventis. JB is partly supported by the UCLH/UCL Biomedical Research Centre. He has performed a consulting or advisory role for Roche, Merck Serono, and

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