Phase I/II study of refametinib (BAY 86-9766) in combination with gemcitabine in advanced pancreatic cancer: association of clinical outcomes with tumour *KRAS* mutational status

Running head: Refametinib and gemcitabine in pancreatic cancer

Jean-Luc Van Laethem*[,1], Hanno Riess[2], Jacek Jassem[3], Michael Haas[4], Uwe M Martens[5], Colin Weekes[6], Marc Peeters[7], Paul Ross[8], John Bridgewater[9], Bohuslav Melichar[10], Stefano Cascinu[11], Piotr Saramak[12], Patrick Michl[13], David Van Brummelen[14], Alberto Zaniboni[15], Wollf Schmiegel[16], Svein Dueland[17], Marius Giurescu[18], Vittorio L Garosi[19], Katrin Roth[18], Anke Schulz[18], Henrik Seidel[18], Prabhu Rajagopalan[20], Michael Teufel[20], Barrett H Childs[20]

[1]Department of Gastroenterology, Erasme University Hospital, Route de Lennik 808 1070, Brussels, Belgium; [2]Medical Department, Division of Hematology, Oncology and Tumor Immunology, Charity Hospital, Virchow-Klinikum Campus, Augustenburger Platz 1, 13353 Berlin, Germany; [3]Department of Oncology and Radiotherapy, Medical University of Gdansk, M. Skłodowskiej-Curie 3a street, Gdansk 80-210, Poland; [4]Department of Hematology and Oncology, University of Munich Medical Center, Marchioninistraße 15, 81366 Munich,, Germany; [5]Department of Hematology and Oncology, Cancer Center Heilbronn-Franken, Am Gesundbrunnen 20-26, 74078 Heilbronn, Germany; [6]Division of Medical Oncology, University of Colorado Cancer Center, 1665 Aurora Ct, Aurora, CO 80045, USA; [7]Department of Oncology, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem, Belgium; [8]Department of Medical Oncology, Guy's & St Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH, UK; [9]Department of Oncology, UCL Cancer Institute, 72 Huntley Street, London, WC1E 6DD, UK; [10]Department of Oncology, Palacky University Medical School and University Hospital Olomouc, Křížkovského 8, 771 47

Olomouc, Czech Republic; [11]Department of Medical Oncology, A.O.U. United Hospitals, Polytechnic University of Marche, Piazza Roma, 22, Ancona, Italy; [12]Department of Oncological Gastroenterology, Maria Skłodowska-Curie Memorial Cancer Center, ul. W.K.Roentgena 5, 02-781 Warsaw, Poland; [13]Department of Gastroenterology, Endocrinology, Metabolism and Infectiology, University Hospital of Giessen and Marburg, Baldingerstraße, 35043 Marburg, Germany; [14]Department of Radiotherapy, UZ Brussels, Avenue du Laerbeek 101, 1090 Brussels, Belgium; [15]Department of Medical Oncology, Poliambulanza Foundation Hospital Institute, Via Bissolati, 57, Brescia, Italy; [16]Department of Gastroenterology and Hepatology, Medical University Hospital Bochum, Alexandrinenstraße 1, Bochum 44791, Germany; [17]Department of Oncology, Oslo University Radium Hospital, Trondheimsveien 235, Bjerke 0514, Oslo, Norway; [18]Bayer Pharma AG, Müllerstraße 178, Berlin 13353, Germany; [19]Bayer S.p.A., Viale Certosa 126-130, 20156 Milan, Italy; [20]Bayer HealthCare Pharmaceuticals, 100 Bayer Blvd, Whippany, NJ 07981, USA

*Correspondence: Professor Jean-Luc Van Laethem, Erasme University Hospital, CP 572/10, route de Lennik 808, 1070 Brussels, Belgium. Tel: +32 2 555 37 12; Fax: +32 2 555 46 97; E-mail: JL.VanLaethem@erasme.ulb.ac.be

ABSTRACT

Background: Activating *KRAS* mutations are reported in up to 90% of pancreatic cancers. Refametinib potently inhibits MEK1/2, part of the MAPK signalling 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 levels were analysed for pharmacokinetics. *KRAS* mutational status was determined from circulating tumour DNA.

Results: Ninety patients overall received treatment. The maximum tolerated dose was refametinib 50 mg twice daily plus standard gemcitabine (1000 mg/m^2 weekly). The combination was well tolerated, with no pharmacokinetic interaction. Treatment-emergent toxicities included thrombocytopenia, fatigue, anaemia and oedema. 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, 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 words: advanced cancer, gemcitabine, MEK inhibitor, pancreas, refametinib

Trial registration ID: ClinicalTrials.gov number NCT01251640 (http://clinicaltrials.gov/show/NCT01251640)

INTRODUCTION

Pancreatic cancer is among the leading causes of cancer-related mortality worldwide (Ferlay *et al*, 2013), and activating *KRAS* mutations are reported in up to 90% of pancreatic cancers (Kanda *et al*, 2012; Morris *et al*, 2010). 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 (Conroy *et al*, 2011; National Comprehensive Cancer Network 2014; Von Hoff *et al*, 2013). However, the survival improvement with gemcitabine monotherapy is modest (Burris *et al*, 1997). Previous phase II and III trials of gemcitabine combined with other cytotoxic agents have shown acceptable safety but inconsistent survival improvement versus monotherapy (Cunningham *et al*, 2009; Goncalves *et al*, 2012; Herrmann *et al*, 2007; Moore *et al*, 2007; Nakai *et al*, 2012). The promising activity of cytotoxic combinations has also been associated with high toxicity (Conroy *et al*, 2011; Von Hoff *et al*, 2013).

Refametinib (BAY 86-9766; Bayer Pharma AG, Berlin, Germany) is an orally available, potent, selective, allosteric (non-adenosine triphosphate competitive) inhibitor of MEK1/2 (Iverson *et al*, 2009). Refametinib has demonstrated both single-agent activity (Puehler *et al*, 2010) and synergistic activity in combination with gemcitabine (Schmieder *et al*, 2011) 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.

METHODS

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. This study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization guideline E6 Good Clinical Practice. All patients provided written, informed consent before participation.

Study design. This open-label, non-randomised, multicentre study 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 (ORR; confirmed complete response and confirmed partial response) per independent radiological review. Secondary outcome measures included disease control rate (complete response, partial response and stable disease), progression-free survival (PFS), overall survival (OS), 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 intravenous gemcitabine 1000 mg/m^2 weekly on day 1; continuous treatment with oral 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. Enrolment 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 opened 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 enrolment to the higher dose level continued. If two or more patients out of a maximum of six patients 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 anaemia; grade 4 neutropenia lasting more than 10 days; grade 3 or 4 neutropenia with fever greater than 38ºC; thrombocytopenia or grade 3 or 4 thrombocytopenia associated with serious bleeding; signs of serious bleeding; grade 3 or higher non-haematological toxicity; grade 3 or higher diarrhoea if refractory to maximal anti-diarrhoeal 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 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/m^2 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 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 end points; 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.

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 [RECIST] 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 bone marrow, hepatic and renal function; and normal cardiac function as estimated by echocardiography.

Assessments. Screening included demographics and baseline characteristics, echocardiography, ophthalmic examination, plasma and tumour biopsy for genotyping and biomarker analysis, and tumour evaluation (blinded) by computed tomography or magnetic resonance imaging (RECIST 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 hours post-infusion at cycle 1, days 1 and 22, and up to 8 hours post-dose at cycle 1, days 21 and 22. Tumour assessments were performed every 8 weeks during treatment until progressive disease or the end of treatment. Confirmatory scans were performed 4 or more weeks after an objective tumour response (complete response or partial response) was documented. 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 (AEs) and concomitant medications were assessed continuously from screening onwards. Following the end of treatment, patients entered a 30-day safety follow-up including AE documentation. Survival follow-up was performed monthly up to 8 months after the first treatment of the last patient.

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).

Biomarker studies. Circulating tumour DNA in plasma was analysed for *KRAS* mutational status by beads, emulsions, amplification and magnetics (BEAMing) technology (Covance Inc., Princeton, NJ, USA), with an assay cut-off of 0.02% mutant allele for positivity. Circulating microRNA from plasma was analysed by quantitative polymerase chain reaction using a human microRNA panel (Exiqon, Woburn, MA, USA). Tumour biopsies were

collected where available, as freshly frozen or formalin-fixed and paraffin-embedded. Histological analysis comprised haematoxylin and eosin staining and Ki67 immunolabelling. Targeted archival tumour gene next-generation sequencing was performed using the FoundationOne® panel (Foundation Medicine, Cambridge, MA, USA). Gene expression was analysed using RNA isolated from tumour 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 (Kim *et al*, 2013) with Bowtie2 (Langmead and Salzberg 2012). Gene-level read counts and reads per kilobase of transcript per million values were calculated with Expressionist® Refiner Genome (Genedata, Lexington, MA, USA).

Statistical analysis. Phase I data were analysed descriptively. The primary efficacy end point in phase II tested the null hypothesis that the overall response rate would be ≤7% 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 analysed 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 end points in phase II were analysed 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.

RESULTS

Baseline demographics and disease characteristics. Of the 24 patients enrolled in phase I, 20 were treated and evaluable for safety assessment (Supplementary Figure S1A). 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; 80 were treated and evaluable for safety assessment (Supplementary Figure S1B), 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; Supplementary Figure S1B), 39 (65%) had *KRAS* mutations, as determined from circulating tumour DNA. Frequently observed *KRAS* mutations included G12D, G12V and G12R; mutations in codons 38 or 436 were not observed. Molecular tumour characterisation was performed in 23 out of 30 archival samples (77%) with sufficient tumour content. Tumour exome sequencing revealed *KRAS* mutations (G12D, G12R, G12V, Q61H, Q61R, A59G) in 15 out of 16 patient samples containing sufficient DNA (Supplementary Figure S2). 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.

Nineteen samples with sufficient tumour content had sufficient RNA for analysis of gene expression. Messenger RNA expression data for all genes and for genes with published *KRAS* pathway signatures (Loboda *et al*, 2010) 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 (Supplementary Figure S3).

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-96-5-p, miR-214-3p and miR-877 (Supplementary Figure S4). The false discovery rate for each analysis was 0.35.

Exposure and safety. 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 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/m^2 (range: 500–1000; relative dose intensity: 90%); 95% of patients (76 out of 80) received 751–1000 mg/m^2 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 AEs not associated with progressive disease (39%) or radiological progression (33%) (Supplementary Table S1). All patients experienced at least one treatment-emergent AE; most experienced at least one grade 3 (49%) or grade 4 (23%) treatment-emergent AE. The most common grade 3 or 4 treatment-emergent AE was neutropenia (39%; 14% grade 4). Overall, 66% of patients experienced at least one serious AE, considered refametinib-related in 24% of patients and gemcitabine-related in 26% of patients. No grade 5 AEs were considered refametinib-related, although one patient (1.3%) had a grade 5 AE considered gemcitabine-related. Frequent treatment-emergent AEs, occurring in ≥20% 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]).

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 (Supplementary Figure S5A). Refametinib and metabolite M-17 pharmacokinetic parameters were generally comparable with historical data in patients with other cancer types (Weekes *et al*, 2013) (Supplementary Table S2). Gemcitabine exposure was comparable when administered without (cycle 1, day 1) and with (cycle 1, day 22) refametinib (Supplementary Figure S5B). The pharmacokinetic parameters of gemcitabine and metabolite difluorodeoxyuridine are shown in Supplementary Table S3.

Efficacy. Of the 60 patients evaluated for response by independent radiological review, none had confirmed complete response and 14 (23%) had confirmed partial response, giving an ORR of 23%; the disease control rate was 73% (Table 3). The null hypothesis of ORR ≤7% could thus be rejected. Seven patients had unconfirmed partial response (12%) and no patients had unconfirmed complete response.

Median PFS was 6.3 months and median OS was 8.9 months (Figure 1).

Response by *KRAS* mutational status. Of the 60 patients evaluated for response by independent radiological review and for *KRAS* mutational status in circulating tumour DNA, *KRAS* mutations were detected in 39 (65%). Of these patients, 11 (28%) had partial response (including unconfirmed partial response) 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 response (including unconfirmed partial response) 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 tumour response (Figure 2).

A greater proportion of patients without detectable *KRAS* mutations (11 out of 20 [55%]) showed best change in target lesion size ≥30% compared with patients with detectable *KRAS* mutations (13 out of 31 [43%]; blinded assessment) (Figure 3A).

Median PFS was 5.3 months and 8.8 months (Figure 3B), and median OS was 6.6 months and 18.2 months (Figure 3C), 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 baseline, 29 showed a ≥50% decrease (Supplementary Figure S6), 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) (Supplementary Figure S7).

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 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 (Weekes *et al,* 2013). The combination appeared generally feasible, with the most frequent AEs 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 (Conroy *et al,* 2011; Cunningham *et al,* 2009; Herrmann *et al,* 2007; Moore *et al,* 2007). In total, seven patients developed pneumonitis, a known toxicity of gemcitabine (Barlési *et al,* 2004), 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) (Infante *et al,* 2014).

The primary efficacy end point in phase II was reached, with an ORR of 23% for the refametinib plus gemcitabine combination; more than twice as high as historical reports of gemcitabine monotherapy (range: 5.4–10.5%) (Burris *et al,* 1997; Nakai *et al,* 2012; Rothenberg *et al,* 1996). The overall disease control rate was consistent with historical reports of gemcitabine monotherapy (73% vs 29.8–47.2%) (Burris *et al,* 1997; Nakai *et al,*

2012; Rothenberg *et al*, 1996). Baseline demographics and disease characteristics were broadly similar to those seen in previous trials (Nakai *et al*, 2012; Rothenberg *et al*, 1996).

Response, PFS and OS were similar to those reported for albumin-bound paclitaxel plus gemcitabine (Von Hoff *et al*, 2013). Partial response and OS were slightly lower than reported for the oxaliplatin, irinotecan, fluorouracil and leucovorin regimen, although PFS was similar (Conroy *et al*, 2011). ORR and OS were also similar to those recently reported for the combination of trametinib and gemcitabine (ORR: 22%; OS: 8.4 months), along with the proportion of patients with detectable *KRAS* mutations (72%) (Infante *et al*, 2014). In the trametinib and gemcitabine study, OS 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 OS was also greater in patients without detectable *KRAS* mutations (18 vs 6.6 months, respectively), as were median PFS and ORR (8.8 vs 5.3 months and 48% vs 28%, respectively).

The proportion of patients with detectable *KRAS* mutations as determined from circulating tumour DNA was similar to that in a previous study in pancreatic cancer (62.6%) (Kinugasa *et al*, 2015). In the latter study, OS 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 tumour 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 ORR in patients with mutant *KRAS* compared with wild-type *KRAS* (11% vs 26%, respectively) (Kim *et al*, 2011). Subgroup analysis revealed longer OS with gemcitabine and erlotinib in patients with wild-type *KRAS* (9.7 vs

5.2 months), with no OS difference between *KRAS* mutational subgroups treated with other gemcitabine-based regimens (7.0 vs 7.0 months) (Kim *et al,* 2011). Conversely, subgroup analysis of a phase III study (Moore *et al,* 2007) reported similar OS 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) (da Cunha Santos *et al,* 2010).

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 OS has been reported (Ogura *et al,* 2013).

C-MYC amplification was prevalent in mutant *KRAS* tumours, consistent with previous observations, suggesting *C-MYC* pathway activation in these patients (Birnbaum *et al,* 2011). These data suggest that targeting *C-MYC* pathways may provide an alternative therapeutic strategy in the treatment of pancreatic adenocarcinoma (Lin *et al,* 2014).

KRAS mutational status also tended to correlate with miR-96-5 and miR-214-3 expression, roles for which have been described as a tumour suppressor in pancreatic adenocarcinoma (Yu *et al,* 2010) and in the regulation of growth and invasion of stem-like cells in a hepatocellular carcinoma model (Xia *et al,* 2012), respectively. However, the significance level must be interpreted with caution because of the sample size analysed (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 tumour specimens and circulating tumour DNA is generally very high, discordance between the mutational status in tumour and

circulating tumour DNA from fresh plasma may occur and deserves further investigation (Higgins *et al,* 2012; Ignatiadis *et al,* 2015; Infante *et al,* 2014). Discordance was observed here in three samples between *KRAS* mutational status as determined by exome sequencing of tumour biopsies and BEAMing technology of fresh plasma. Although BEAMing technology is highly sensitive (Li *et al,* 2006), sensitivity was not formally tested and false negatives could not be conclusively excluded in this small sample.

Refametinib is metabolised by liver enzymes CYP3A4 and CYP2C19, and is a substrate for glucuronidation by UGT2B7. Gemcitabine is metabolised 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 end point of phase II was met: the combination showed a relatively high ORR 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 tumour 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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CONFLICTS OF INTEREST

HR has performed a consulting or advisory role for Boehringer Ingelheim, Bayer, GlaxoSmithKline, Bristol-Myers Squibb, Roche and Sanofi. 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 AstraZeneca, and has received travel, accommodation and other expenses from Merck Serono and the European Society for

Medical Oncology. BM has received honoraria from, and has performed a consulting or advisory role for, Bayer. SC has performed a consulting or advisory role for Roche, Sanofi and Celgene. PM has received honoraria from Ipsen, Bayer and Celgene, and has received research funding from Ipsen. DVB has received travel, accommodation and other expenses from Amgen. MG, KR, AS and HS are employees of Bayer Pharma AG. VLG is an employee of Bayer S.p.A. PRa is an employee of Bayer HealthCare and has stock or other ownership interests in Bayer HealthCare. MT and BHC are employees of Bayer HealthCare. J-LVL, UMM, CW, PS, AZ, WS and SD have no conflicts of interest to declare.

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Titles and legends to figures

Figure 1. Overall median PFS (A) and overall median OS (B) (primary analysis set). Abbreviation: CI = confidence interval.

Figure 2. Tumour response and mutant allele frequency (primary analysis set).

Figure 3. 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. Abbreviations: CI = confidence interval; HR = hazard ratio; NE = not evaluable.