A prospective, phase II, double blind multicentre randomised controlled trial comparing gemcitabine plus vandetanib with gemcitabine plus placebo in locally advanced or metastatic pancreatic carcinoma

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SUMMARY

Background

Vandetanib is a novel tyrosine kinase inhibitor of vascular endothelial growth factor receptor-2, rearranged during transfection (RET), and epidermal growth factor receptor, all of which are in involved in the pathogenesis of pancreatic cancer. We investigated the clinical efficacy of vandetanib in patients with advanced pancreatic cancer.

Methods

Treatment-naïve adult patients with locally advanced or metastatic pancreatic ductal adenocarcinoma with an ECOG performance status of 0–2 were recruited into a phase II double-blind multicentre randomised placebo-controlled trial. Patients were randomly assigned (1:1) to receive 300mg/day vandetanib once daily or placebo. In addition all patients were to have gemcitabine (1000mg/m^2 30min intravenous infusion, weekly for seven weeks followed by a one week break then a cycle of weekly treatment for three weeks with a one-week break), until disease progression. The primary outcome measure was overall survival.

Findings

142 patients were randomised and analysis was undertaken with 131 deaths after a median follow up of 24·9 months. The median (95% confidence interval [CI]) overall survival in the 70 patients randomised to gemcitabine and placebo was 8·95 (6·55-11·7) months and 8·83 (7·11-11·6) months in the 72 patients randomised to gemcitabine and vandetanib (hazard ratio = 1·21, 95% CI = 0·85, 1·73; log rank X^2_{1df} = 1·1; P = 0·303). Compared to the control arm the median (95% CI) survival in patients randomised to gemcitabine and vandetanib was 11·92 (10·89 – NA) months for the 14 patients developing a grade $\geq$2 rash, and 7·76 (4·34 – 1·15) months for the 58 patients who did (log rank X^2_{2df} = 7·23; P= 0·027).

Interpretation

The addition of vandetanib to gemcitabine did not improve overall survival in advanced pancreatic cancer.

Funding

Cancer Research UK and Astra Zeneca

INTRODUCTION

With poor survival and around 338,000 new cases diagnosed worldwide, pancreatic cancer seems set to become the second leading cause of cancer mortality; unless new therapies for advanced pancreatic cancer can be developed (1). The survival improvement from systemic chemotherapies has been small relative to the advances seen in other adenocarcinomas. A regimen comprising folinic acid, 5-fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX) produces the largest increase in median overall survival for patients with metastatic disease, from 6·8 months with gemcitabine to 11·1 months with FOLFIRINOX and a corresponding increase in one year survival from 20·6% to 48·4%, respectively (2). This regimen is associated with significant toxicity and many patients are not sufficiently fit to tolerate it and gemcitabine monotherapy remains a standard option for such patients. The median overall survival for patients with metastatic disease treated with gemcitabine and nab-paclitaxel was 8·5 months compared to 6·7 months for gemcitabine monotherapy, with 35% of patients alive at one year with the combination compared to 22% for gemcitabine alone (3).

The combination of the epidermal growth factor receptor (EGFR) inhibitor erlotinib with gemcitabine showed a marginally-improved median survival in patients with locally advanced and metastatic disease of 6·2 months compared to 5·9 months with gemcitabine alone (4). Patients treated with erlotinib and experiencing ≥ grade 2 rash, which is a presumed marker of more effective EGFR inhibition had a better median overall survival of 10·5 months (4). The addition of the vascular endothelial growth factor (VEGF) inhibitor bevacizumab to the gemcitabine-erlotinib backbone also improved progression free survival but not overall survival (5).

Vandetanib is a multi-targeted tyrosine kinase inhibitor of the epithelial growth factor receptor (EGFR), vascular epithelial growth factor (VEGF) receptor-2 (VEGFR-2) and the receptor encoded by the proto-oncogene called REarranged during Transfection (RET). Vandetanib is a once-daily oral agent and the only RET inhibitor currently available that selectively targets RET, VEGFR, and EGFR signalling (6, 7). RET is the receptor for the glial-derived neurotrophic factor (GDNF) family ligands (GDFLs). Vandetanib inhibits RET auto-phosphorylation, RET-dependent extracellular signal-regulated kinase (ERK) phosphorylation and tumour pathogenesis (7). It inhibits oncogenic RET isoforms and wild type RET tyrosine kinase with equal potency. Neural invasion through GDNF secretion is a prominent feature of pancreatic cancer (8). Neuro-invasive pancreatic cancer cells in contact with nerves become elongated and migrate along the nerves; attracted along a GDNF gradient (9). Not all pancreatic cancer cells show high levels of migration in response to GDNF (10). Pancreatic cancer cells, which migrate in response to GDNF, also proliferate upon GDNF treatment. Cells responding robustly to GDNF were shown to be heterozygous for the rs1799939 (p.G691S) RET allele and over-expression of rs1799939 increased invasion. The p.G691S polymorphism was present in 37% of primary cancers and in 31% of the matched normal pancreas. Levels of RET expression were the same in those with and without the polymorphism. The p.G691S polymorphism has been similarly implicated in the biology of desmoplasia melanoma, another neurotrophic disease (11). In addition RET is overexpressed in 50-65% of pancreatic cancers (10, 12, 13).

Pre-clinical data in vitro, showed that the combination of vandetanib with gemcitabine provided synergistic cytotoxicity (14). Vandetanib increased the expression of deoxycytidine kinase (dCK) and the dCK/RRM1xRRM2 ratio. dCK is required to phosphorylate gemcitabine allowing its cytotoxic and cytostatic effects. Gemcitabine-sensitive pancreatic cancer cells may have higher levels of dCK than resistant cells and gemcitabine resistance is associated with down-regulation of dCK (15). In a highly metastatic orthotropic pancreatic cancer model, tumour weight was significantly less with the combination of gemcitabine and vandetanib than in those animals treated with either agent alone (16). The combination

also significantly increased apoptosis in the primary tumour. Gemcitabine alone did not impact on the development of metastases but none of five combination-treated animals developed liver metastases, whilst all gemcitabine-treated mice developed nodal metastases compared with only one of five combination-treated animals.

We report here the final results of a randomised phase II study comparing gemcitabine plus placebo with gemcitabine plus vandetanib in patients with advanced pancreatic cancer. We also report here the biomarker analysis of outcome according to p.G691S RET polymorphism status and additionally examine selected SNPs that have been proposed as being predictive of the activity of other angiogenesis inhibitors as well as RET tissue expression by immunohistochemistry.

METHODS

Study Design

The vandetanib in pancreatic cancer (ViP) trial was a phase II placebo-controlled blinded randomised trial to compare gemcitabine plus vandetanib against gemcitabine plus placebo in patients with locally-advanced or metastatic pancreatic ductal adenocarcinoma. Patients were recruited from 18 UK hospitals, which were centrally coordinated by the Cancer Research United Kingdom Liverpool Cancer Trials Unit (LCTU). The trial was reviewed and endorsed by the West London REC 2 Research Ethics Committee (MREC REF: 11/LO/0097).

Participants

Patients were eligible if they were at least 18 years old and diagnosed with locally advanced or metastatic carcinoma of the pancreas. Patients had to have an Eastern Cooperative Oncology Group (ECOG) score of zero, one or two and a documented life expectancy greater than 3 months. Patients undergoing curative or definitive locally directed therapies were excluded as were any patients who had undergone major surgery or radiotherapy within 4 weeks previous to randomisation. Patients were further excluded if they had received previous chemotherapy for locally advanced or metastatic disease. Adjuvant chemotherapy for

resected pancreatic cancer was permitted provided that chemotherapy was completed > 12 months previously. All patients entering the study gave their written informed consent following a full explanation of the study and after reading the patient information sheet.

Randomisation and Masking

Fisher Clinical Services (Fisher Clinical Services, Horsham, United Kingdom) were contracted to manage the drug (randomisation, dispensing, and discontinuation) and unblinding of patients. Patients were randomised to each treatment group on a 1:1 basis according to computer generated permuted blocks of variable size. Patients were stratified at randomisation by their disease stage (locally advanced versus metastatic) and their ECOG performance status (0/1 versus 2). Prior to randomisation, staff at the LCTU verified patient details and eligibility criteria before being forwarded to Fisher to complete the randomisation process. Masking was achieved by using tablets with identical appearance in numbered bottles. Fisher allocated patients to each treatment group and directly informed the Pharmacy at each site, which numbered bottle to distribute to which patients. Only staff at Fisher were unblinded to treatment allocation prior to the end of the study. Patients were unmasked only in the event of a possible Suspected Unexpected Serious Adverse Reaction (SUSAR). An independent clinical coordinator via direct communication carried out unblinding with Fisher.

Procedures

Gemcitabine was administered at 1000mg/m^2 weekly as a 30-minute infusion for seven continuous weeks followed by a one week break. Following this, gemcitabine was prescribed on a cycle of three continuous weeks followed by a one week break. Vandetanib was prescribed orally once a day at 300mg/day. Placebo was prescribed to replicate the vandetanib prescription. Treatment continued until disease progression, intolerable toxicity despite supportive measures and dose modifications or withdrawal of consent. Patients were followed up continually until either death or end of study.

Outcomes

The primary outcome measure was overall survival. Secondary outcome measures were progression-free survival, objective response rate, disease control rate, toxicity and patient pain assessments. Toxicity assessments were assessed following the Common Terminology Criteria for Adverse Events (CTCAE) Version 4·02 definitions. Patient response to therapy was measured using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1·1 (17)

Best radiological response was defined as complete response, partial response, stable disease or progressive disease. Objective response was defined as any patient with a complete or partial response. Disease control was defined as any patient with stable disease, a partial response or a complete response. Pain assessments were made using a 100 point visual analogue scale. The trial was subject to 100% source data verification of all outcome data.

Translational Methodology

Carbohydrate Antigen (CA) 19·9 levels were measured at the participating site hospital clinical laboratories C-reactive protein (CRP) levels were measured at the Royal Liverpool Hospital Clinical Laboratories (upper limit of normal = 5 mg/l). All other analyses were undertaken centrally in the LCTU GCPLabs.

Single nucleotide polymorphism (SNP) analyses for RET p.G691S, IL-8 rs4073, VEGF-A rs699947 and FLT1 rs9582036 were undertaken using predesigned TaqMan® MGB probes (ThermoFisher Scientific, United Kingdom). Genomic DNA was extracted from patient blood using an automated MagNA Pure Compact Instrument (Roche Diagnostics, Germany) with the MagNA Pure Compact Nucleic Acid Isolation Kit I, according to the manufacturer's instructions. Polymerase chain reactions (PCRs) were performed on a Roche Lightcycler 480. Patients were classified as heterozygous or homozygous for each allele.

Immunohistochemistry for RET was undertaken on formalin-fixed paraffin-embedded (FFPE) tissue biopsies. Antigen retrieval and de-paraffinisation of sections was performed in pH9 Target Retrieval Solution (Dako, United Kingdom) using a PT link. Sections were incubated for 1 hour at room temperature with anti-RET antibody (clone EPR2871, ab134100, Abcam, United Kingdom) diluted 1:20 in Antibody

Diluent (Dako, United Kingdom). A further 1 hour incubation followed with Envision™ anti-rabbit HRP secondary antibody (Dako, United Kingdom) before visualisation with DAB chromogen. All slides were stained simultaneously to account for batch variation, alongside healthy kidney tissue sections for use as controls.

Sections containing tumour cells were scored independently by two investigators, including a specialist histopathologist (FC). Both were blind to the patient data. Where there was disagreement a consensus was reached. Scores were given on a scale of 0-3 based on intensity of staining. In cases where heterogeneous staining was observed, the lowest score was given for that patient.

Statistical Analysis

Study design and sample size calculations were carried out with reference to similar trials performed in the same type of patient group (4, 18, 19). From these, it was estimated that a survival rate of 51% at 6 months would be observed for the control group. It was estimated that with the addition of vandetanib an increase in 6 month survival rate to 67%, an absolute improvement of 16% corresponding to a hazard ratio of 0·6 would be considered sufficiently clinically relevant to warrant progression to phase III studies. Using a one-sided α level of 0·1, a total of 100 deaths were required to obtain 90% Power resulting in a total sample size of 120 patients (60 in each treatment group). The study design incorporated a single interim analysis to assess futility after 50 deaths had been observed. The inclusion of this analysis reduced the overall type I error rate from 0·1 to 0·096 for assessment of the primary endpoint at the point of final analysis. During the course of the study, the decision to extend recruitment to recruit 140 patients was made to account for patient drop-out and ensure sufficient quantities of good quality translational materials were collected. The impact was to extend the target number of deaths to 109 and to increase the trial power to 91·8%.

Overall survival was measured as the time from randomisation until death by any cause. Patients still alive at the point of final analysis were censored at the date last seen alive. Progression free survival was

measured as the time from randomisation until disease progression. Patients alive and without progression at the point of final analysis were censored at the date last seen alive. Survival estimates were obtained using the Kaplan-Meier (20) method and compared across treatment groups using a stratified log-rank test (21). The effect of treatment allocation is expressed as a hazard ratio (gemcitabine plus vandetanib versus gemcitabine plus placebo) with an associated 95% confidence interval. Secondary analysis was carried out by adjusting the treatment effect using multivariable regression techniques based on Cox proportional hazards models (22). Stratification factors and treatment effects are included in all models. Univariate factors with a log rank significance of P<0·25 are considered for inclusion. A forward step-wise regression approach is used with terms included based on Akaike Information Criteria (AIC) (23). The use of translational factors as possible indicator of treatment efficacy were assessed via Cox proportional hazards models including a treatment effect as an interaction term with each translational factor.

Treatment administration was reported as the median (range) of cycles of each drug received. The proportion of grade 3-4 toxicity was compared across treatments. The number of patients observing high grade events across each AE was compared using a X^2 test.

All statistical analyses were carried out using the statistical package R (version 3.2). All analyses were carried out on an intention to treat principle, retaining patients in their randomised groups irrespective of any protocol violations. A one-sided P-value of 0·096 (with 80·8% CI) was considered significant for the analysis of the primary endpoint and assessment of the treatment effect. For all other analyses a nominal two sided P < 0·05 was used (with 95% CI).

The trial was assessed at regular intervals by an Independent Data and Safety Monitoring Committee (IDSMC), which was responsible for assessing the trial in terms of safety and efficacy. The IDSMC was un-blinded to treatment allocation throughout the full course of the trial. The trial was registered with the UKCRN (2007-004299-38).

Role of the Funding Source

ViP was an investigator-initiated (non-commercial) trial, and no direct payments were available to cover the costs associated with patient recruitment, treatment administration, follow-up visits, data collection or travel expenses. The trial was part of the Cancer Research UK and AstraZeneca collaboration portfolio. As part of this collaboration there was unrestricted funding to support the trial from AstraZeneca to the Cancer Research UK Liverpool CTU, University of Liverpool (Chief Investigator for funding purposes: Professor John P Neoptolemos). The trial was endorsed by Cancer Research UK, consequently with endorsement from the National Cancer Research Network (NCRN, UK) and UK Clinical Research Network (UKCRN). The funder of the study had no role in the study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

The final protocol (v.6) and dates of amendments are available on-line at:

https://www.lctu.org.uk/public/

RESULTS

A total of 381 patients were screened for the trial of which 142 were randomised between the 24th October 2011 and 7th October 2013. Two further patients were recruited beyond the recruitment target as they had already returned written informed consent at the point at which randomisation was complete. One patient was lost-to-follow up in the study and one patient withdrew consent. Follow-up data were included up to 15th July 2015 when the final database was locked for analysis with 131 deaths (target 109 deaths) after a median follow up of 24·9 (24·3, NA) months. The CONSORT diagram is shown in Figure 1.

Seventy patients were randomised to the gemcitabine plus placebo arm and 72 were randomised to the gemcitabine plus vandetanib arm. The baseline characteristics of all randomised patients are shown in Table 1. Forty-one patients (29%) had locally advanced disease and 101 (71%) had metastatic disease. The median age was 67 and 58% of patients were female; 11% of participants had an ECOG performance status

of 2. Baseline characteristics were mostly well balanced between arms, including baseline CA19·9 and CRP levels but there were significantly more patients in the vandetanib and gemcitabine arm with body tumours and more patients who were current and ex-smokers.

One-hundred and thirty-one patients had died at the time of analysis, 61 (87%) in the placebo group and 70 (97%) in the vandetanib group. There was no difference in overall survival between the treatment groups (Figure 2a). The median (95% CI) overall survival was 8·95 (6·55-11·7) months and 8·83 (7·11-11·6) months for the gemcitabine-placebo and gemcitabine-vandetanib arms respectively (hazard ratio = 1·21, 80.8% CI = 0·95, 1·53; log rank test (X^2_{1df}) = 1·1 , P = 0·303). Six and 12 month median (95% CI) survival estimates were 62% (52% - 75%) and 34% (25% - 48%) for the gemcitabine-placebo arm and 61% (51% - 74%) and 36% (27% - 49%) for the gemcitabine-vandetanib arm respectively. There was no evidence of a differential effect for the addition of vandetanib to gemcitabine by stage of disease. In patients with locally advanced disease the median survival was 10·9 (0·16 - 20·5) months in the gemcitabine plus placebo arm and 12·1 (9·97 - 16·1) months in the gemcitabine plus vandetanib patients arm [HR: 1·13 (0·59 - 2·19), P-value: 0·713]. In metastatic patients, the median survival was 7·20 (4·74 - 11·9) months in the gemcitabine plus placebo arm and 7·11 (4·21 - 11·2) in the gemcitabine plus vandetanib arm [HR: 1·13 (0·59 - 2·19); P = 0·713]

The results of univariable analysis of baseline characteristics as survival factors are shown in Table 2. In multivariable analysis ECOG performance status, tumour histology and CA19·9 and CRP levels were independent prognostic factors (Table 3). Following adjustment, the treatment effect changes little (HR = 1·32; 95% CI = 0·91, 1·89, P=0·14).

Table 4 shows the results of the prognostic and predictive power of the levels of CA19·9 and CRP, the SNPs in RET (p.G691S), VEGFR1 (rs9582036), VEGF (rs699947) and IL8 (rs4073) and the results of immunohistochemistry scoring superimposed on ECOG performance status and histology. Continuous data were dichotomised at their observed median for illustration but all Cox models retain them as

continuous covariables. Seventy-six (62·5%; Hardy-Weinberg = 63·8%) of 122 patients were germ line common homozygous (GG) for the RET p.G691S SNP, 43 (35·2%; Hardy-Weinberg = 32·2%) were heterozygous (GA) and three (2·5%; Hardy-Weinberg = 4·0%) were rare homozygous (AA). There was sufficient tissue from 66 (46·5%) patients for accurate RET expression immunohistochemistry scoring of which 40 (60·6%) had positive scores (examples are shown in Figure 5). Core biopsies were mostly homogeneous producing a single score but in eighteen cases there was a discrepancy in the scores. We present here the results using the lowest score to be representative which showed no association with survival in either treatment arm (Table 4). A sensitivity analysis showed that taking the highest score to be representative in the 18 cases with heterogeneous scores did not significantly affect the results of the first analysis.

One-hundred and thirty-six patients had progressed or died at the time of analysis, 65 (93%) in the gemcitabine-placebo group and 71 (99%) in the gemcitabine-vandetanib group. The median (95% CI) progression-free survival estimates were 6·09 (5-9.9) months and 8·04 (·454-10·3) months for the gemcitabine-placebo and gemcitabine-vandetanib arms, respectively (Figure 2b; stratified log-rank test =1·11, 95% CI = 0·88-1·41, P = 0·554).

Nineteen patients achieved an objective radiological response, nine (13%) on the gemcitabine-placebo arm and 10 (14%) in the gemcitabine-vandetanib arm (odds ratio = 1·06, 95% CI = 0·39-2·88, P =0·916) and the disease control rates were 75/142 (53%) and 82/142 58% respectively (odds ratio =1·419, 95% CI = 0·507 - 3·972, P= 0·505).

There were no treatment related deaths in either arm. Selected Grade 3-4 adverse events are presented in Table 5. Due to the small sample size and the low occurrence rates, P values were not that meaningful for many of the adverse events and so have been omitted. Neutropenia was more common in patients receiving vandetanib (p = 0·055), as was prolonged QT interval (p = 0·028) and rash (p=0·058). There was an increased incidence of grade 3 chronic kidney disease (a reduction of estimated glomerular filtration

rate to 15 – 29 mls/min) in five (7%) of patients receiving gemcitabine and vandetanib compared to none in patients receiving gemcitabine plus placebo. Grade 2 chronic kidney disease (a reduction of estimated glomerular filtration rate to 30 – 50 mls/min) was seen in three (5%) patients in the gemcitabine plus placebo arm and 14 (20%) in the gemcitabine and vandetanib arm. There was no significant difference in grade 3-4 diarrhoea. Grade $\geq$2 rash was more prevalent in patients treated with vandetanib; 19% (14/72) on the gemcitabine plus vandetanib versus 6 % (4/70) on the gemcitabine plus placebo arm (P = 0·021). The median (IQR) time to the development of grade $\geq$2 rash was 131 (88, 195) days. Exploratory analysis showed that patients developing a rash on the combination of gemcitabine plus vandetanib had improved survival compared with those patients receiving gemcitabine plus vandetanib and not developing a rash and compared with those patients receiving gemcitabine alone. The median survival for the 14 patients receiving vandetanib and developing grade $\geq$2 rash was 11·92 (10·89 – NA) months, for the 58 patients receiving vandetanib and not developing grade $\geq$2 rash this was 7·76 (4·34 – 11·5) months and for the patients receiving gemcitabine plus placebo this was 8·95 (6·55 – 11·7) months (log rank X^2_{2df} = 7·23; P= 0·027). Median progression-free survival was also greater in patients receiving vandetanib and developing a rash $\geq$2 of 11·15 (10·2 – 21·12) months, compared to those without a grade $\geq$2 rash of 4·85 (3·78 – 8·98) months and 6·09 (5·0 – 9·9) months for those on gemcitabine plus placebo. With gemcitabine plus placebo as the reference group the hazard ratio for overall survival for the gemcitabine plus vandetanib without development of rash $\geq$2 was 1·41 (0·98 - 2·04; P = 0·065) and for gemcitabine plus vandetanib with the development of a rash $\geq$2, this was 0·655 (0·35 - 1·22; P = 0·181. The test for interaction for survival by treatment arm and rash was significant (P = 0·0162). Landmark analysis was carried out after excluding patients who died within 3 months and gave hazard ratios of 1.48 (0.974, 2.238) for gemcitabine plus vandetanib without development of rash $\geq$2 and 0.79 (0.419, 1.49) for gemcitabine plus vandetanib with the development of a rash >2.

The median (IQR) number of gemcitabine administrations was 12 (7, 21) in patients receiving gemcitabine and placebo and 13 (7, 22) in those receiving gemcitabine plus vandetanib. The percentage (95% CI) of gemcitabine delivered at full protocol dose was 70% (50·6 - 85·9) in patients receiving gemcitabine and placebo and 63·2% (51·9 -73·7) in those receiving gemcitabine plus vandetanib.

DISCUSSION

This trial showed that the addition of vandetanib to gemcitabine therapy did not improve the overall survival of patients with advanced pancreatic cancer when compared with gemcitabine alone. The combination of gemcitabine and vandetanib was generally well tolerated. Although grade 3-4 neutropenia was increased this did not translate to greater febrile neutropenia and the increased rash and QT prolongation in patients receiving vandetanib are already known side effects. This study included patients with both locally-advanced disease as well as metastatic patients as this was a signal-seeking randomised phase II study and patients were stratified at study entry for stage of disease and there was no mechanistic reason why there should be a differential response based on stage.

In vitro studies suggest that the p.G691S RET polymorphism may distinguish pancreatic cancer cells that proliferate, migrate and invade neural tissue under the influence of GDNF, from those that do not (10). There was no evidence however in the current study, that patients who were germ line heterozygous for the p.G691S RET polymorphism obtained any incremental clinical benefit from the addition of vandetanib. Furthermore, there was no evidence that tissue expression of RET was a predictive biomarker for the addition of vandetanib. None of the VEGF pathway SNPs investigated in the current study showed any association with vandetanib outcomes. Previously it had been shown that the rs9582036 SNP was the only one of 138 VEGF pathway SNPs to be associated with survival in patients with pancreatic cancer receiving bevacizumab in combination with gemcitabine and erlotinib but not in the control arm (24). This SNP was in high linkage disequilibrium with a SNP that enhanced VEGFR1 mRNA translation and VEGFR1 expression. The VEGF SNP rs699947 was selected based on its association with survival in patients treated with

paclitaxel/bevacizumab and not in those with paclitaxel alone in the randomized E2100 trial in breast cancer (25). This SNP has also been associated with improved outcome in patients with pancreatic cancer treated with chemoradiation plus sorafenib (26). Finally, the IL8 polymorphism rs4073 was previously shown to be associated with overall survival in renal cancer patients treated with pazopanib and sunitinib (27) and also associated with response to ifosfamide and bevacizumab in ovarian cancer patients (28).

Exploratory analysis showed that the development of a rash typical of that seen in patients treated with other EGFR inhibitor was predictive of both overall and progression free survival. Patients on vandetanib with gemcitabine who developed a rash had a doubling of survival compared with those not developing a rash on vandetanib and those patients receiving placebo with gemcitabine. This adds to the existing evidence for the development of rash as a surrogate biomarker of biological activity in patients receiving EGFR tyrosine kinase inhibitors in pancreatic cancer (4, 5).

Vandetanib combines anti-RET activity together with VEGFR2 and EGFR inhibition. The benefit of EGFFR blockade and of additional VEGF inhibition in pancreatic cancer is modest but we hypothesised that the addition of RET inhibition to dual EGFR/VEGFR2 blockade would provide further therapeutic benefit. In spite of the pre-clinical data suggesting that RET could be a valid target in pancreatic cancer we found no evidence of a RET-based predictive biomarker. Furthermore inhibition of neural invasion may be less important once the primary has already locally invaded peri-neural tissue and/or the cancer has already metastasized. Whilst, the development of rash with agents targeting EGFR appears to be highly predictive of an improved outcome, attempts to dose to rash has so far not enhanced response in pancreatic cancer (29). Further research may be directed towards tyrosine kinase inhibitors in pancreatic cancer identifying biomarkers that could predict the development of a rash to vandetanib and also other tyrosine kinase inhibitors in pancreatic cancer.

PANEL: RESEARCH IN CONTEXT

Evidence Before This Study

We searched PubMed for all randomised trials in advanced pancreatic cancer. We also searched PubMed for any experimental work pertaining to RET in pancreatic cancer and for all clinical trials in which vandetanib, a RET, VEGFR2 and EGFR inhibitor, or any other RET inhibitors had been used in any cancer. Our review showed that the outcome of patients with pancreatic cancer remains extremely poor with median survivals even with the most aggressive regimens being under a year. For many patients gemcitabine monotherapy remains standard of care because of their inability to tolerate the more aggressive regimens. Thus, well-tolerated combinations with gemcitabine are urgently needed. RET is the receptor for the GDNF family of ligands. Neural invasion via a GDNF gradient is a prominent feature of pancreatic cancer causing pain and mediating the spread of the disease. Pancreatic cancer cells which carry the p.G691S polymorphism migrate more rapidly towards GDNF. Pre-clinical models have shown synergistic cytotoxicity for the gemcitabine/vandetanib combination in pancreatic cancer and reduced metastasis formation compared with gemcitabine alone in mouse models. A phase III trial of vandetanib in patients with advanced medullary thyroid cancer which over-expresses RET showed a survival benefit compared with placebo (30). Hence, we performed a randomised phase II trial of gemcitabine with or without vandetanib in advanced pancreatic cancer.

Added value of this study

Tissue acquisition for biomarker discovery was a crucial component of the trial and we were able to perform a comprehensive evaluation of the predictive and prognostic power of the p.G691S polymorphism along with a number of SNPs that have been associated with responses to agents targeting the VEGF pathway. We also analysed the predictive impact of RET immunostaining.

Implication of all the available evidence

There was no evidence that the addition of vandetanib to gemcitabine improved outcome compared to gemcitabine alone in patients with advanced pancreatic cancer. There was also no evidence of any sub-group effect with respect to RET or VEGF-related biomarkers. Further exploration of RET inhibition in advanced pancreatic cancer would not appear to be warranted. Whether further investigation of RET inhibition in earlier stage disease before significant invasion and migration occurs remains unknown. Since vandetanib is a multikinase inhibitor it is possible that as yet unidentified subgroups may benefit from its addition to gemcitabine. The development of rash typical of that seen in patients treated with other EGFR inhibitors were predictive of both overall and progression free survival in patients on vandetanib with gemcitabine. Further studies to investigate potential predictive biomarkers are required.

AUTHOR CONTRIBUTIONS

The Academic Chief Investigator Professor Gary Middleton and the principal grant holder Professor John P Neoptolemos had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

All of the authors gave final approval of the version to be published. Development of the study design was supported and conducted through National Cancer Research Institute (NCRI) of the UK Pancreatic Cancer Sub-Group and the Cancer Research UK Liverpool Cancer Trials Unit (of the Liverpool Clinical Trials Unit) and was led by the ViP working party comprising G Middleton, J Neoptolemos, D Cunningham, J Valle, J Wadsley, D Propper, F Coxon, P Ross, S Madhusudan, and P Corrie. Richard Jackson was responsible for detailed statistical analysis. Drs W Greenhalf, Anthony Evans, E Costello and John P Neoptolemos were responsible for the translational aspects of the trial. Charlotte L. Rawcliffe was the senior trial coordinator responsible for central administration ensuring ethical standards for collection and verification of data. The results were interpreted by the ViP working party, which prepared the initial draft and were responsible for collating changes proposed by all of the authors into the final draft paper before final approval by all participants ViP Study Group.

The Independent Data and Safety Monitoring Committee

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Conflicts Of Interest

Professor Gary Middleton has provided consultancy to, and received research funding from, KAEL-GemVax Co.

Professor John Neoptolemos was an NIHR Senior Investigator and was part funded by the NIHR Biomedical Research Centre at the Royal Liverpool University, Liverpool, UK; received funding for the trial from AstraZeneca (to Cancer Research UK Liverpool CTU) in the form of an unrestricted educational grant in support of the ViP trial; consultancy provided to Boehringer Ingelheim Pharma GmbH & Co. KG, Novartis Pharma AG, KAEL GemVax and Astellas, has received payment for lectures from Amgen and Mylan an educational travel grants from NUCANA and other research grants from Taiho Pharma (Japan), KAEL GemVax (Korea), AstraZeneca, Clovis Oncology and Ventana and Pharma Nord during the course of the trial.

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Professor Juan Valle has previously received honoraria and research funding from Lilly Oncology

Dr. Jonathan Wadsley receives honoraria from Roche; Merck Serono, Cellgene, Bayer, Sanofi Aventis, Novartis, Bristol Myers Squibb and SIRTex and research funding from Merck Serono and Sanofi Aventis.

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31. FIGURES AND TABLES

Figure 1: Consort Diagram.

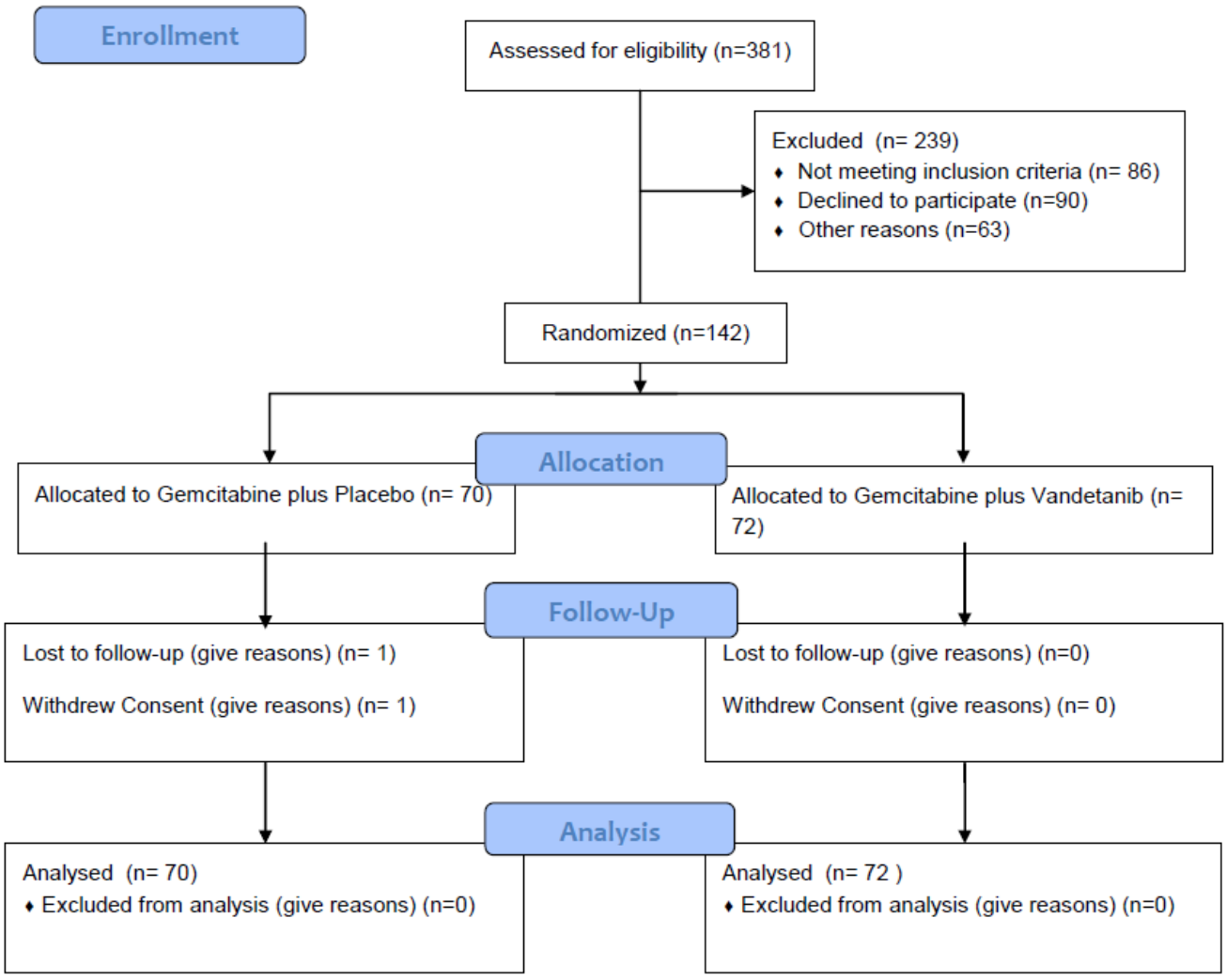
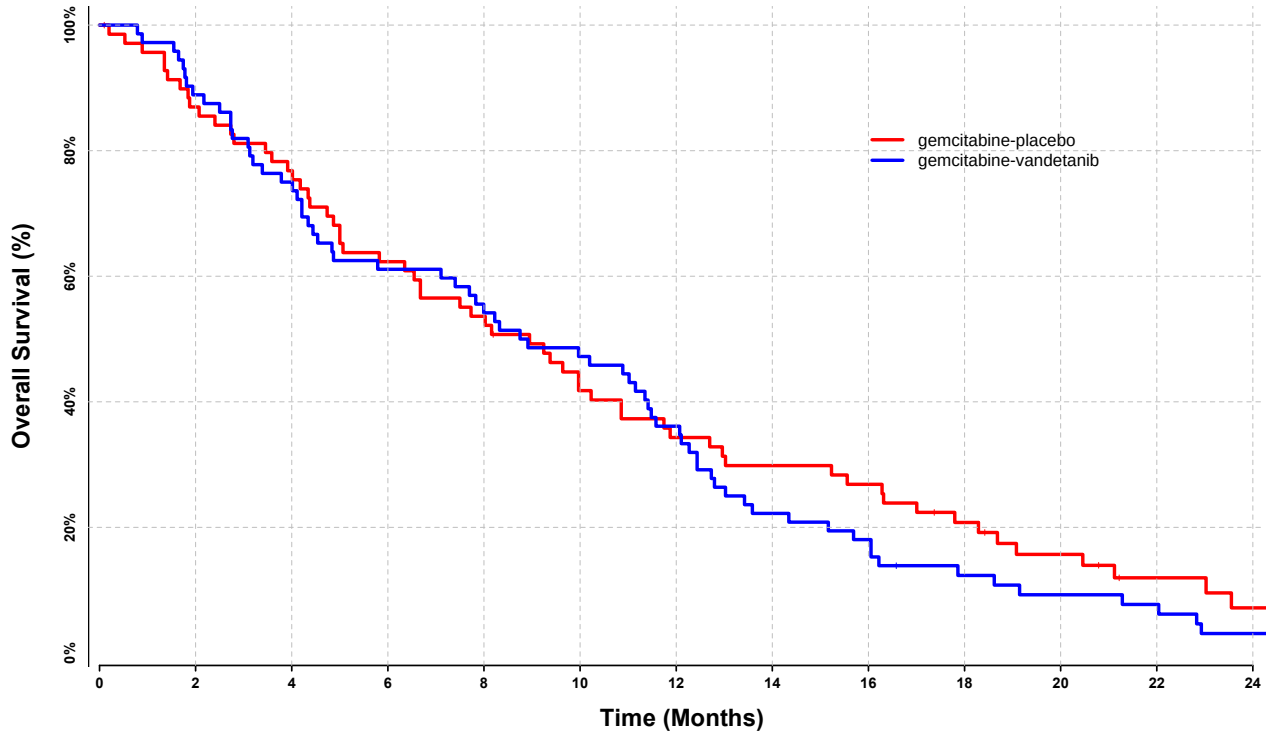
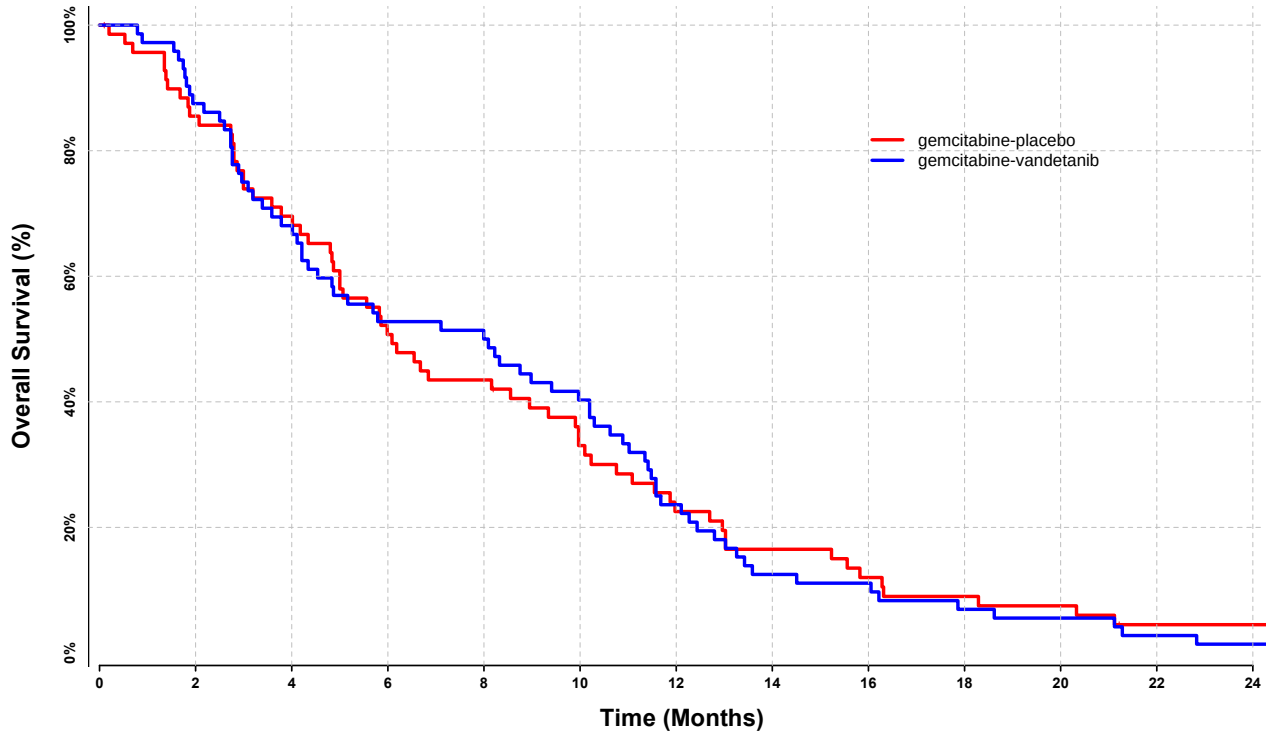


Figure 2: (a) Overall survival and estimates by treatment arm. (b) Progression free survival estimates by treatment arm.



Numbers at risk

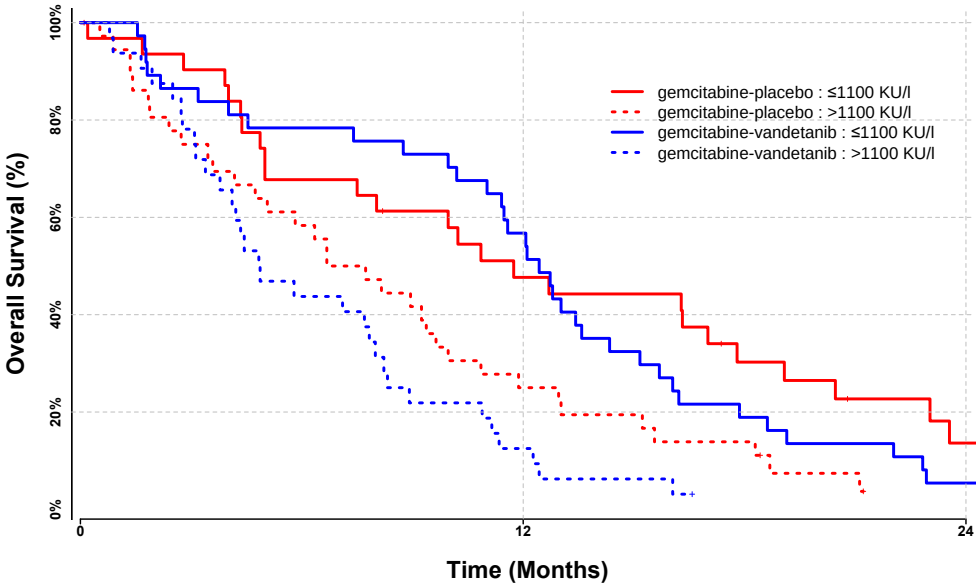
gemcitabine-placebo	70	60	53	43	37	28	23	20	18	13	9	5	3
gemcitabine-vandetanib	72	64	54	44	39	34	26	16	13	8	6	5	2



Numbers at risk

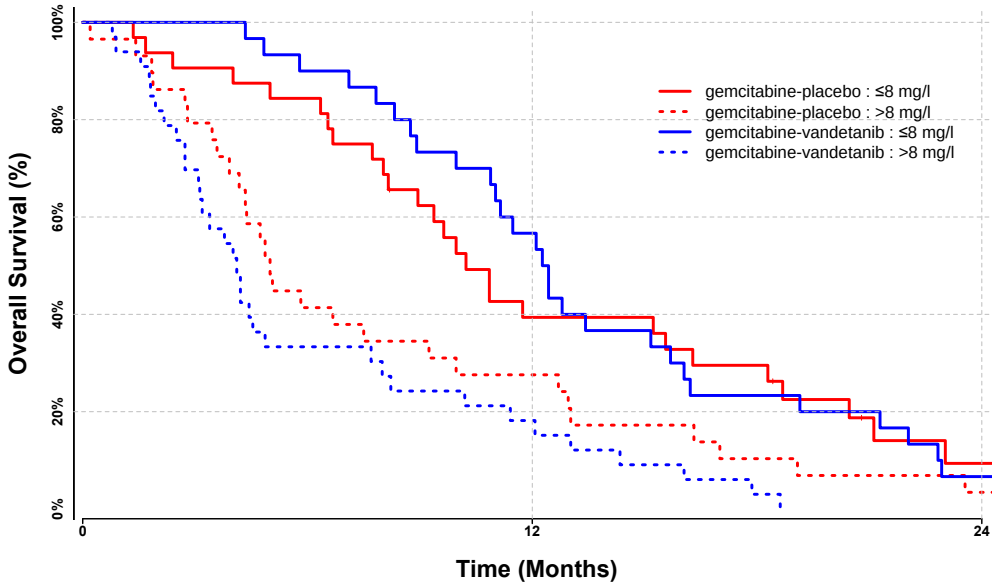
gemcitabine-placebo	70	59	48	35	30	22	15	11	8	6	5	2	2
gemcitabine-vandetanib	72	63	49	38	36	29	17	9	8	5	4	2	1

Figure 3: (a) Overall survival by CA19.9 levels and treatment arm. (b) Overall survival by CRP levels and treatment arm.



Numbers at risk

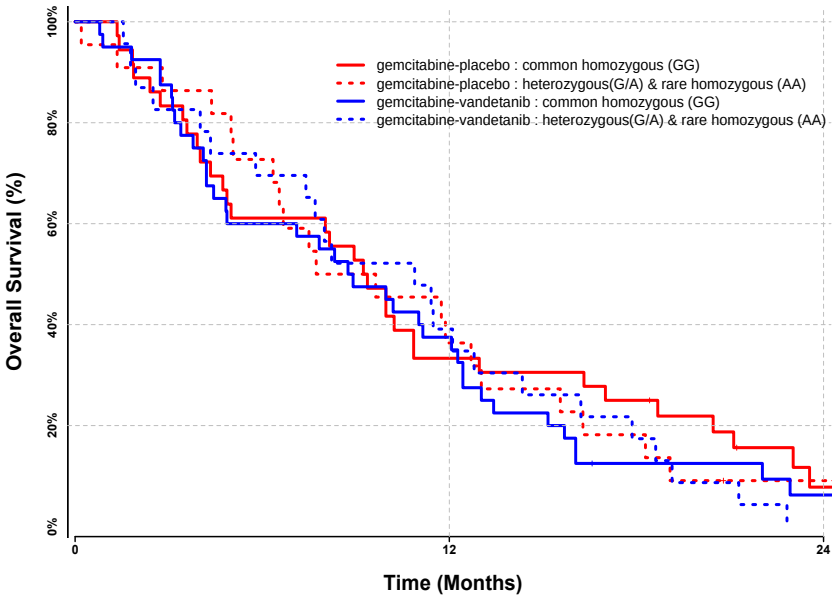
gemcitabine-placebo : ≤1100 KU/l	32	14	3
gemcitabine-placebo : >1100 KU/l	36	9	0
gemcitabine-vandetanib : ≤1100 KU/l	37	21	2
gemcitabine-vandetanib : >1100 KU/l	32	4	0



Numbers at risk

gemcitabine-placebo : ≤8 mg/l	32	12	2
gemcitabine-placebo : >8 mg/l	29	8	1
gemcitabine-vandetanib : ≤8 mg/l	30	17	2
gemcitabine-vandetanib : >8 mg/l	33	6	0

Figure 4: Overall survival by RET polymorphism and treatment arm.



Numbers at risk

gemcitabine-placebo : common homozygous (GG)	36	12	2
gemcitabine-placebo : heterozygous(G/A) & rare homozygous (AA)	22	8	1
gemcitabine-vandetanib : common homozygous (GG)	40	15	2
gemcitabine-vandetanib : heterozygous(G/A) & rare homozygous (AA)	23	9	0

Figure 5: Examples of RET staining by immunohistochemistry in pancreas tissue biopsies. Scale bar = 50µm.

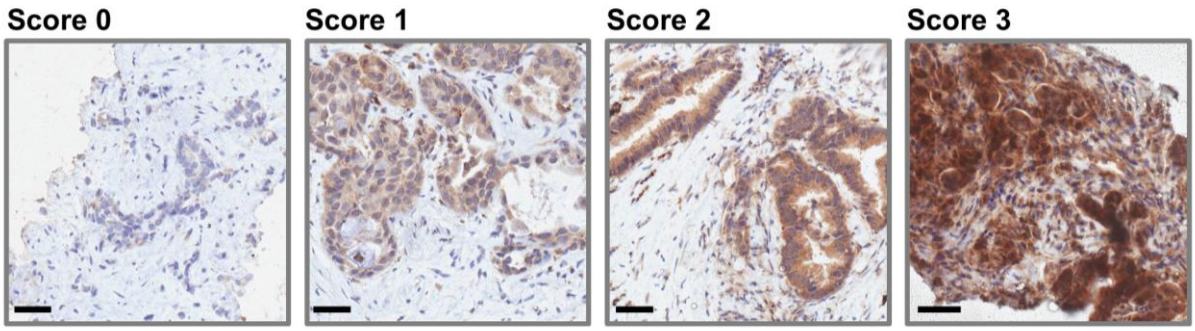


Figure 6. Overall survival by development of rash, grade ≥2.

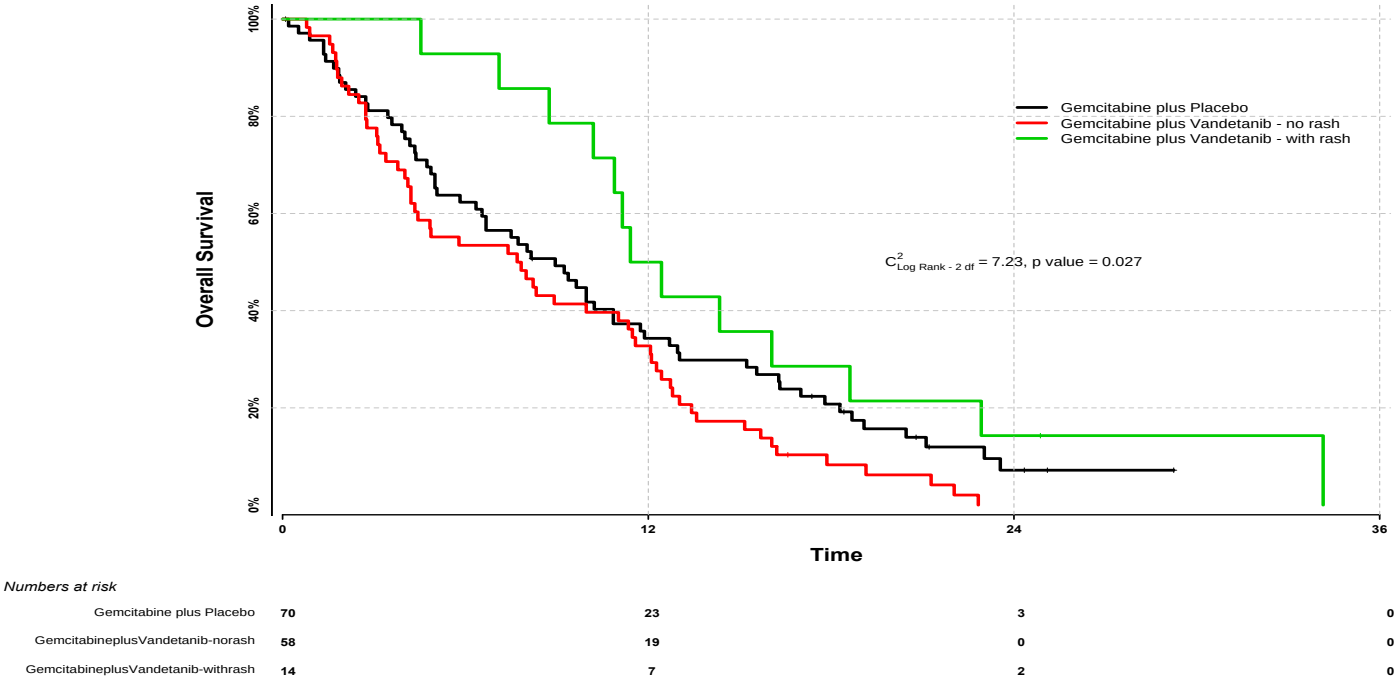


Table 1: Baseline characteristics of the intention to treat population

Clinical Characteristic	**Gemcitabine plus Placebo (n=70)**	**Gemcitabine plus Vandetanib (n=72)**	**TOTAL**	**P-value**
Gender, number (%)				
Male	30 (43%)	29 (40%)	59 (42%)	0·888
Female	40 (57%)	43 (60%)	83 (58%)	
Age, median (inter-quartile range) years	67·5 (61, 73)	66·5 (61, 73)	67 (61, 73)	0·558
Disease Stage, number (%)				
Locally advanced	20 (29%)	21 (29%)	41 (29%)	
Metastatic	50 (71%)	51 (71%)	101 (71%)	1
ECOG Performance Status, number (%)				
0	19 (27%)	21 (29%)	40 (28%)	0·965
1	43 (61%)	43 (60%)	86 (61%)	
2	8 (11%)	8 (11%)	16 (11%)	
Tumour Histology, number (%)				
Pancreatic ductal adenocarcinoma	62 (89%)	66 (92%)	128 (90%)	
Undifferentiated carcinoma of the pancreas	8 (11%)	6 (8%)	14 (10%)	0·736
Tumour Site, number (%)				
Head	47 (67%)	31 (43%)	78 (55%)	
Uncinate	5 (7%)	4 (6%)	9 (6%)	0·017
Body	13 (19%)	24 (33%)	37 (26%)	
Tail	5 (7%)	13 (18%)	18 (13%)	
Tumour Differentiation, number (%)				
Well	7 (10%)	6 (8%)	13 (9%)	0·6701
Moderate	12 (17%)	16 (22%)	28 (20%)	
Poor	15 (21%)	16 (12%)	30 (22%)	
Unknown	29 (41%)	30 (42%)	59 (42%)	
Cannot be assessed	7 (10%)	4 (6%)	11 (8%)	
Smoking Status, number (%)				
Current Smoker	10 (15%)	19 (28%)	29 (21%)	0·026
Ex-Smoker	23 (34%)	30 (43%)	53 (39%)	
Never Smoked	34 (51%)	20 (29%)	54 (40%)	
CA19-9*, median (inter-quartile range) mg/l	1259·5 (264·75,6080·25)	1018 (199, 6104)	1100 (223, 6104)	0·659
CRP*, median (inter-quartile range) mg/l	8 (0, 23)	10 (0, 46)	8.5 (0, 28·5)	0·175

Table 2: Univariable analysis of prognostic variables. * Hazard ratios obtained by modelling continuous data on the log transformed scale **Prognostic Variable**	**Number of patients (Deaths)**	**Median overall survival**	**Hazard ratio (95% confidence interval)**	**P**
Arm				
Gemcitabine plus placebo	70 (61)	8·95 (6·55-11·7)		
Gemcitabine plus vandetanib	72 (70)	8·83 (7·11, 11·6)	1·17 (0·83, 1·65)	0·376
Gender				
Female	59 (55)	8·.32 (5·.82, 11·4)		
Male	83 (76)	9·38 (6·68, 11·9)	0·94 (0·67, 1·34)	0·744
Age				
<65 years	60 (53)	10·08 (4·87, 12·70)		
>65 years	82 (78)	8·75 (6·68, 11·0)	1·01 (0·99, 1·03)	0·546
Disease Stage				
Locally advanced	41 (37)	11·53 (9·97, 15·16)		
Metastatic	101 (94)	7·11 (4·74, 9·97)	1·42 (0·97, 2·08)	**0·071**
ECOG Performance Status				
0	40 (36)	11·58 (8·16, 15·2)		
1	86 (79)	8·91 (7·40, 11·3)	1·32 (0·88, 1·97)	
2	16 (16)	4·59 (3·91, 10·2)	2·46 (1·34, 4·51)	**0·012**
Tumour Histology				
Pancreatic ductal adenocarcinoma	128 (117)	8·95 (7·70, 11·5)		
Undifferentiated carcinoma of the pancreas	14 (14)	6·23 (3·39, 11·6)	2·06 (1·17, 3·64)	**0·013**
Tumour Site				
Body	37 (36)	8·22 (5·82, 12·3)		
Head	78 (70)	9·97 (7·73, 11·6)	0·84 (0·56, 1·26)	
Tail	18 (16)	9·67 (3·59,NA)	0·81 (0·45, 1·46)	
Uncinate	9 (9)	5·00 (2·4, NA)	1·33 (0·64, 2·77)	0·52
Tumour Differentiation				
Well	13 (11)	13·59 (12·43, NA)		
Moderate	28 (24)	10·69 (8·22, 19·14)	1·47 (0·70, 3·07)	
Poor	31 (29)	6·25 (4·38, 11·58)	2·47 (1·20, 5·08)	
Unknown	59 (57)	7·11 (4·54, 9·97)	2·490 (1·22, 4·72)	
Cannot be assessed	11 (10)	10·86 (8·03, NA)	1·37 (0·57, 3·31)	**0·018**
Smoking Status				
Never	29 (27)	11.02 (7.83, 13.6)		
Past	53 (51)	9.61 (7.11, 13.4)	0.96 (0.60, 1.53)	
Present	54 (47)	8.32 (5.07, 11.2)	0.98 (0.61, 1.58)	**0.983**
CA19-9, KU/I				
< 1100	69 (61)	12·1 (10·86, 15·16)		
> 1100	63 (60)	7·11 (4·84, 8·95)	1·14 (1·05, 1·24)*	**0·001***
CRP, mg/l				

Table 2: Univariable analysis of prognostic variables. * Hazard ratios obtained by modelling continuous data on the log transformed scale **Prognostic Variable**	**Number of patients (Deaths)**	**Median overall survival**	**Hazard ratio (95% confidence interval)**	**P**
< 8	62 (56)	11·48 (9·.97, 15·23)		
> 8	62 (61)	4·41 (3·.01, 6·68)	1·46 (1·26, 1·69)*	**<0·001***

Table 3: Multivariable analysis by overall survival. *CRP and *CA19.9 included on the log transformed scale

Factor	**HR (95% CI**	**P-value**
ECOG Performance Status		
Fully Active		
Ambulatory (Work Able)	1·44 (0·94, 2·19)	0·091
Ambulatory (Not Work Able)	2·29 (1·17, 4·48)	**0·003**
Tumour Histology, number (%)		
Pancreatic ductal adenocarcinoma		
Undifferentiated carcinoma of the pancreas	1·88 (1·04, 3·41)	**0·038**
CA19.9 levels*	1·15 (1·06, 1·24)	**0.001**
CRP levels*	1·42 (1·21, 1·66)	**<0·001**
Arm, number (%)		
Gemcitabine plus placebo		
Gemcitabine plus vandetanib	1·32 (0·91, 1·89)	**0·14**

Table 4: Overall survival by prognostic translational factors and the SNPs in RET (rs1799939 and p.G691S), VEGFR1 (rs9582036), VEGF rs699947) and IL8 (rs4073). *P-value denotes interaction effect.

Prognostic Variable	Number of patients (Deaths)	Median overall survival (months)	Hazard ratio	P	Gemcitabine plus placebo: Number of patients (Deaths)	Median overall survival (months)	Hazard ratio	P	Gemcitabine plus vandetanib: Number of patients (Deaths)	Median overall survival (months)	Hazard ratio	P	Interaction: Hazard ratio	P*
CA19-9 KU/I*														
<1100	69 (61)	12·11 (10·86, 15·16)			32 (25)	11·70 (7·50, 19·08)			37 (36)	12·43 (11·41, 15·16)			1·51 (0·87, 2·60)	
>1100	68 (65)	6·51 (4·44, 8·32)	1·15 (1·06, 1·24)	**0·001**	36 (34)	7·20 (4·74, 9·97)	1·19 (1·05, 1·34)	0·005	32 (31)	4·85 (4·11, 8·22)	1·10 (0·98, 1·23)	0·105	1·70 (0·99, 2·93)	0·318
CRP														
< 8	62 (56)	11·48 (9·97, 15·23)			32 (27)	10·20 (8·95, 18·29)			30 (29)	12·35 (11·02, 16·05)			1·21 (0·68, 2·15)	
> 8	62 (61)	4·41 (4·01, 6·68)	1·43 (1·.22, 1·67)	**<0·001**	29 (28)	5·00 (4·34, 9·97)	1·37 (1·03, 1·81)	0·028	33 (33)	4·11 (3·12, 7·99)	1·51 (1·23, 1·86)	<0·001	1·09 (0·59, 1·99)	0·635
RET p·G691S														
Major Allele	76 (70)	9·10 (7·11, 11·00)			36 (32)	9·31 (4·87, 13·00)			40 (38)	8·83 (4·84, 12·40)			1·20 (0·73, 1·97)	
Heterozygous (G/A)	45 (43)	9·64 (7·40, 12·80)	1·15 (0·77, 1·73)	0·49	22 (20)	8·68 (6·55, 15·60)	1·16 (0·63, 2·13)	0·635	23 (23)	10·89 (7·40, 16·20)	1·18 (0·66, 2·10)	0·583	1·30 (0·66, 2·59)	0·933
IL-8 rs4073														
AA	28 (25)	8·37 (4·21, 15·60)			10 (8)	5·13 (2·80, NA)			18 (17)	9·36 (4·44, 16·1)			0·97 (0·40, 2·39)	
TT/AT	100 (94)	9·64 (8·03, 11·50)	1·06 (0·67, 1·70)	0·8	50 (45)	9·.38 (7·50, 12·70)	0·82 (0·38, 1·79)	0·613	50 (49)	10·54 (7·40, 12·40)	1·30 (0·70, 2·42)	0·411	1·50 (0·97, 2·33)	0·389
VEGFA rs699947														
AA	32 (31)	8·85 (7·50, 12·10)			11 (10)	8·95 (6·35, NA)			21 (21)	8·75 (5·79, 12·70)			0·95 (0·43, 2·13)	
AC/CC	96 (88)	9·64 (7·70, 11·90)	0·68 (0·44, 1·05)	0·083	49 (43)	9·38 (6·55, 13·00)	0·62 (0·30, 1·28)	0·194	47 (45)	10·20 (7·11, 13·00)	0·76 (0·43, 1·33)	0·331	1·31 (0·83, 2·04)	0·44
VEGF (FLT1) rs9582036														
AA	69 (66)	8·32 (6·68, 11·00)			30 (27)	8·55 (6·35, 13·00)			39 (39)	8·32 (4·87, 12·10)			1·64 (0·94, 2·84)	
AC/CC	59 (53)	10·86 (7·73, 12·70)	1·03 (0·69, 1·52)	0·901	30 (26)	10·23 (5·00, 16·30)	1·51 (0·82, 2·80)	0·187	29 (27)	11·41 (8·22, 14·30)	0·67 (0·39, 1·15)	0·147	0·88 (0·50, 1·55)	0·093
Imunohisto-chemistry Score														
0	26 (25)	7·30 (4·74, 12·30)			10 (9)	6·28 (4·74, NA)			16 (16)	7·40 (4·11, 14·3)			0·87 (0·33, 2·29)	
1	28 (26)	5·77 (4·21, 10·20)	1·47 (0·79,	0·224	14 (13)	8·34 (4·38, 19·1)	1·31 (0·49,	0·573	14 (13)	4·44 (4·01, 15·2)	1·51 (0·60, 3·82)	0·385	1·32 (0·52, 3·37)	

			2·75)				3·62)						
2/3	12 (11)	11·92 (7·83, NA)	0·85 (0·40, 1·80)	0·669	7 (6)	13·03 (1·84, NA)	0·60 (0·18, 2·00)	0·402	5 (5)	8·32 (7·83, NA)	1·13 (0·36, 3·58)	0·837	4·11 (0·59, 28·78)	0·770

Table 5: Summary of Adverse Events

Toxicity	Grade 3/4 adverse events	
	Gemcitabine plus Placebo	Gemcitabine plus Vandetanib
GGT increased	32 (46%)	29 (40%)
Neutrophil count decreased	22 (31%)	35 (49%)
Platelet count decreased	16 (23%)	20 (28%)
Fatigue	15 (21%)	17 (24%)
White blood cell decreased	13 (19%)	12 (17%)
Hypertension	11 (16%)	9 (12%)
ALT increased	11 (16%)	8 (11%)
Hyponatremia	8 (11%)	10 (14%)
ALP increased	10 (14%)	8 (11%)
Lethargy	7 (10%)	9 (12%)
Lymphocyte count decreased	6 (9%)	9 (12%)
Diarrhea	4 (6%)	7 (10%)
Blood bilirubin increased	3 (4%)	4 (6%)
Abdominal Pain	5 (7%)	2 (3%)
Anorexia	4 (6%)	3 (4%)
Prolonged QT interval	0 (0%)	6 (8%)
AST	2 (3%)	4 (6%)
Anaemia	3 (4%)	3 (4%)
Dyspnoea	2 (3%)	3 (4%)
Chronic kidney disease	0 (0%)	5 (7%)
Rash maculo-papular	0 (0%)	5 (7%)
Investigations - Other	1 (1%)	4 (6%)
Nausea	2 (3%)	3 (4%)
Ascites	1 (1%)	3 (4%)
Vomiting	3 (4%)	1 (1%)
Hyperglycemia	1 (1%)	2 (3%)
Abdominal pain	0 (0%)	3 (4%)
Hypokalemia	2 (3%)	1 (1%)
Hypomagnesemia	1 (1%)	2 (3%)
Hypoalbuminemia	2 (3%)	1 (1%)
Hypoxia	1 (1%)	1 (1%)
Syncope	1 (1%)	1 (1%)
Renal and urinary disorders	1 (1%)	1 (1%)
Hypotension	0 (0%)	2 (3%)
Thromboembolic event	1 (1%)	1 (1%)
Pain	0 (0%)	2 (3%)
Dizziness	0 (0%)	2 (3%)
Leukocytosis	0 (0%)	2 (3%)
Oedema limbs	1 (1%)	1 (1%)
Papulopustular rash	0 (0%)	2 (3%)

Toxicity	Grade 3/4 adverse events	
	Gemcitabine plus Placebo	Gemcitabine plus Vandetanib
Creatinine increased	1 (1%)	1 (1%)
Hyperthyroidism	1 (1%)	0 (0%)
Blurred vision	1 (1%)	0 (0%)
Vertigo	1 (1%)	0 (0%)
Colitis	1 (1%)	0 (0%)
Obstruction gastric	1 (1%)	0 (0%)
Bile duct stenosis	1 (1%)	0 (0%)
Surgical and medical procedures	1 (1%)	0 (0%)
Left ventricular systolic dysfunction	0 (0%)	1 (1%)
Encephalopathy	0 (0%)	1 (1%)
Pulmonary edema	0 (0%)	1 (1%)
Purpura	0 (0%)	1 (1%)
Respiratory, thoracic and medical diseases	0 (0%)	1 (1%)
Dyspepsia	0 (0%)	1 (1%)
Pleural effusion	0 (0%)	1 (1%)
Bronchial infection	1 (1%)	0 (0%)
Pain in extremity	1 (1%)	0 (0%)
Skin ulceration	0 (0%)	1 (1%)
Gastrointestinal disorders Ð Other	0 (0%)	1 (1%)
Biliary tract infection	0 (0%)	1 (1%)
Depression	1 (1%)	0 (0%)
Infections and infestations - Other	0 (0%)	1 (1%)
Cough	1 (1%)	0 (0%)
Fever	0 (0%)	1 (1%)
Skin - other	0 (0%)	1 (1%)
Back pain	0 (0%)	1 (1%)
Constipation	0 (0%)	1 (1%)
Hyperkalemia	0 (0%)	0 (0%)
Mucositis oral	0 (0%)	0 (0%)
Hypocalcemia	0 (0%)	0 (0%)