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Phase II Trial of Cetuximab and Conformal Radiotherapy Only in Locally Advanced Pancreatic Cancer with Concurrent Tissue Sampling Feasibility Study[1]

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Abstract

BACKGROUND: Preclinical data have indicated the anti–epidermal growth factor receptor (EGFR) agent cetuximab (Erbitux) as a radiosensitizer in pancreatic cancer, but this has not been specifically addressed in a clinical study. We report the results of an original study initiated in 2007, where cetuximab was tested with radiotherapy (RT) alone in locally advanced pancreatic cancer in a phase II trial (PACER). *METHODS:* Patients (n = 21) received cetuximab loading dose (400 mg/m^2) and weekly dose (250 mg/m^2) during RT (50.4 Gy in 28 fractions). Toxicity and disease response end point data were prospectively assessed. A feasibility study of on-trial patient blood and skin sampling was incorporated. *RESULTS:* Treatment was well tolerated, and toxicity was low; most patients (71%) experienced acute toxicities of grade 2 or less. Six months posttreatment, stable local disease was achieved in 90% of evaluable patients, but only 33% were free from metastatic progression. Median overall survival was 7.5 months, and actuarial survival was 33% at 1 year and 11% at 3 years, reflecting swift metastatic progression in some patients but good long-term control of localized disease in others. High-grade acneiform rash (P = .0027), posttreatment stable disease (P = .0059), and pretreatment cancer antigen 19.9 (CA19.9) level (P = .0042) associated with extended survival. Patient skin and blood samples yielded sufficient RNA and good quality protein, respectively. *CONCLUSIONS:* The results indicate that cetuximab inhibits EGFR-mediated radioresistance to achieve excellent local control with minimal toxicity but does not sufficiently control metastatic progression in all patients. Translational studies of patient tissue samples may yield molecular information that may enable individual treatment response prediction.

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Introduction

Unresectable, locally advanced pancreatic cancer (LAPC) is a devastating disease with high morbidity. Median survival in randomized trials remains low at only 7 to 14 months [1,2]. The main goals of treatment are to balance palliation of symptoms and prolong life while minimizing side effects. There is no consensus on the optimal treatment regimen, and no specific treatment is recommended for LAPC in the United Kingdom [3]. Current treatment approaches may include best supportive care, radiotherapy (RT) only, single or multiagent chemotherapy, chemotherapy followed by chemoradiotherapy (CRT), or immediate concurrent CRT. Only sufficient inhibition of the local tumor in the absence of metastatic progression (downstaging) such that resection is possible allows prolonged survival in a minority of patients.

Although metastatic spread is a dominant concern, which has led to systemic chemotherapy as the cornerstone of treatment, control of local disease is important to prevent pain, gastrointestinal (GI) obstruction, ulceration, bleeding, cholangitis, and ultimately death due to local disease advance alone [4]. Modern RT has important roles in controlling local disease: to prevent disease symptoms, increase the possibility of resection, and extend survival [5–9]. However, when combined with chemotherapeutics to maximize disease control, the effectiveness of treatment is limited by the radiation doses that can be given safely, due to the risk of toxicity in surrounding radiosensitive abdominal structures and the toxicity of the chemotherapy. Alternative molecular targeted agents that enhance the RT response with minimal toxicity and permit RT dose escalation are therefore of interest. Conformal RT alone has low toxicity and reasonable survival outcome compared with other standard treatment approaches [10], providing scope to test such agents independently.

Epidermal growth factor receptor (EGFR) overexpression and oncogenic mutation of the downstream K-RAS signaling protein are common in pancreatic cancers and have been associated with increased tumor aggressiveness and poor survival [11–17]. Furthermore, recent experimental data have shown interdependence of EGFR signaling and oncogenic K-RAS in pancreatic oncogenesis [18,19]. EGFR-mediated radioresistance has been evidenced by EGFR activation and stimulation of DNA repair by ionizing radiation [20,21], and the ability of anti-EGFR agents to enhance radiation in pancreatic cancer has been demonstrated in animal studies [22,23]. Cetuximab (Erbitux®; Merck Serono Ltd, Feltham, UK) is an EGFR-specific chimeric IgG1 monoclonal antibody that inhibits EGFR-mediated signal transduction [24] and radiation-induced DNA repair [25]. In animal models of pancreatic cancer, cetuximab has been shown to improve the treatment efficacy of gemcitabine and radiation (gemcitabine + RT) [23,26,27]. An independent radiosensitizing effect of cetuximab has been shown in a phase III head and neck cancer trial, where cetuximab + RT conferred a survival advantage compared with RT alone [28]. However, although phase I/II studies of cetuximab in combination with CRT approaches in localized pancreatic cancer have shown some encouraging results [29–33], the inclusion of systemic chemotherapeutics has confounded interpretation of the contribution of cetuximab. As a result, it has remained unclear whether cetuximab acts as an effective independent radiosensitizer or if EGFR-mediated radioresistance plays a role in treatment efficacy in patients with pancreatic cancer. To specifically address these clinical science questions, a trial was initiated to evaluate cetuximab and conformal RT (cetuximab + RT) only for the first time in patients with LAPC. The aim of the pancreatic cancer cetuximab and radiotherapy (PACER) trial was to assess efficacy and safety of the regimen with long-term follow-up. During the trial, a translational substudy (PACER-TRANS) was instigated to investigate the feasibility of on-trial patient blood and skin sampling for future biomarker analysis.

Methods

Study

This study was a single-arm, multicenter, phase II, prospective national clinical trial (NCT00599833). It was supported by Cancer Research UK (CRUK), Elekta Oncology, and Merck KGA (Darmstadt, Germany), facilitated by the National Institute for Health Research Cancer Research Network, approved by the Greater Manchester Research Ethics Committee (06/Q1407/157), and authorized by the Medicines and Healthcare products Regulatory Agency (EudraCT 2006-001742-13). Hospital research and development approval was obtained from the coordinating center (The Christie NHS Foundation Trust) and other participating centers (Leeds Teaching Hospitals NHS Trust and Betsi Cadwaladr University Health Board). The study was additionally approved by the United Kingdom National Cancer Research Institute as part of the upper GI studies group portfolio.

The trial was cautiously designed in 2006 with support from the European Cancer Organisation Flims clinical trial design workshop, using published evidence available at that time. A key concern was the possibility of rapid disease progression, because of the lack of cytotoxic chemotherapy agent and potential for the regimen to have low activity. To minimize risk, the primary clinical end point selected was progression-free survival (PFS) 6 months after the start of treatment, and a Simon "Minimax" two-stage trial model was used to minimize sample size and false rejection error [34]. This approach allowed interim analysis to terminate the trial early if necessary. The efficacy of the cetuximab + RT treatment was compared with that of patients with LAPC who received other CRT regimens at The Christie Hospital (Manchester, United Kingdom), using 6-month PFS data determined by preceding audit. The specifications of the trial were $\alpha = 0.05$, power = 0.8, $p0 = 0.25$, and $p1 = 0.45$, where $p0$ and $p1$ are the 6-month PFS rate under the null and alternative hypotheses; i.e., if PFS < 25%, accept the null hypothesis that the regimen is less effective than other CRT treatments and terminate trial early; if 25% < PFS < 45%, conclude that the regimen is no better or worse than other current treatments; and if PFS > 45%, accept the alternative hypothesis that the regimen has greater efficacy than other CRT treatments. An independent data monitoring committee advised initial recruitment of 21 patients to ensure at least 17 eligible patient data sets for primary outcome assessment on interim analysis (assuming dropout rate of 20%). If PFS and toxicity were considered acceptable, recruitment could then continue up to 44 patients to ensure at least 36 evaluable patients in total. RT dose escalation to 54 Gy in 30 fractions could also be considered. Secondary end points of the trial were acute toxicity profile, tumor and overall disease posttreatment Response Evaluation Criteria in Solid Tumors (RECIST) response, freedom from local progression (FFLP), and overall survival (OS) up to 3 years posttreatment. Amendments made to the trial after recruitment was initiated included incorporating additional centers to enhance recruitment and establishing a feasibility translational substudy (PACER-TRANS) of patient blood and skin sampling for subsequent protein and RNA analysis.

Patients

All patients had biopsy-confirmed inoperable LAPC. The inability to resect patients was determined by multidisciplinary team decision (radiologist, surgeon, and treating oncologist) as due to tumor-related factors before surgical approach or following invasive diagnostic laparoscopy or attempted surgical approach. All patients gave written informed consent and underwent an eligibility screening examination. This included full blood count, biochemistry profile, plasma cancer antigen 19.9 (CA19.9), isotope renogram and baseline abdominal computed tomography (CT) scan, and chest X-ray/thoracic CT scans taken within 4 weeks of starting treatment. Inclusion criteria included Karnofsky performance status (KPS) $\geq$ 60 plus adequate hematological, hepatic, and renal functions, including hemoglobin (Hb) $\geq$ 10 g/dl, white blood cells (WBC) $\geq 3.0 \times 10^9$/l, absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l, bilirubin $\leq$ 1.5 × upper limit of normal (ULN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq$ 1.5 × ULN, alkaline phosphotase (ALP) $\leq$ 4 × ULN, creatinine $\leq$ 1.5 × ULN, and creatinine clearance > 50 ml/min. Adequate biliary drainage with no evidence of active uncontrolled infection was required. Permitted prior treatments were noncurative operation (e.g., palliative bypass procedure or R2 resection with macroscopic, residual, localized disease evident on CT scan) and/or biliary stent insertion. Exclusion criteria included neuroendocrine histology or pancreatic lymphomas, prior malignancy, chemotherapy, or any previous EGFR-targeted therapy, or extensive disease unable to be covered in a radically treatable RT volume.

Treatment

Patients were treated in specialist United Kingdom cancer centers with modern RT units—The Christie Hospital, St James' Institute of Oncology (Leeds, United Kingdom), and the North Wales Cancer Treatment Centre (Rhyl, United Kingdom). A quality assurance program was implemented to ensure a high level of accuracy, and consistency was achieved in RT planning and delivery between centers. This included a clear RT protocol, dummy run, and central individual case monitoring and review [35].

RT-planning CT scans were acquired with 50-mm slice thickness in the treatment (supine) position after oral and IV contrast. The *clinical target volume* was defined as the primary pancreatic tumor plus CT-defined involved lymph nodes only. To ensure target coverage with motion, anterior-posterior and lateral margins of 17 mm and a superior-inferior margin of 35 mm were applied [36] to produce the planning target volume (PTV). The maximum PTV allowable was 1000 ml, and the liver, spinal cord, and kidneys were considered as organs at risk: maximum allowable radiation dose to the spinal cord was 38 Gy, no more than 50% of the total liver volume was planned to receive more than 30 Gy (V_{30} < 50%), and no more than 30% and 50% of the contralateral and ipsilateral kidney, respectively, were planned to receive more than 20 Gy (V_{20} < 30% and 50%). Treatment was planned isocentrically with the PTV encompassed within the 95% isodose. A three- or four-field multicollimation technique was used without tissue density heterogeneity correction. Patients received 50.4 Gy to the 100% isodose in 28 fractions Monday to Friday for 5 1/2 weeks, starting in week 2. Daily patient positioning was verified using tattoos and orthogonal laser beams; treatment verification was performed daily using portal imaging/cone beam CT for the first three fractions and weekly thereafter, with a maximum variation tolerance of 5 mm.

During week 1, an initial loading dose (400 mg/m^2) of cetuximab was given intravenously (IV) over a 1 hour period, preceded by IV chlorphenamine (10 mg). Patients were monitored closely for signs of cetuximab sensitivity, and infusion was extended to 2 hours if necessary. During weeks 2 to 7, cetuximab (250 mg/m^2) was administered once per week for 1 to 2 hours, again preceded by chlorphenamine (10 mg) 2 to 4 hours before RT.

Patient Assessment

Baseline tumor assessment was either from diagnostic CT scans (if taken within 4 weeks of starting treatment) or from RT-planning CT scans. During treatment, clinical, hematological, and biochemical assessments (including CA19.9 and magnesium levels) were undertaken weekly. Acute toxicity was scored using the European Organisation for the Research and Treatment of Cancer (EORTC) Common Toxicity Criteria for Adverse Events version 3 [37]. A diagnostic contrast-enhanced CT scan was planned 26 weeks from the start of treatment. Scans were brought forward for patients with clinical signs of progression, and alternative treatment was offered according to standard departmental protocol. For the purposes of the study, RECIST criteria only were used to define primary tumor response and disease progression [38]. Sites of primary disease progression were determined by date of RECIST progression and defined by radiologic description, documented clinical evidence, and (where appropriate) postmortem report. Patients were followed up on a 3-monthly basis for the first year. Thereafter, follow-up was continued on a 6-monthly basis, for a minimum of 3 years or until death. At each follow-up visit, patients underwent clinical, hematological, and biochemical assessment, with CT scans undertaken when clinically indicated.

Blood Samples and Skin Biopsies

Twenty milliliters of blood samples was taken before treatment, weekly during treatment, and at the first follow-up, at the same time as routine sampling for trial toxicity analyses. Blood plasma was separated by centrifugation and stored at −20°C for subsequent proteomic analysis.

Three-millimeter skin punch biopsies were taken following intradermal local anesthetic. Basal fat was removed, and the skin tissue was placed into sterile RNA*later* (Life Technologies Corporation, Carlsbad, CA). Mechanical disaggregation and homogenization were carried out in TRIzol (Life Technologies Corporation). Phenol/chloroform extraction and a PureLink centrifugation column (Life Technologies Corporation) were used for RNA purification. Samples were analyzed for quantity, integrity, and purity using an Agilent 2100 Bioanalyzer microfluidics platform (Agilent Technologies Inc, Santa Clara, CA) and a NanoDrop light spectrophotometer (Thermo Fisher Scientific Inc, Waltham, MA).

Statistics

Statistical analyses were performed using Stata version 9.2 (StataCorp, College Station, Texas). FFLP, PFS, and OS data were analyzed using Kaplan-Meier methodology. Survival times were calculated from the first day of treatment to the date of objectively confirmed local disease progression for FFLP, to the date of progression/death due to disease progression for PFS, and to the date last seen or date of death for OS. The log-rank test statistic was used to determine difference in survival probability according to degree of acneiform rash and posttreatment disease status. Cox proportional hazards model or the χ^2 test was used

as appropriate to determine the influence of pretreatment factors (CA19.9, KPS, and Hb levels) on PFS, OS, and posttreatment RECIST disease status.

Results

Patients

Twenty-one eligible patients were recruited to the first stage of the trial between October 2007 and March 2010. Slow accrual was experienced due to high eligibility attrition (~80%). The main reasons were lack of confirmative biopsy; disease too advanced to be encompassed within RT field; patient's informed refusal; poor KPS or inadequate hematological, hepatic, or renal function; or detection of metastatic disease on staging/planning CT scan. As a result of slow patient accrual, funding constraints, and dissolution of the Manchester research department, patient recruitment was stopped at the end of the first stage of the trial.

Patient and tumor characteristics are summarized in Table 1. Patient toxicity and skin biopsy data are given in Tables 2 and 3, respectively. Three patients underwent prior surgery: one a diagnostic laparoscopy, one a failed trial dissection, and one a duodenal and biliary bypass. All patients had T3/4 N0/1 disease (stage II/III) of at least 3 cm in the longest dimension. No patients had "borderline resectable" disease, showed symptoms of neuroendocrine disease, could/would not undergo surgery, nor were lost to follow-up. Comprehensive individual patient data, including toxicity and outcome information, are given in Table 4.

RT Plan Characteristics

Twenty of 21 (95%) patients received IV and oral contrast for their RT-planning CT scan (contrast omitted in one patient due to temporarily raised creatinine). Median PTV volume was 392.4 cm^3 (range = 212.2-712.3). Median liver volume receiving ≥30 Gy was 12.0% (0%-50%), and median maximal dose to the spinal cord was 20.4 Gy (range = 8.7-36.9 Gy). Median volumes of the left and right kidneys receiving ≥20 Gy were 1.4% (range = 0%-28.2%) and 2.0% (range = 0%-28%), respectively.

Table 1. Patient and Tumor Characteristics.

Patient and Tumor Characteristics	*n* (%)
Gender	
Male	13 (62%)
Female	8 (38%)
KPS	
70-80	5 (24%)
90-100	16 (76%)
Tumor location	
Head	17 (81%)
Body	3 (14%)
Head and body	1 (5%)
Histology	
Adenocarcinoma	19 (90%)
Not specified	2 (10%)
Prior treatment	
Biliary stent	15 (72%)
Other surgery	3 (14%)
CA19.9	
Below ULN: <30 U/ml	6 (29%)
Raised: >30 U/ml (ULN)	15 (71%)
Raised: Median (range; U/ml)	511 (136-26328)
Hb	
Normal	15 (71%)
Anemia (Hb < 12/14 g/dl)	6 (29%)
Age	
Median (range)	65 (47-80) yr
Weight	
Median (range)	65.5 (46.3-100.2) kg

Table 2. Incidence of Acute Toxicity ≥ Grade 2.

Toxicity	Number of Patients (*n* = 21)		
	Grade 2	Grade 3	Grade 4
Skin			
Acneiform rash	11	1	-
Pruritis/itching	1	-	-
Rash/desquamation	3	-	-
Dry skin	3	-	-
GI			
Anorexia	6	1	-
Abdominal bloating	4	-	-
Diarrhea	-	1	-
Nausea	3	-	-
Vomiting	-	1	-
Enteritis	1	-	-
Gastritis	1	-	-
Oral mucositis	2	-	-
Bleeding	-	1	-
Other			
Fatigue	6	2*	-
Pain	5	2*	-
Anemia	2		
Hyperbilirubinemia	1	-	-
Thrombocytopenia	1	-	-
Pericarditis	-	1*	-
Hyperglycemia	-	1	-
Infection	-	-	1*

*Pericarditis with associated fatigue in one patient and liver abscess with associated abdominal pain in another patient were reported as SAEs but judged unlikely or not related to treatment.

Treatment Compliance and Toxicity

All 21 patients tolerated cetuximab infusion well. Seventeen patients (81%) received the full planned course of cetuximab, and 20 (95%) received the full planned RT dose. Single cetuximab doses were omitted for three patients because of acute pericarditis, grade 3 acneiform rash, and liver abscess, respectively, all of which resolved before the next due dose. Doses were also omitted in another patient from week 4 due to study withdrawal. This followed clinical deterioration consistent with local and metastatic disease progression. The patient was changed to a palliative RT course such that they received a total dose of 28.8 Gy in 16 fractions.

Acute toxicity was low (≤grade 2) in most (71%) patients. The most common all-grade acute toxicities were acneiform rash (95%), fatigue (86%), nausea (67%), diarrhea and anorexia (both 62%), pruritus, desquamation, and dry skin (all 57%). Acute toxicities ≥ Grade 2 are summarized in Table 2. Eleven incidences of grade 3 and one grade 4 serious adverse events (SAEs) were reported in six patients during the 7 weeks of active treatment, although four SAEs occurring in two patients were considered unlikely to be related to treatment (pericarditis and associated fatigue in one patient; liver abscess and associated abdominal pain in another). Two grade 3 SAEs occurred after treatment was completed: one was hypomagnesemia and hypocalcemia at 8 weeks, which was considered definitely related to cetuximab treatment (known side effect); the other was GI bleeding at 14 weeks, which was possibly related to RT treatment.

Patient Follow-Up and Outcome Measures

Following treatment (and subsequent photodynamic therapy as part of a phase 1 trial), one patient had a Whipple procedure with complete excision of residual pancreatic adenocarcinoma, plus adjuvant chemotherapy. Four patients received palliative chemotherapy on disease progression, and one received additional chemotherapy when entering another trial (TeloVac). One patient, who developed early bony metastases, received palliative spinal RT. Posttreatment CT scans were brought forward for seven patients. As a result, the median time from start of treatment to posttreatment scan was 23.9 weeks. Three recruited patients were ineligible for progression/survival outcome on "per protocol" analysis. One was excluded because they failed to complete treatment (withdrawn), and two were retrospectively radiologically determined to have had metastatic spread before treatment, as lung lesions were detected on posttreatment chest CT, which were apparent but indeterminable on pretreatment abdominal CT/chest X-ray.

Of the remaining 18 assessable patients, 14 patients were evaluable for posttreatment primary target lesion response (Figure 1). Nonevaluability was because the primary target lesion was ill defined on CT, inhibiting definitive measurement (two patients), or the planned posttreatment CT scan was not performed due to patient condition/disease progression (two patients). Local control was high. Only one patient (P18) showed a tumor size increase consistent with RECIST local progression (>20%) and concurrent development of metastatic disease at 3.7 months. In the majority of remaining patients, the primary tumor either reduced in size (range = 2-38 mm) or stayed the same. Two patients (P19 and P21) showed tumor size decreases consistent with RECIST partial response (>30%).

Local control remained high posttreatment with 90% FFLP at 6 months and 1 year (Figure 2*A*). However, crude posttreatment (median = 23.9 weeks) overall RECIST response rates showed 12 of 17 (71%) patients with progressive disease (PD), and only 5 of 17 (29%) with stable disease (SD). The predominant initial site of progression was intraabdominal (liver, duodenum, stomach, and ascites), but a more widespread pattern of first progression was noted in patients with posttreatment PD: three had subcutaneous nodules in the umbilical area, and another had bone metastasis. Median PFS was 5.1 [95% confidence interval (CI) = 4.6-6.2] months (Figure 2*B*). Actuarial PFS was 33.3% (95% CI = 13.7-54.5) at 6 months and 22.2% (95% CI = 6.9-42.9) at 1 year. There was a trend for patients who developed grade 2 or more acneiform rash to have better PFS (Figure 2*C*; $P = .0651$).

Median OS was 7.5 (95% CI = 6.4-12.7) months (Figure 3*A*). Actuarial OS was 33% at 1 year and 11% at 2 and 3 years (two patients were alive and progression free at 3 years). Log-rank analysis showed that the degree of acneiform rash (Figure 3*B*; $P = .0027$) and

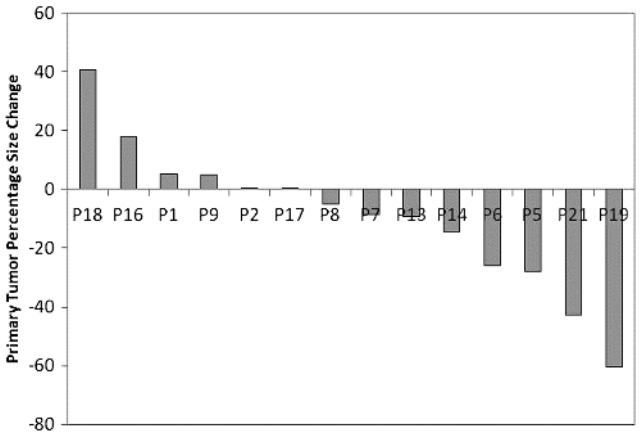

Figure 1. Waterfall plot of percentage of change in primary tumor longest dimension in 14 evaluable patients with LAPC following cetuximab + RT treatment (median = 23.9 weeks posttreatment). Tumor size was objectively assessed on CT. Individual patient data are given from worst to best response.

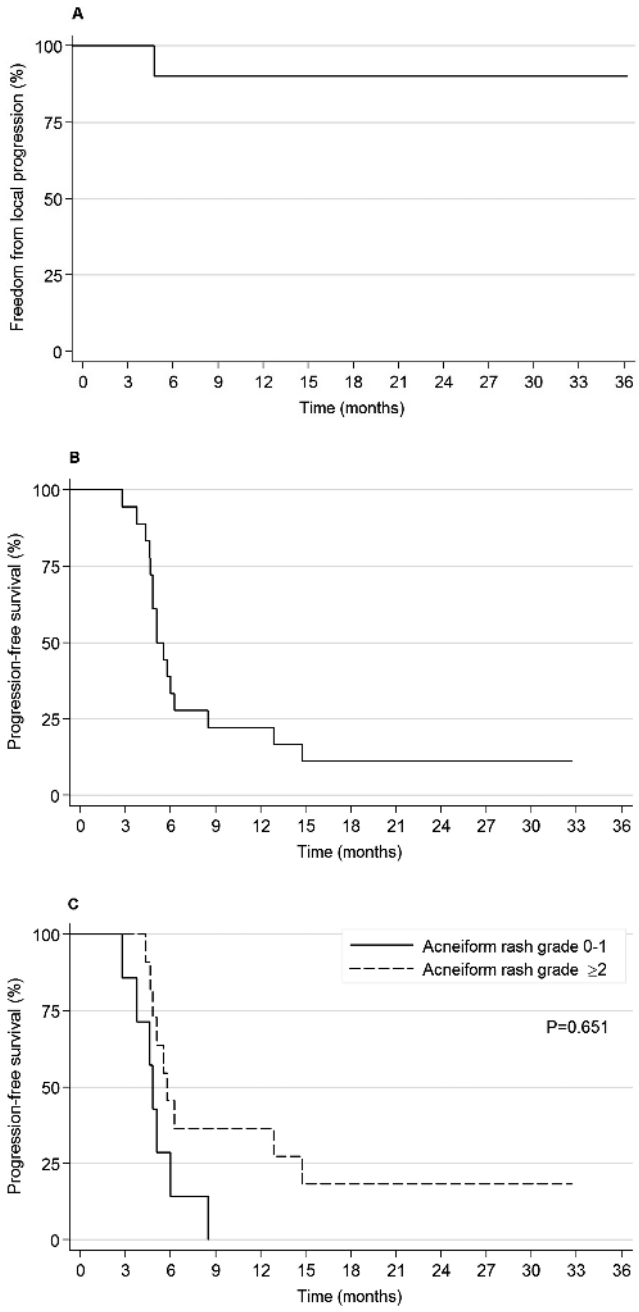

Figure 2. Kaplan-Meier disease progression analysis in 17 patients with LAPC following treatment with cetuximab + RT. (A) FFLP. (B) PFS. Median (95% CI) = 5.1 (4.6-6.2) months. (C) PFS and acneiform rash (log-rank $P = .651$).

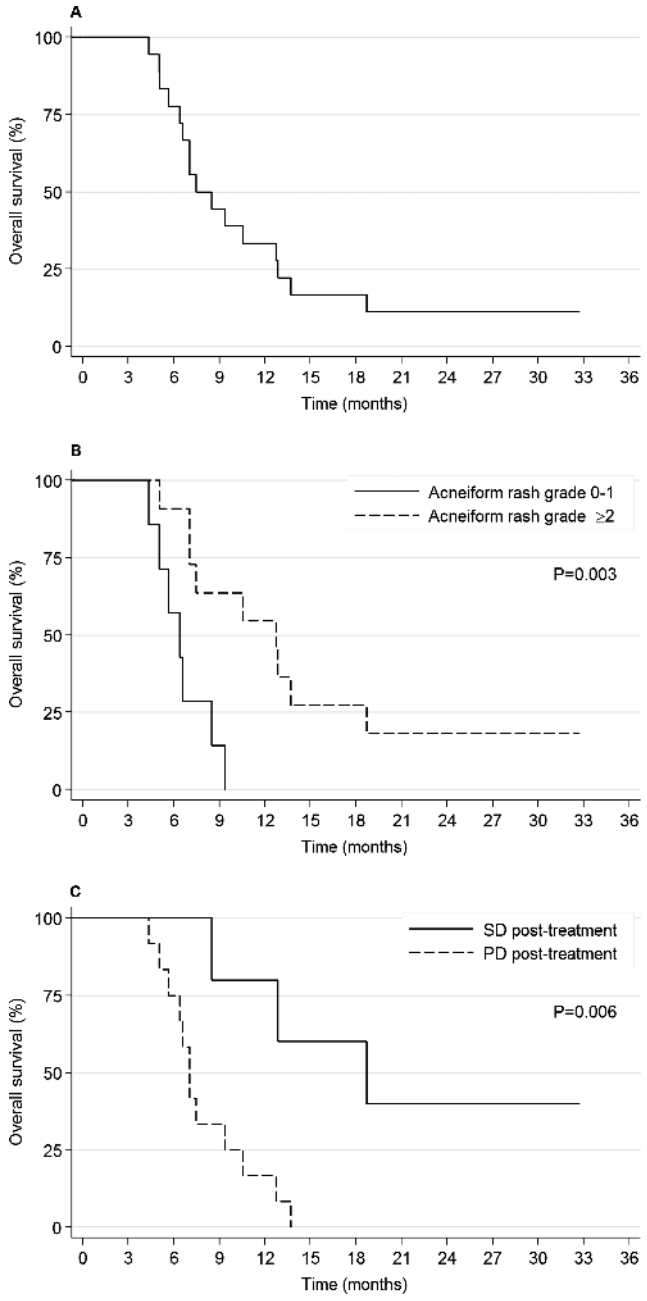

Figure 3. Kaplan-Meier disease survival analysis in 17 patients with LAPC following treatment with cetuximab + RT. (A) OS. Median (95% CI) = 7.5 (6.4-12.7) months. (B) OS and acneiform rash (log-rank $P = .0027$). (C) OS and posttreatment SD (log-rank $P = .0059$).

posttreatment RECIST SD status (Figure 3*C*; $P = .0059$) were prognostic for OS. Cox proportional hazards model showed that pretreatment CA19.9 level > 1000 U/ml was prognostic for reduced OS ($P = .042$). There were no statistical associations between pretreatment anemia and KPS with PFS, OS, or posttreatment SD.

Biopsy Substudy

Of the 10 patients approached, 5 consented to donate blood samples, and 2 consented to skin biopsies. Blood samples from three patients were processed and analyzed in a proteomics-based biomarker study [39]. RNA sample parameters from skin biopsies are shown in Table 3. Samples obtained varied in yield, purity, and integrity of obtained RNA.

Discussion

There is no consensus on the best treatment for LAPC, and patient outcomes remain poor. Improved biologic understanding of the disease and investigation of novel mechanism-based therapeutics are required to facilitate the development of more effective treatments with reduced toxicity. PACER is the first study to investigate cetuximab+RT alone in pancreatic cancer and indicates the efficacy of cetuximab as an EGFR-targeted radiosensitizer, consistent with preclinical studies. The results are important and relevant to current advances in pancreatic cancer as it provides benchmark data, in the absence of chemotherapy, for comparing and evaluating other RT + cetuximab–containing regimens. PACER was also the first trial of patient normal tissue sampling feasibility during an experimental treatment for pancreatic cancer. The key limitation of the study was the inability to continue to recruit in the second stage, which impacts on the study size and power.

Cetuximab was well tolerated; the prophylactic use of IV chlorphenamine is likely to have prevented the infusion reactions and other severe adverse events reported by others [29,40]. Only one (4.8%) patient experienced one short-term incidence of grade 3 acneiform rash. Toxicity was considerably lower compared with other CRT regimens, and no patient experienced any hematological toxicity > grade 2 [7,8,10,33,41]. Despite relatively large PTVs (median = 392.4 cm^3), only two patients (9.5%) suffered with any GI toxicity > grade 2. This may have been aided by limited-field RT, but unlike a previous study that also omitted uninvolved nodes, we found no statistical relationship between large PTV (>260 cm^3) and GI toxicity [8].

Excellent local control was achieved. Large decreases in tumor size were seen in some patients, and one patient (5.5%) subsequently underwent Whipple resection. Although rarely reported for chemotherapy regimens, local control was higher than the ~70% achieved in a trial arm of gemcitabine only [7], and no patients showed local

Table 3. RNA Yield and Purity from Skin Biopsies.

Sample	Tissue Weight (mg)	RNA Yield (ng/μl)	Absorption (260/280)	Absorption (260/230)	RIN
P4-IN* posttreatment	10	104.5	1.55	0.21	N/A
P4-OUT* posttreatment	11	46.8	2.07	2.15	8.4
P6-IN pretreatment	4.4	37.8	1.83	1.03	6.9
P6-OUT pretreatment	2.3	50.9	1.93	1.3	3.2
P6-IN posttreatment	22	103.8	1.94	1.34	8.1
P6-OUT posttreatment	40	155.7	1.97	1.53	7.9

260/280, ratio of sample absorption at wavelength 260 nm and at 280 nm; 260/230, ratio of sample absorption at wavelength 260 nm and at 230 nm; RIN, RNA integrity number.
*P4 and P6 donated skin samples from inside (IN) and outside (OUT) the RT field pretreatment and/or posttreatment.

Table 4. Individual Patient Data.

Patient	Treatment Complete?	PTV (cm^3)	Toxicity ≥ Grade 2	Posttreatment Response			Progression (RECIST)		Additional Therapy	Survival
				Primary Tumor		Overall	Time	Sites		
				Change*	RECIST	RECIST				
P1	Yes	506.2	Anorexia, nausea, fatigue, and tumor pain	+5.3%	SD	PD	6.0 mo	Liver, omentum, and ascites	None	6.6 mo
P2	Yes	456	Acneiform rash and desquamation	No change	SD	PD	4.3 mo	Skin	None	7.0 mo
P3	Yes	300.5	None	N/A	N/A	N/A	N/A	Lung	Palliative chemotherapy	26.8 mo
P4	Yes	346.5	None	N/A	N/A	PD	4.8 mo	Stomach and duodenum	None	6.4 mo
P5	Yes	440	Abdominal bloating and raised AP and γGT	−28.0%	SD	PD	2.8 mo	Lung, liver, omentum, and skin	Palliative chemotherapy	5.7 mo
P6	Yes	225.2	Acneiform rash and pruritis	−26.0%	SD	SD	46.8 mo	N/A	None	46.8 mo
P7	Yes	536	Acneiform rash, dry skin, and fatigue	−8.7%	SD	SD	12.8 mo	Liver	None	12.8 mo
P8	Yes	367	Acneiform rash, nausea, and fatigue	−5.0%	SD	PD	5.5 mo	Liver and skin	None	13.7 mo
P9	Yes	483.3	Acneiform rash, anorexia, fatigue, abdominal bloating, and hyperbilirubinemia	+5.0%	SD	PD	4.6 mo	Liver, peritoneum, omentum, and ascites	None	7.0 mo
P10	Yes	364.9	Acneiform rash, desquamation, anorexia, and tumor pain	N/A	N/A	N/A	5.1 mo	Liver	None	5.1 mo
P11	No (withdrawn from study)	333.8	Anorexia, GI bleeding, and anemia	N/A	N/A	N/A	N/A	Ascites	Palliative RT	2.0 mo
P12	Yes	712.3	Acneiform rash, anorexia, enteritis, fatigue, and tumor pain	N/A	N/A	N/A	N/A	Lungs	None	8.2 mo
P13	Yes	616.0	Acneiform rash, dry skin, vomiting, anorexia, nausea, fatigue, and tumor pain	−9.3%	SD	SD	-	(none at 36 mo)	Photodynamic therapy, Whipple resection, and adjuvant chemotherapy	Still alive at 36 mo
P14	Yes	260.7	Acneiform rash, oral mucositis,and abdominal bloating	−14.3%	SD	SD	14.8 mo	Liver	Palliative chemotherapy	18.7 mo
P15	Yes	212.2	None	N/A	N/A	PD	5.1 mo	Peritoneum	None	9.4 mo
P16	No (missed wk 3 cetuximab)	322	Acneiform rash, dry skin, fatigue, tumor pain, anemia, and pericarditis	+17.9%	SD	PD	6.2 mo	Liver and peritoneum	Palliative chemotherapy	10.5 mo
P17	No (missed wk 4 cetuximab)	511.7	Acneiform rash, tumor pain, and abdominal bloating	No change	SD	PD	5.8 mo	Liver	Palliative chemotherapy	12.7 mo
P18	Yes	381.7	None	+40.6%	PD	PD	3.7 mo	Local (liver)	None	4.3 mo
P19	No (missed wk 6 cetuximab)	673.9	Anorexia, fatigue, abdominal pain, thrombocytopenia, and liver abscess	−32.8%	PR	SD	8.5 mo	Locoregional (lungs)	Chemotherapy (TeloVac trial)	8.5 mo
P20	Yes	392.4	Acneform rash, diarrhea, and hyperglycemia	N/A	N/A	PD	4.8 mo	Bone	Palliative RT	7.5 mo
P21	Yes	633.1	Oral mucositis	−42.6%	PR	PD	4.6 mo	Liver, lung, peritoneum, and ascites	None	5.0 mo

AP indicates alkaline phosphatase; N/A, data not available or not applicable; (gray shading), excluded from outcome analysis according to initial study eligibility/trial protocol; γGT, gamma glutamyl transpeptidase.
*% change in length of tumor longest dimension.

disease progression only, unlike the 30% reported from autopsies of patients with pancreatic cancer [4]. Compared with other CRT regimens, a similarly high crude rate of stable local disease posttreatment (95%) has been reported in a trial of cetuximab + gemcitabine + intensity-modulated RT [33]. The 90% FFLP at 1 year was better than that reported by other contemporary CRT trials that used similar size measures of local control, including 64% reported in a trial of full-dose gemcitabine + RT [8] and 77% reported in a trial of induction cetuximab + gemcitibine + oxaliplatin, followed by cetuximab + capecitabine + RT [29]. The RT methodology, using limited field, IV contrast to minimize tumor CT underestimation, and margins incorporating on-treatment motion [36,42,43], may have aided the high rate of local control.

The PFS rate of 33% at 6 months meant that the trial could have proceeded to the second stage and indicated that the regimen was comparable to standard CRT regimens. In light of the low toxicity obtained, the RT dose may have been increased in the second stage as intended in the trial protocol. Although the actuarial PFS at 12 months (22.2%) was similar or better than recent trials of gemcitabine + RT [7,41], the median PFS at 6 months (33%) was low compared with other recent CRT trials [5,7,8,41,43]. Notably, five patients (28%) fared particularly badly and progressed before 20 weeks. Early metastatic progression is not uncommon in LAPC, and the presence of metastases occult to CT detection is believed to be responsible; e.g., 15% to 33% of patients are reported to develop metastatic disease during induction chemotherapy [44]; and in a recent randomized trial of gemcitabine + intensity-modulated RT, ~20% of patients overall progressed before 20 weeks [7]. The rapid progression seen in some patients and the lower median PFS suggest the presence of occult metastases and aggressive disease growth and dissemination, which was not effectively controlled by the predominately localized effects of cetuximab + RT.

The median OS was at the lower end of the range (7-14 months) reported from other recent CRT- and chemotherapy-trialled regimens [1,2]. This is most likely due to the swift metastatic progression seen in some patients. Nevertheless, OS was respectable for late-stage unresectable patients, and analysis was informative. Both OS and response rate were greater than those reported in previous trial arms of RT treatment alone, despite having more patients with higher stage disease in this study [10,45]. Survival was also considerably better than that reported for best supportive care only in patients with LAPC [9]. Although 1-year survival at 33% was less than the 40% to 70% reported for other CRT treatments associated with higher toxicities [5–9,46], it compares well with ~18% 1-year survival reported by CRUK for patients with all-stage (and all-treatment) pancreatic cancer [47]. Most importantly, 11% of patients survived more than 3 years, which was considerably more than that reported for other higher toxicity CRT regimens, including 5-fluorouracil + RT, gemcitabine + RT, and capecitabine + RT [5,7–9,41,46]. The association of posttreatment SD with survival lends support to other studies showing the importance of local control for patient outcome [5–8]. The association of cetuximab-induced acneiform rash with survival has been shown before [29] and indicates that cetuximab + RT efficacy was linked to patient cetuximab sensitivity. The association between high pretreatment CA19.9 level and CRT outcome confirms other findings [48,49] and suggests that increased tumor load or aggressive phenotype reduced cetuximab + RT efficacy.

Taken together, the results indicate cetuximab as an effective radiosensitizer in pancreatic cancer, consistent with preclinical data [22,23,25–27]. The treatment enabled very good control of local disease without substantially increasing toxicity, which importantly resulted in prolonged survival in some patients. The radiosensitizing efficacy of cetuximab may have particular relevance for improving local control and, in particular, increasing resectability rates in LAPC [50,51]. However, the ability of cetuximab + RT to inhibit metastatic growth and dissemination was insufficient in most patients and appeared to be related to individualized cetuximab sensitivity and tumor propensity for metastasis. There are three lines of support in accordance with these findings: first, patient sensitivity and skin reactions to cetuximab are known to vary; second, translational studies have indicated metastatic pancreatic cancer development as either progressive stages of a linear model of metastases or distinct morphologic/genetic cancer subtypes [52]; and third, testing cetuximab in combination with RT in locally advanced disease has shown encouraging results [29,30,32,33], whereas cetuximab tested with chemotherapy agents in advanced pancreatic cancer has not [53–55]. Susceptibility to EGFR-targeted therapy in pancreatic cancer has been suggested to be mediated by tumor K-RAS mutational status [13,15,17], as assumed in colorectal cancer, but recent evidence has shown interdependence rather than mutually exclusive action of oncogenic K-RAS and EGFR signaling to drive pancreatic oncogenesis [18,19]. A radiosensitizing mechanism independent of K-RAS status is therefore possible [27,56,57]. Alternatively, both metastatic potential and cetuximab sensitivity could be influenced by cellular or nuclear levels/activity of EGFR [11,12] or avain erythroblastic leukaemia viral oncogene homolog 2 (ERBB2) signaling activation [58].

It is likely that improved cohort PFS and OS would be gained with the use of induction chemotherapy before cetuximab + RT treatment—resulting from selecting out patients destined to progress early with aggressive occult metastatic disease [44] or by incorporating concurrent cytotoxic chemotherapy agents to the cetuximab + RT regimen—to improve control of metastatic disease but at the expense of increasing toxicity [29,32,33]. For CRT approaches that include cetuximab, the RT methodology, inclusion of chlorphenamine/non-steroidal anti-inflammatory drugs (NSAIDs)/steroids, and choice of chemotherapy agent appear to be important to reduce adverse events [29,32,33]. It is additionally important to recognize that some patients responded well to cetuximab + RT alone. However, there is currently no pretreatment method to identify these patients. Improved staging could enhance patient selection for localized treatment, e.g., with high-sensitivity fluorodeoxyglucose-positron emission tomography (FDG-PET), laparoscopy, or circulating tumor cell assay to detect CT occult disease. Our results also suggest that high pretreatment CA19.9 levels as an indicator of aggressive disease and initial testing of patient cetuximab sensitivity by rash induction may help to select patients who would be most responsive to cetuximab radiosensitization. Prediction of individual patient response would have ultimate benefit for the selection of patients for the most appropriate therapeutic approach, highlighting the need to return to translational studies to elucidate mechanisms and markers of cetuximab + RT efficacy.

To date, biologic investigation of pancreatic cancer and therapeutic response has been largely confined to pathologic material or *in vitro* models. Normal skin samples may be useful for assessment of EGFR expression or predicting cetuximab sensitivity. However, we found that patients were generally unwilling to consent to skin sampling, plus the processing was difficult and resulted in variable RNA quality. Blood samples were easily obtainable during routine treatment monitoring and resulted in valuable longitudinal samples

suitable for ongoing proteomics studies. We would therefore recommend blood sampling to be prioritized, with integration of consent into trials. In an initial blood proteomics study of three patients studied here, differentially expressed proteins were observed between patients at baseline [39], indicating patient variability that may be useful for determining molecular treatment sensitivity. In addition to EGFR, K-RAS and ERBB2, other molecules associated with cetuximab resistance, metastatic development, or aggressive phenotype include SMAD family member 4/deleted in pancreatic carcinoma locus 4 (SMAD4/DPC4) [4,29] and thus may have relevance in the elucidation of cetuximab + RT mechanism of action or prediction of individual treatment response.

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References

[1] Crane CH, Varadhachary G, Settle SH, Fleming JB, Evans DB, and Wolff RA (2009). The integration of chemoradiation in the care of patient with localized pancreatic cancer. *Cancer Radiother* **13**, 123–143.

[2] Sultana A, Tudur Smith C, Cunningham D, Starling N, Tait D, Neoptolemos JP, and Ghaneh P (2007). Systematic review, including meta-analyses, on the management of locally advanced pancreatic cancer using radiation/combined modality therapy. *Br J Cancer* **96**, 1183–1190.

[3] NICE (2001). *Guidance on the Use of Gemcitabine for the Treatment of Pancreatic Cancer*. National Institute of Clinical Excellence (NICE).

[4] Iacobuzio-Donahue CA, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Vilardell F, Wang Z, Keller JW, Banerjee P, et al. (2009). *DPC4* gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol* **27**, 1806–1813.

[5] Ben-Josef E, Schipper M, Francis IR, Hadley S, Ten-Haken R, Lawrence T, Normolle D, Simeone DM, Sonnenday C, Abrams R, et al. (2012). A phase I/II trial of intensity modulated radiation (IMRT) dose escalation with concurrent fixed-dose rate gemcitabine (FDR-G) in patients with unresectable pancreatic cancer. *Int J Radiat Oncol Biol Phys* **84**, 1166–1171.

[6] Chang JS, Wang ML, Koom WS, Yoon HI, Chung Y, Song SY, and Seong J (2012). High-dose helical tomotherapy with concurrent full-dose chemotherapy for locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* **83**, 1448–1454.

[7] Loehrer PJ Sr, Feng Y, Cardenes H, Wagner L, Brell JM, Cella D, Flynn P, Ramanathan RK, Crane CH, Alberts SR, et al. (2011). Gemcitabine alone *versus* gemcitabine plus radiotherapy in patients with locally advanced pancreatic cancer: an Eastern Cooperative Oncology Group trial. *J Clin Oncol* **29**, 4105–4112.

[8] Murphy JD, Adusumilli S, Griffith KA, Ray ME, Zalupski MM, Lawrence TS, and Ben-Josef E (2007). Full-dose gemcitabine and concurrent radiotherapy for unresectable pancreatic cancer. *Int J Radiat Oncol Biol Phys* **68**, 801–808.

[9] Shinchi H, Takao S, Noma H, Matsuo Y, Mataki Y, Mori S, and Aikou T (2002). Length and quality of survival after external-beam radiotherapy with concurrent continuous 5-fluorouracil infusion for locally unresectable pancreatic cancer. *Int J Radiat Oncol Biol Phys* **53**, 146–150.

[10] Cohen SJ, Dobelbower R Jr, Lipsitz S, Catalano PJ, Sischy B, Smith TJ, and Haller DG (2005). A randomized phase III study of radiotherapy alone or with 5-fluorouracil and mitomycin-C in patients with locally advanced adenocarcinoma of the pancreas: Eastern Cooperative Oncology Group study E8282. *Int J Radiat Oncol Biol Phys* **62**, 1345–1350.

[11] Uegaki K, Nio Y, Inoue Y, Minari Y, Sato Y, Song MM, Dong M, and Tamura K (1997). Clinicopathological significance of epidermal growth factor and its receptor in human pancreatic cancer. *Anticancer Res* **17**, 3841–3847.

[12] Yamanaka Y, Friess H, Kobrin MS, Buchler M, Beger HG, and Korc M (1993). Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. *Anticancer Res* **13**, 565–569.

[13] Bournet B, Muscari F, Guimbaud R, Cordelier P, and Buscail L (2013). *KRAS* mutations and their correlation with survival of patients with advanced pancreatic cancer. *Pancreas* **42**, 543–544.

[14] da Cunha Santos G, Dhani N, Tu D, Chin K, Ludkovski O, Kamel-Reid S, Squire J, Parulekar W, Moore MJ, and Tsao MS (2010). Molecular predictors of outcome in a phase 3 study of gemcitabine and erlotinib therapy in patients with advanced pancreatic cancer: National Cancer Institute of Canada Clinical Trials Group study PA.3. *Cancer* **116**, 5599–5607.

[15] Kim ST, Lim do H, Jang KT, Lim T, Lee J, Choi YL, Jang HL, Yi JH, Baek KK, Park SH, et al. (2011). Impact of *KRAS* mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. *Mol Cancer Ther* **10**, 1993–1999.

[16] Oliveira-Cunha M, Hadfield KD, Siriwardena AK, and Newman W (2012). EGFR and KRAS mutational analysis and their correlation to survival in pancreatic and periampullary cancer. *Pancreas* **41**, 428–434.

[17] Kullmann F, Hartmann A, Stöhr R, Messmann H, Dollinger MM, Trojan J, Fuchs M, Hollerbach S, Harder J, Troppmann M, et al. (2011). KRAS mutation in metastatic pancreatic ductal adenocarcinoma: results of a multicenter phase II study evaluating efficacy of cetuximab plus gemcitabine/oxaliplatin (GEMOXCET) in first-line therapy. *Oncology* **81**, 3–8.

[18] Ardito CM, Grüner BM, Takeuchi KK, Lubeseder-Martellato C, Teichmann N, Mazur PK, Delgiorno KE, Carpenter ES, Halbrook CJ, Hall JC, et al. (2012). EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* **22**, 304–317.

[19] Navas C, Hernández-Porras I, Schuhmacher AJ, Sibilia M, Guerra C, and Barbacid M (2012). EGF receptor signaling is essential for K-*Ras* oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* **22**, 318–330.

[20] Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Raju U, Milas L, Chen DJ, Kehlbach R, and Rodemann HP (2005). Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. *J Biol Chem* **280**, 31182–31189.

[21] Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Kehlbach R, and Rodemann HP (2011). Nuclear epidermal growth factor receptor modulates cellular radiosensitivity by regulation of chromatin access. *Radiother Oncol* **99**, 317–322.

[22] Kimple RJ, Vaseva AV, Cox AD, Baerman KM, Calvo BF, Tepper JE, Shields JM, and Sartor CI (2010). Radiosensitization of epidermal growth factor receptor/HER2–positive pancreatic cancer is mediated by inhibition of Akt independent of ras mutational status. *Clin Cancer Res* **16**, 912–923.

[23] Morgan MA, Parsels LA, Kollar LE, Normolle DP, Maybaum J, and Lawrence TS (2008). The combination of epidermal growth factor receptor inhibitors with gemcitabine and radiation in pancreatic cancer. *Clin Cancer Res* **14**, 5142–5149.

[24] Harding J and Burtness B (2005). Cetuximab: an epidermal growth factor receptor chemeric human-murine monoclonal antibody. *Drugs Today (Barc)* **41**, 107–127.

[25] Dittmann K, Mayer C, and Rodemann HP (2005). Inhibition of radiation-induced EGFR nuclear import by C225 (cetuximab) suppresses DNA-PK activity. *Radiother Oncol* **76**, 157–161.

[26] Bruns CJ, Harbison MT, Davis DW, Portera CA, Tsan R, McConkey DJ, Evans DB, Abbruzzese JL, Hicklin DJ, and Radinsky R (2000). Epidermal growth factor receptor blockade with C225 plus gemcitabine results in regression of human pancreatic carcinoma growing orthotopically in nude mice by antiangiogenic mechanisms. *Clin Cancer Res* **6**, 1936–1948.

[27] Buchsbaum DJ, Bonner JA, Grizzle WE, Stackhouse MA, Carpenter M, Hicklin DJ, Bohlen P, and Raisch KP (2002). Treatment of pancreatic cancer

xenografts with Erbitux (IMC-C225) anti-EGFR antibody, gemcitabine, and radiation. *Int J Radiat Oncol Biol Phys* **54**, 1180–1193.

[28] Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, Jones CU, Sur /surname>R, Raben D, Jassem J, et al. (2006). Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* **354**, 567–578.

[29] Crane CH, Varadhachary GR, Yordy JS, Staerkel GA, Javle MM, Safran H, Haque W, Hobbs BD, Krishnan S, Fleming JB, et al. (2011). Phase II trial of cetuximab, gemcitabine, and oxaliplatin followed by chemoradiation with cetuximab for locally advanced (T4) pancreatic adenocarcinoma: correlation of *Smad4(Dpc4)* immunostaining with pattern of disease progression. *J Clin Oncol* **29**, 3037–3043.

[30] Arnoletti JP, Frolov A, Eloubeidi M, Keene K, Posey J, Wood T, Greeno E, Jhala N, Varadarajulu S, Russo S, et al. (2011). A phase I study evaluating the role of the anti-epidermal growth factor receptor (EGFR) antibody cetuximab as a radiosensitizer with chemoradiation for locally advanced pancreatic cancer. *Cancer Chemother Pharmacol* **67**, 891–897.

[31] Pipas JM, Zaki BI, McGowan MM, Tsapakos MJ, Ripple GH, Suriawinata AA, Tsongalis GJ, Colacchio TA, Gordon SR, Sutton JE, et al. (2012). Neoadjuvant cetuximab, twice-weekly gemcitabine, and intensity-modulated radiotherapy (IMRT) in patients with pancreatic adenocarcinoma. *Ann Oncol* **23**, 2820–2827.

[32] Demols A, Mahin C, Marechal R, Delaunoit T, Borbath I, Hendlisz A, Jacquy C, Mitine C, and Van Laethem J (2008). Cetuximab plus chemoradiation combined therapy for locally advanced inoperable pancreatic adenocarcinoma: a phase I study. *J Clin Oncol* **26**(15S). American Society of Clinical Oncology Meeting Proceedings Abstract 4629.

[33] Munter M, Timke C, Abdollahi A, Freiss H, Jaeger D, Heeger S, Buchler M, Debus J, Huber P, and Krempien R (2008). Final results of a phase II trial (PARC-Study ISCRTN56652283) for patients with primary inoperable locally advanced pancreatic cancer combining intensity modulated radiotherapy (IMRT) with cetuximab and gemcitabine. *J Clin Oncol* **26**(15S). American Society of Clinical Oncology Meeting Proceedings Abstract 4613.

[34] Simon R (1989). Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* **10**, 1–10.

[35] Poortmans PM, Davis JB, Ataman F, Bernier J, and Horiot JC (2005). The quality assurance programme of the Radiotherapy Group of the European Organisation for Research and Treatment of Cancer: past, present and future. *Eur J Surg Oncol* **31**, 667–674.

[36] Whitfield G, Jain P, Green M, Watkins G, Henry A, Stratford J, Amer A, Marchant T, Moore C, and Price P (2012). Quantifying motion for pancreatic radiotherapy margin calculation. *Radiother Oncol* **103**, 360–366.

[37] National Cancer Institute (Cancer Therapy Evaluation Program) (2006). Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Published August 2006. Available from: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf (Accessed 27 Feb 2014).

[38] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, et al. (2009). New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* **45**, 228–247.

[39] Zhou C, Simpson KL, Lancashire LJ, Walker MJ, Dawson MJ, Unwin RD, Rembielak A, Price P, West C, Dive C, et al. (2012). Statistical considerations of optimal study design for human plasma proteomics and biomarker discovery. *J Proteome Res* **11**, 2103–2113.

[40] Chakravarthy AB, Tsai CJ, O'Brien N, Lockhart AC, Chan E, Parikh A, Berlin JD, and Merchant N (2012). A phase I study of cetuximab in combination with gemcitabine and radiation for locally advanced pancreatic cancer. *Gastrointest Cancer Res* **5**, 112–118.

[41] Mukherjee S, Hurt CN, Bridgewater J, Falk S, Cummins S, Wasan H, Crosby T, Jephcott C, Roy R, Radhakrishna G, et al. (2013). Gemcitabine-based or capecitabine-based chemoradiotherapy for locally advanced pancreatic cancer (SCALOP): a multicentre, randomised, phase 2 trial. *Lancet Oncol* **14**, 317–326.

[42] Arvold ND, Niemierko A, Mamon HJ, Fernandez-del Castillo C, and Hong TS (2011). Pancreatic cancer tumor size on CT scan *versus* pathologic specimen: implications for radiation treatment planning. *Int J Radiat Oncol Biol Phys* **80**, 1383–1390.

[43] Jackson AS, Jain P, Watkins GR, Whitfield GA, Green MM, Valle J, Taylor MB, Dickinson C, Price PM, and Saleem A (2010). Efficacy and tolerability of limited field radiotherapy with concurrent capecitabine in locally advanced pancreatic cancer. *Clin Oncol (R Coll Radiol)* **22**, 570–577.

[44] Johung K, Saif MW, and Chang BW (2012). Treatment of locally advanced pancreatic cancer: the role of radiation therapy. *Int J Radiat Oncol Biol Phys* **82**, 508–518.

[45] Moertel CG, Frytak S, Hahn RG, O'Connell MJ, Reitemeier RJ, Rubin J, Schutt AJ, Weiland LH, Childs DS, Holbrook MA, et al. (1981). Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil: the Gastrointestinal Tumor Study Group. *Cancer* **48**, 1705–1710.

[46] Crane CH, Abbruzzese JL, Evans DB, Wolff RA, Ballo MT, Delclos M, Milas L, Mason K, Charnsangavej C, Pisters PWT, et al. (2002). Is the therapeutic index better with gemcitabine-based chemoradiation than with 5-fluorouracil-based chemoradiation in locally advanced pancreatic cancer? *Int J Radiat Oncol Biol Phys* **52**, 1293–1302.

[47] Cancer Research UK. CRUK pancreatic cancer survival statistics. Electronic Article available from: http://www.cancerresearchuk.org/cancer-info/cancerstats/types/pancreas/survival/.

[48] Mayahara H, Ito Y, Morizane C, Ueno H, Okusaka T, Kondo S, Murakami N, Morota M, Sumi M, and Itami J (2012). Salvage chemoradiotherapy after primary chemotherapy for locally advanced pancreatic cancer: a single-institution retrospective analysis. *BMC Cancer* **12**, 609.

[49] Rudra S, Narang AK, Pawlik TM, Wang H, Jaffee EM, Zheng L, Le DT, Cosgrove D, Hruban RH, Fishman EK, et al. (2012). Evaluation of predictive variables in locally advanced pancreatic adenocarcinoma patients receiving definitive chemoradiation. *Pract Radiat Oncol* **2**, 77–85.

[50] Morganti AG, Massaccesi M, La Torre G, Caravatta L, Piscopo A, Tambaro R, Sofo L, Sallustio G, Ingrosso M, Macchia G, et al. (2010). A systematic review of resectability and survival after concurrent chemoradiation in primarily unresectable pancreatic cancer. *Ann Surg Oncol* **17**, 194–205.

[51] Truty MJ, Thomas RM, Katz MH, Vauthey JN, Crane C, Varadhachary GR, Wolff RA, Abbruzzese JL, Lee JE, and Fleming JB (2012). Multimodality therapy offers a chance for cure in patients with pancreatic adenocarcinoma deemed unresectable at first operative exploration. *J Am Coll Surg* **215**, 41–51; discussion 51-42.

[52] Tuveson DA and Neoptolemos JP (2012). Understanding metastasis in pancreatic cancer: a call for new clinical approaches. *Cell* **148**, 21–23.

[53] Philip PA, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, et al. (2010). Phase III study comparing gemcitabine plus cetuximab *versus* gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group–directed intergroup trial S0205. *J Clin Oncol* **28**, 3605–3610.

[54] Cascinu S, Berardi R, Labianca R, Siena S, Falcone A, Aitini E, Barni S, Di Costanzo F, Dapretto E, Tonini G, et al. (2008). Cetuximab plus gemcitabine and cisplatin compared with gemcitabine and cisplatin alone in patients with advanced pancreatic cancer: a randomised, multicentre, phase II trial. *Lancet Oncol* **9**, 39–44.

[55] Kullmann F, Hollerbach S, Dollinger MM, Harder J, Fuchs M, Messmann H, Trojan J, Gäbele E, Hinke A, Hollerbach C, et al. (2009). Cetuximab plus gemcitabine/oxaliplatin (GEMOXCET) in first-line metastatic pancreatic cancer: a multicentre phase II study. *Br J Cancer* **100**, 1032–1036.

[56] Toulany M, Dittmann K, Baumann M, and Rodemann HP (2005). Radiosensitization of Ras-mutated human tumor cells *in vitro* by the specific EGF receptor antagonist BIBX1382BS. *Radiother Oncol* **74**, 117–129.

[57] Toulany M, Kasten-Pisula U, Brammer I, Wang S, Chen J, Dittmann K, Baumann M, Dikomey E, and Rodemann HP (2006). Blockage of epidermal growth factor receptor-phosphatidylinositol 3-kinase-AKT signaling increases radiosensitivity of K-*RAS* mutated human tumor cells *in vitro* by affecting DNA repair. *Clin Cancer Res* **12**, 4119–4126.

[58] Yonesaka K, Zejnullahu K, Okamoto I, Satoh T, Cappuzzo F, Souglakos J, Ercan D, Rogers A, Roncalli M, Takeda M, et al. (2011). Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. *Sci Transl Med* **3**, 99ra86.