

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

J. P. Arnoletti · A. Frolov · M. Eloubeidi · K. Keene · J. Posey · T. Wood ·
Edward Greeno · N. Jhala · S. Varadarajulu · S. Russo · J. Christein ·
R. Oster · D. J. Buchsbaum · S. M. Vickers

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Abstract

Purpose (1) To determine the safety of the epidermal growth factor receptor (EGFR) antibody cetuximab with concurrent gemcitabine and abdominal radiation in the treatment of patients with locally advanced adenocarcinoma of the pancreas. (2) To evaluate the feasibility of pancreatic cancer cell epithelial–mesenchymal transition (EMT) molecular profiling as a potential predictor of response to anti-EGFR treatment.

Methods Patients with non-metastatic, locally advanced pancreatic cancer were treated in this dose escalation study with gemcitabine (0–300 mg/m²/week) given concurrently with cetuximab (400 mg/m² loading dose, 250 mg/m² weekly maintenance dose) and abdominal irradiation (50.4 Gy). Expression of E-cadherin and vimentin was assessed by immunohistochemistry in diagnostic endoscopic ultrasound fine-needle aspiration (EUS-FNA) specimens.

Results Sixteen patients were enrolled in 4 treatment cohorts with escalating doses of gemcitabine. Incidence of grade 1–2 adverse events was 96%, and incidence of 3–4 adverse events was 9%. There were no treatment-related mortalities. Two patients who exhibited favorable treatment response underwent surgical exploration and were intraoperatively confirmed to have unresectable tumors. Median overall survival was 10.5 months. Pancreatic cancer cell expression of E-cadherin and vimentin was successfully determined in EUS-FNA specimens from 4 patients.

Conclusions Cetuximab can be safely administered with abdominal radiation and concurrent gemcitabine (up to 300 mg/m²/week) in patients with locally advanced adenocarcinoma of the pancreas. This combined therapy modality exhibited limited activity. Diagnostic EUS-FNA specimens could be analyzed for molecular markers of EMT in a minority of patients with pancreatic cancer.

J. P. Arnoletti · A. Frolov · J. Christein
Department of Surgery, University of Alabama at Birmingham,
Birmingham, AL, USA

M. Eloubeidi · S. Varadarajulu
Department of Gastroenterology, University of Alabama
at Birmingham, Birmingham, AL, USA

K. Keene · D. J. Buchsbaum
Department of Radiation Oncology, University of Alabama
at Birmingham, Birmingham, AL, USA

J. Posey · T. Wood
Department of Medical Oncology, University of Alabama
at Birmingham, Birmingham, AL, USA

E. Greeno
Division of Hematology, Oncology and Transplantation,
University of Minnesota, Minneapolis, MN 55455, USA

N. Jhala
Department of Pathology, University of Alabama
at Birmingham, Birmingham, AL, USA

S. Russo
Department of Radiation Oncology,
East Carolina University, Green Ville, NC, USA

R. Oster
Department of Biostatistics, University of Alabama
at Birmingham, Birmingham, AL, USA

S. M. Vickers (✉)
Surgery Department, University of Minnesota,
420 Delaware Street SE, Mayo Mail Code 195,
Minneapolis, MN 55455, USA
e-mail: vickers@umn.edu

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Introduction

Approximately 42,500 new cases of pancreatic carcinoma occur in the United States per year with only 5–10% of 5-year overall survival [1]. Standard treatment for locally advanced unresectable disease has historically consisted of 5-fluorouracil (5-FU, either bolus or continuous infusion) and external beam radiation (doses of 50 to >60 Gy). The addition of radiosensitizing chemotherapy, delivered with lower external beam radiation doses of 35–50 Gy or higher doses of ~60 Gy using CT-guided treatment planning resulted in modest local control with median patient survival of 8–12 months [2–4].

Since then, chemoradiation for locally advanced pancreatic cancer has evolved toward more dose-intensive regimens combining modern radiotherapy techniques (continuous course radiotherapy, increased dose) with systemic chemotherapeutic agents, mainly gemcitabine [5–8]. Local failure is common after chemoradiation for locally advanced pancreatic cancer, and escalated doses of radiation are limited by normal tissue toxicity, which in turn has limited further attempts to escalate radiation dose. Recently, improved understanding of radiation-induced effects at a cellular and molecular level have allowed investigation of many potential mechanisms of improving radiosensitization, including targeting growth factors and downstream signal transduction pathways [9].

The epidermal growth factor receptor (EGFR) has become a molecular target for pharmacologic intervention and selective inhibitors of EGFR, including monoclonal antibodies, have been developed. EGFR is overexpressed in approximately 50–60% of pancreatic adenocarcinomas and up to 46% of metastases [10]. These findings have led to the use of EGFR inhibitors in the treatment of patients with pancreatic cancer [11, 12].

Pre-clinical studies have consistently demonstrated that EGFR down-regulation inhibits cell proliferation and modifies cellular response to radiation and/or chemotherapy [13].

Cetuximab is an anti-EGFR antibody which has been shown to have effective synergy when combined with radiation therapy in the treatment of patients with squamous cell carcinomas of the head and neck as well as other types of cancer [14, 15]. Several pre-clinical studies have confirmed that cetuximab, in combination with gemcitabine and radiation therapy is effective in promoting pancreatic cancer cell apoptosis and inhibition of pancreatic cancer xenograft growth [16]. Cetuximab has also displayed an acceptable toxicity profile and exhibited promising activity

when combined with gemcitabine in the treatment of patients with pancreatic cancer [11].

The objective of this phase I open label, non-randomized dose escalation study was to determine the safety of concurrent gemcitabine with cetuximab and abdominal radiation in patients with histologically proven, non-metastatic, locally advanced, adenocarcinoma of the pancreas. The primary endpoint of the study was to define the maximum tolerated dose (MTD) of gemcitabine given concurrently with cetuximab and radiation therapy in this group of patients.

Pre-clinical reports point to epithelial–mesenchymal transition (EMT) as a potential indicator of lack of response to EGFR inhibition [17]. EMT is characterized by loss of cell adhesion, repression of E-cadherin expression, and increased cell mobility [18]. A secondary objective of this Phase I trial was to analyze pancreatic cancer diagnostic specimens obtained by endoscopic ultrasound guided fine needle aspiration (EUS-FNA) for markers of EMT, assessing the feasibility of this approach for the evaluation of potential predictors of therapeutic response to EGFR inhibition.

Methods

Patient enrollment and treatment was carried out at the University of Alabama at Birmingham and the University of Minnesota between March of 2006 and August 2008. The clinical trial protocol was reviewed and approved by the Institutional Review Board at both institutions, and all study participants provided informed consent. Final analysis of patient data was performed on May 19, 2009.

Patient eligibility

Participants in this clinical trial were adult patients with Karnofsky Performance Status ≥ 60 and biopsy-proven primary (non-recurrent) adenocarcinoma of the pancreas who had not received any prior therapy. Pre-entry triple phase (pancreatic protocol) computed tomography (CT scan) of the chest, abdomen, and pelvis with oral and intravenous contrast was performed in all patients within 4 weeks of initiation of therapy. All study participants were deemed to have locally advanced pancreatic cancer without radiologic evidence of distant metastasis. The definition of locally advanced pancreatic cancer was adopted following guidelines published elsewhere [19, 20]. Patients were evaluated by an experienced pancreatic surgeon and deemed ineligible for pancreatic resection with curative intent. All patients underwent required pre-treatment laboratory evaluations within 14 days of initiation of therapy.

Patients with non-metastatic, locally advanced, adenocarcinoma of the pancreas, received external beam radiation therapy (50.4 Gy in 28 fractions) with concurrent gemcitabine (0, 150, 225, or 300 mg/m² IV infusion over 30 min on days 1, 8, 22, and 29) and cetuximab (400 mg/m² loading dose and 250 mg/m² maintenance dose over 120 and 60 min, respectively, on days 1, 8, 15, 22, and 29) beginning on the first day of radiation. Four patient treatment cohorts (1–4) were established, based on the gemcitabine dose administered (0–300 mg/m²). Cetuximab infusion preceded gemcitabine infusion by 2 h. Post-chemoradiation chemotherapy began after a 6-week rest period, and consisted of gemcitabine 1,000 mg/m² IV during weeks 12, 13, and 14, and 16, 17, and 18.

Treatment schema was as follows:

	Week																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Cetuximab	X	X	X	X	X													
Gemcitabine	X	X		X	X							X	X	X		X	X	X
Radiation Tx	X	X	X	X	X	X												

Radiation therapy

External beam radiation therapy (RT) with megavoltage linear accelerators (>6MV) was used to deliver multiple (≥ 4) field techniques using either a 3D conformal or IMRT technique. All patients underwent CT simulation in the supine position with arms above their heads.

Target Definitions: The gross tumor volume (GTV) included all gross disease and involved pathologic lymph nodes as defined by CT. Elective lymph nodes were not specifically targeted, and the clinical target volume (CTV) was equal to the GTV. The edges of the initial fields were defined superiorly at the intervertebral T10–11 space, inferiorly at the intervertebral L3–4 space, laterally and anteriorly with a 2-cm margin around the pre-operative tumor volume (including all areas of gross disease), and posteriorly by splitting the anterior vertebral bodies in half. The planning target volume (PTV) accounted for setup error and patient motion. This was an expansion of 2 cm from the GTV for the initial PTV (PTV1) and 1.5 cm for the boost PTV (PTV2).

Dose Prescription: RT began on the first day of week 1. The PTV1 was prescribed 1.8 Gy/day $\times$ 25 fractions for a total of 45 Gy. The PTV2 was prescribed 5.4 Gy delivered in 1.8 Gy fractions per day for 3 additional fractions (total of 50.4 Gy in 28 fractions). The dose was prescribed to the 100% isodose line and the PTV was to be covered by the 95% isodose line. Dose homogeneity did not exceed +10%, and the maximum dose point was located within the tumor volume.

Follow-up

After a 6-week rest period following post-radiation chemotherapy, patients underwent restaging CT scan of the chest, abdomen and pelvis. Patients with no evidence of disease progression and/or favorable response to therapy were re-evaluated for surgical resection and surgical exploration was allowed no earlier than 6 weeks after completion of chemoradiation. Patients with evidence of progression or unable to undergo surgical resection with curative intent were followed for toxicity and survival. Patients were followed on protocol for a minimum of 12 months, with regularly scheduled CT scans, clinical evaluations, and laboratory work every 3 months. Patient survival was measured from the time of EUS-FNA diagnosis. Results reported are at the time of study closure on May 19, 2009.

Immunohistochemical analysis of EMT markers expression

Cell blocks were prepared from EUS-FNA material submitted in Hank's solution, using fibrin clot method. Serial 5- μ m sections were cut 1 day prior to immunostaining and mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA). Immunohistochemical stains for E-cadherin and vimentin were performed on the cell block using routine clinical diagnostic laboratory protocols. All immunohistochemical stains were performed with appropriate positive and negative controls. All negative control slides (omitted primary antibodies) were negative for staining.

Results

Sixteen patients were enrolled in the trial, eight were women (50%) and 3 patients were African-American (19%). Median patient age was 63 years (range 41–80). Ten patients were enrolled at the University of Alabama at Birmingham, and 6 patients were enrolled at the University of Minnesota. Patient demographics and treatment cohort distribution are summarized in Table 1. Median treatment duration was 71 days (range 4–126 days). All 16 patients presented at least one adverse event (AE). The median number of AEs per patient was 13, with a range of 1 AE to 42 AEs. There were a total of 214 AEs reported over the course of the study with their distribution depicted in Table 2. The most frequent Grade 1 AE was nausea (11/143), followed by fatigue (10/143), diarrhea (7/143), vomiting (7/143), thrombocytopenia (6/143), and rash on various parts of the body (14/143). The most frequent Grade 2 AE was fatigue (4/52), followed by nausea (3/52), and rash on various parts of the body (7/52). The most frequent Grade 3 AE was thrombocytopenia (3/15), hyperkalemia

Table 1 Baseline patient demographics

	Total	Cohort I	Cohort II	Cohort III	Cohort IV
Age at enrollment					
Median	63	69	62	68	49
Min–max	41–80	58–78	51–76	56–80	41–61
<i>N</i>	16	4	3	6	3
Gender					
Female (<i>n</i> , %)	8 (50)	2 (50)	3 (100)	3 (50)	0
Male (<i>n</i> , %)	8 (50)	2 (50)	0	3 (50)	3 (100)
Patient ethnicity					
Caucasian, not Hispanic (<i>n</i> , %)	13 (81)	2 (50)	2 (67)	6 (100)	3 (100)
African American (<i>n</i> , %)	3 (19)	2 (50)	1 (33)	0	0

Table 2 Summary of adverse events (AE) with patient cohort distribution

	Cohort I	Cohort II	Cohort III	Cohort IV	Total
Number of AEs	37	47	83	47	214
Severity, <i>n</i> (% total)					
Mild	28 (13)	33 (15)	51 (24)	31 (15)	143 (67)
Moderate	8 (4)	10 (5)	22 (10)	12 (6)	52 (24)
Severe	0	4 (2)	8 (4)	3 (1)	15 (7)
Life threatening	1 (0.5)	0	2 (1)	1 (0.5)	4 (2)

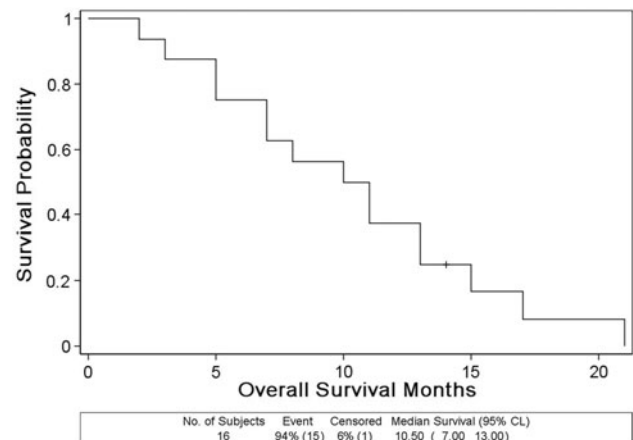
(3/15), and low platelets (2/15). The four Grade 4 AEs were ischemic colitis, perforated duodenum, pulmonary embolism, and thrombocytopenia. None of these were attributed to cetuximab toxicity.

Tumor response and patient survival

There were two patients who exhibited partial response and another two patients who had stable disease while on treatment. Twelve of the sixteen patients had evidence of disease progression according to RECIST criteria on CT controls.

There were fifteen patients who died prior to May 19, 2009. Thirteen patients died of disease progression. One patient died from duodenal perforation following placement of a duodenal stent for palliation of gastric outlet obstruction; a second patient died from ischemic colitis. As mentioned, neither of these latter two deaths was attributed to treatment toxicity. The overall median survival for the study was 10.5 months (7–13 months). The Kaplan–Meier survival curve is displayed in Fig. 1.

There were 2 (12.5%) patients who showed a favorable tumor response and underwent surgical exploration with the intent of curative resection. One patient was confirmed to have locally advanced, unresectable, disease and the other patient was deemed unresectable because of liver cirrhosis.

**Fig. 1** Kaplan–Meier patient overall survival curve

Molecular analysis of diagnostic EUS-FNA specimens

To assess feasibility of molecular immunohistochemical analysis of EUS-FNA specimens, we analyzed two markers of EMT: vimentin and E-cadherin. Gain of vimentin and loss of E-cadherin are paramount features of carcinoma cells losing adhesion and becoming metastatic. There were 13 patients who underwent diagnostic EUS-FNA at our institutions prior to initiation of therapy. The other 3 patients enrolled in the study underwent diagnostic CT-guided needle biopsies at outside institutions and the specimens

were not available for further analysis. Of the 13 pancreatic cancer EUS-FNA specimens obtained, there were 9 which did not have enough cellular remnant on cell block to allow for additional characterization. There were 4/13 EUS-FNA specimens with rich cellular content that enabled us to perform immunohistochemistry after diagnosis had been completed. We were able to detect loss of E-cadherin and gain of vimentin expression in the 4 available specimens (Fig. 2). Another significant feature of this analysis was the marked heterogeneity of EMT marker expression within individual cells from the same specimen. This finding suggests a dynamic EMT process with significant cell-to-cell variation, a feature that would be very difficult to detect in total crude protein lysates from the cytologic aspirate. It was not possible to attempt correlation of EMT status with clinical outcome due to the low number of available specimens.

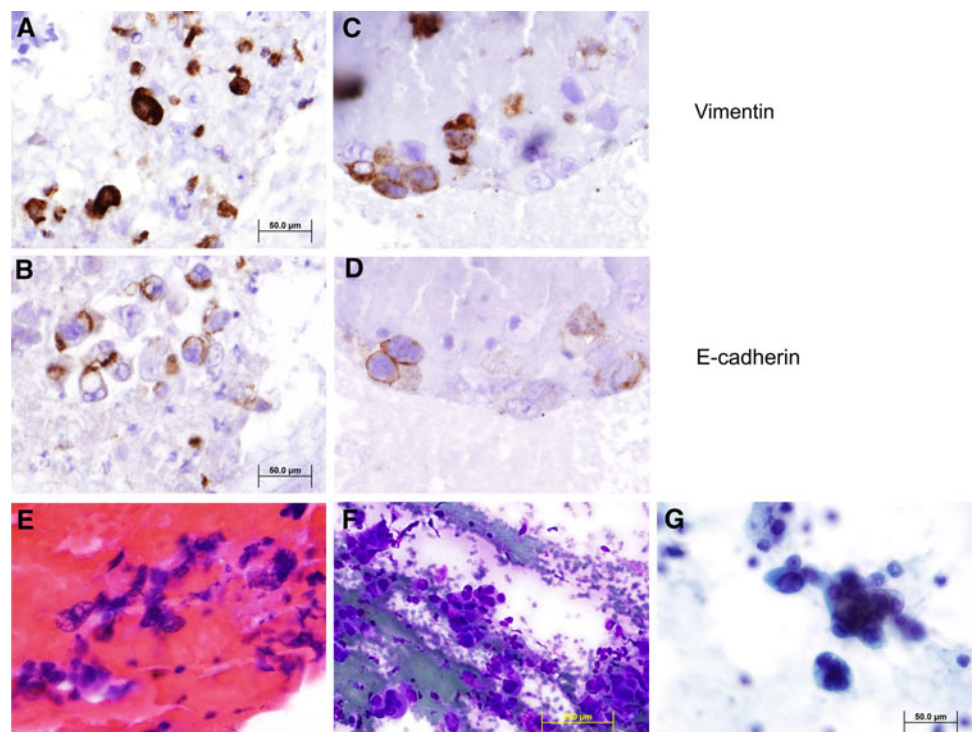
Discussion

Cetuximab is the chimeric counterpart of the murine M225 antibody, directed against the ligand-binding site of the EGFR. It has been shown that cetuximab-mediated EGFR inhibition is synergistic with radiation therapy, although the exact mechanism of action is not clear. Potential mechanisms for radiosensitization include effects on tumor cell biology, immune system interaction, and angiogenesis inhibition [13]. Several phase II trials evaluating cetuximab in

patients with cancers known to overexpress EGFR have been performed [14]. Cetuximab has been delivered as a single agent, or with other therapeutic modalities such as radiotherapy, cisplatin, doxorubicin, and paclitaxel [21]. The most encouraging data are observed in the studies using cetuximab and radiation therapy for patients with squamous cell carcinomas of the head and neck, which have resulted in a 10% increase in 3-year survival with a significant improvement in local control compared to radiation alone [22, 23]. Pre-clinical data suggests that cetuximab increases pancreatic cancer sensitivity to gemcitabine and radiation therapy [9, 16]. A multicenter phase II trial showed promising activity for the combination of gemcitabine with cetuximab in the treatment of advanced pancreatic cancer [11]. A recent phase II multicenter trial, however, failed to show a significant survival benefit for the cetuximab/gemcitabine/oxaliplatin combination in advanced disease [24]. A phase III study failed to demonstrate a clinically significant advantage of the addition of cetuximab to gemcitabine for survival and response in advanced pancreatic cancer [25]. EGFR inhibition seems to have a role, albeit modest, in the treatment of advanced pancreatic cancer as reflected by the approval of the small molecule EGFR inhibitor erlotinib based on the results reported by the National Cancer Institute of Canada Clinical Trials Group [12].

There have been several reports in abstract form of clinical trials combining cetuximab with gemcitabine and radiation therapy in the treatment of pancreatic cancer. A small

Fig. 2 Pancreatic adenocarcinoma EUS-FNA from 2 different patients stained using immunohistochemistry for vimentin (panels A and C) and E-cadherin (panels B and D). Each section shows carcinoma cells with nuclear enlargement and pleomorphism. Panel E is hematoxylin and eosin (H&E), panel F is a Diff-Quick stain and panel G is a Papanicolaou's stain of air-dried material obtained from the same EUS-FNA



phase I trial from Vanderbilt University described significant toxicity and was unable to determine safe drug dosage [26]. Another phase I trial performed in Belgium, however, described good tolerance recommending 45 Gy of radiation therapy in combination with gemcitabine (300 mg/m²) and cetuximab (loading dose 400 mg/m² and then 250 mg/m² weekly) [27]. The results of a phase II randomized study from the University of Heidelberg were reported in abstract form in 2008 [28]. In that study, 68 patients with inoperable, locally advanced pancreatic cancer were treated with concomitant radiotherapy, gemcitabine, and cetuximab. A partial response was seen in 23/68 patients, and 14 patients underwent surgical resection. The reported median survival was 15 months. There was no significant difference in clinical outcome between the two treatment groups.

The objective of our phase I study was to determine the safety of concurrent gemcitabine with cetuximab and abdominal radiation in patients with histologically proven, non-metastatic, locally advanced, adenocarcinomas of the pancreas. At our institutions, we follow strict consensus guidelines as defined by the NCCN and which have been summarized in a recent joint statement from surgical societies [19, 20].

Prior studies have confirmed that full-dose gemcitabine (1,000 mg/m²) with concurrent radiation therapy is well tolerated and active in patients with pancreatic cancer [29]. Because of toxicity concerns at the time of study design, and since our therapeutic regimen also included cetuximab, we reduced gemcitabine dosing in the different cohorts. Our study effectively determined the safety of gemcitabine in doses up to 300 mg/m²/week given concurrently with cetuximab and abdominal irradiation in this group of patients. This combined therapeutic modality exhibited an acceptable toxicity profile with well-tolerated side effects. There were no serious treatment-related AE, and no patient death was attributable to drug toxicity. Treatment response rates and overall patient survival (median 10.5 months) were modest and comparable to what has been previously reported for patients with locally advanced pancreatic cancer. None of our patients were able to undergo surgical resection of their locally advanced pancreatic cancer.

In the past few years, there have been multiple efforts to identify molecular markers that may be indicative of tumor dependence on EGFR signaling and that, therefore, could be utilized to improve patient selection for EGFR-targeted therapeutic strategies. Mutations of *EGFR* in NSCLC and wild-type *K-ras* both in NSCLC and colon cancer have been associated with favorable tumor responses to EGFR inhibition [30, 31]. We and others have previously reported the absence of activating *EGFR* mutations in pancreatic cancer [32]. Up to 90% of pancreatic cancers exhibit *K-ras* mutations and those mutations have been clearly linked to resistance to anti-EGFR agents in colon cancer. Pancreatic

cancer EUS-FNA material was insufficient for *K-ras* mutation analysis in our study.

Based on pre-clinical studies, EMT is a factor that seems to be associated with pancreatic cancer response to anti-EGFR agents, albeit most of the data comes from small molecule EGFR inhibitors such as erlotinib. EMT markers are associated with initiation of the metastatic process and cellular invasion, which shares many phenotypic similarities with EMT, including E-cadherin repression, loss of cellular adhesion, and increased cell mobility [33]. Pancreatic tumor cell lines insensitive to EGFR inhibition typically exhibit loss of E-cadherin while gaining proteins associated with a mesenchymal phenotype such as vimentin. In pancreatic cancer tumor specimens, gain of mesenchymal characteristics has been correlated with advancing tumor stage [34].

The molecular profiling of EUS-FNA for markers of EMT is an attractive concept that could be employed in the characterization of pancreatic cancers in an attempt to predict the likelihood of response to EGFR signaling inhibition. Most patients with pancreatic cancer at our institutions undergo EUS-FNA diagnosis and this is a safe and highly sensitive diagnostic modality. Effective molecular analysis of EUS-FNA specimens requires an expert cytopathologist and additional techniques such as immunohistochemistry and laser-capture microdissection. We were able to obtain adequate specimens for immunohistochemical analysis of EMT in a minority of patients. Our results indicate that such analysis is feasible but requires a high degree of coordination between the endoscopist, cytopathologist, and molecular biologist for adequate processing of the specimen.

We have demonstrated that the anti-EGFR antibody cetuximab can be safely administered with abdominal radiation and concurrent gemcitabine in patients with locally advanced adenocarcinoma of the pancreas. Response rates were modest and no patient was able to undergo curative surgical resection. Diagnostic EUS-FNA specimens could be analyzed for molecular markers of EMT in a minority of patients.

Additional molecular profiling is necessary to identify the pancreatic cancer patient sub-population likely to obtain the maximum benefit from this type of targeted therapy.

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