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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

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Key Words

Pancreatic cancer · Chemotherapy · KRAS mutation · Cetuximab · Gemcitabine · Oxaliplatin

Abstract

Background: Genetic alterations within the epidermal growth factor receptor (EGFR) pathway, including KRAS mutations, have been demonstrated to be associated with response to EGFR inhibitors like cetuximab in colorectal cancers. Mutations in the KRAS gene have been found in 70–90% of pancreatic cancers. Unfortunately, the addition of cetuximab to chemotherapy did not increase response or survival in patients with advanced pancreatic cancer in phase II and phase III studies. The aim of this study was to evaluate the relationship between KRAS mutations and response or survival in patients with metastatic pancreatic cancer treated with cetuximab plus chemotherapy. ***Methods:*** Within a multicenter phase II trial, 64 patients with metastatic pancreatic cancer were treated with cetuximab in combination with gemcitabine and oxaliplatin until disease progression. Analyses of the EGFR pathway, including KRAS mutations, could be performed in 25 patients. Analyses were carried out following microdissection of the tumor. ***Results:*** Fourteen (56%) of the 25 patients examined harbored a point mutation in codon 12 of the KRAS gene. No differences between the groups were noted in median progression-free survival (104 days in KRAS wild-type patients vs. 118 days in patients with KRAS mutations). Overall survival was longer in wild-type patients compared to patients with KRAS mutations (263 vs. 162 days), but the difference did not reach statistical significance. A further analysis of our clinical phase II trial showed that the presence of a rash was significantly correlated with overall survival. ***Conclusions:*** KRAS mutation in codon 12 may be associated with reduced survival compared to KRAS wild type. The role of KRAS mutations for cetuximab therapy in pancreatic cancer warrants further investigation in larger trials to exclude an epiphenomenon. Furthermore, the development of a rash is indicative of clinical benefit.

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Introduction

The prognosis for patients with pancreatic adenocarcinoma is still dismal. Since 1997, gemcitabine has been accepted as the standard palliative chemotherapy for advanced pancreatic cancer, with a median survival of 6 months [1]. Many trials have been conducted evaluating various combination protocols. Meta-analysis of randomized trials revealed that pancreatic cancer patients with good performance status appear to benefit from gemcitabine-based cytotoxic combinations [2]. Nevertheless, data are conflicting as shown in a recently published study by Colucci et al. [3]. In this randomized phase III trial, the addition of weekly cisplatin to gemcitabine failed to demonstrate any improvement as first-line treatment of advanced pancreatic cancer.

Therefore, there is a strong need for effective treatment modalities. With respect to molecular biology, the epidermal growth factor receptor (EGFR) has been shown to play an important role in carcinogenesis of pancreatic cancer [4, 5]. Moore et al. [6] reported a statistically significant overall survival benefit of 0.33 months for erlotinib, an oral reversible inhibitor of EGFR tyrosine kinase, in combination with gemcitabine compared with gemcitabine alone for first-line therapy in patients with advanced pancreatic cancer. Cetuximab, a monoclonal antibody targeting the EGFR, also resulted in promising activity in preclinical and early clinical trials [7, 8]. Thus, we recently conducted a phase II study to assess the efficacy and safety of cetuximab plus the combination of gemcitabine/oxaliplatin in metastatic pancreatic cancer [9]. In 64 patients with metastatic pancreatic carcinoma the addition of cetuximab to the combination of gemcitabine and oxaliplatin was well tolerated and exhibited a high response rate (33%). The median time to progression-free survival was 3.9 months and overall survival was 7.1 months.

The aim of this study was to evaluate the relationship between KRAS mutations and response or survival in patients with metastatic pancreatic cancer treated with cetuximab plus chemotherapy, based on tumor samples available from the recently conducted study [9].

Methods

This study was conducted as part of a multicenter phase II trial of 64 patients with metastatic pancreatic cancer [9]. Eligible patients were treated with cetuximab in combination with gemcitabine and oxaliplatin until disease progression. All participants for mutation analysis gave written informed consent. Ethical approval for the retrospective use of paraffin material for the study was given by the ethical committee at the University of Regensburg.

Tumor material was available for the study from a total of 25/64 (39%) patients, of whom 10 were from Regensburg, 4 from Augsburg, 4 from Halle, 4 from Frankfurt and 3 from Munich. For the remaining cases, there were either no paraffin blocks available or there was not sufficient tumor tissue in the block for molecular analysis. Analyses of KRAS mutations were performed at the Institute of Pathology in Erlangen. All pathologic specimens were cut from formalin-fixed paraffin-embedded tumor blocks, and the HE-stained section was reviewed by one pathologist (A.H.). Tumor cells were manually microdissected from paraffin sections of 10-μm thickness under an inverted microscope to obtain a tumor cell population of 70%.

DNA was isolated using the QIAamp®DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany). Quantity and quality of the DNA were controlled using a spectral photometer (NanoDrop®, peQLab, Erlangen, Germany). Further details were described previously [10].

Statistical analysis was performed using the SPlus software (Insightful Corp., Seattle, Wash., USA). Comparisons of Kaplan-Meier curves were based on the two-sided log-rank test.

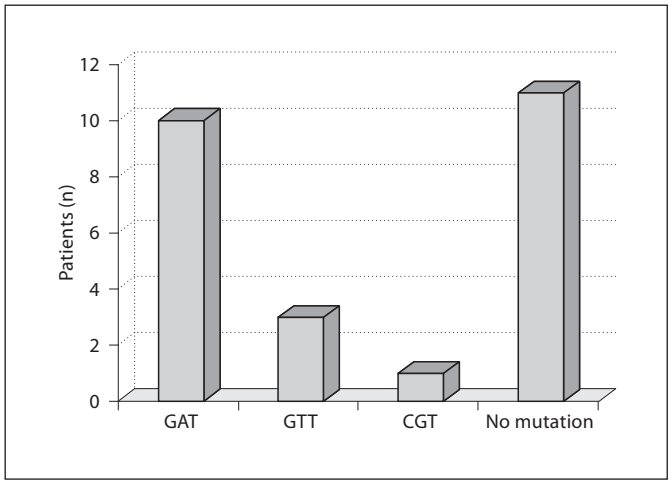

Fig. 1. Distribution of KRAS mutations.

Results

Baseline characteristics of the evaluable patient population are shown in table 1. Fourteen (56%) of the 25 pancreatic adenocarcinomas examined harbored a point mutation in the KRAS gene. All KRAS mutations occurred at codon 12. The distribution of different KRAS mutations is shown in figure 1. The most frequently observed point mutation at codon 12 was GAT Gly12Asp followed by GTT Gly12 Val.

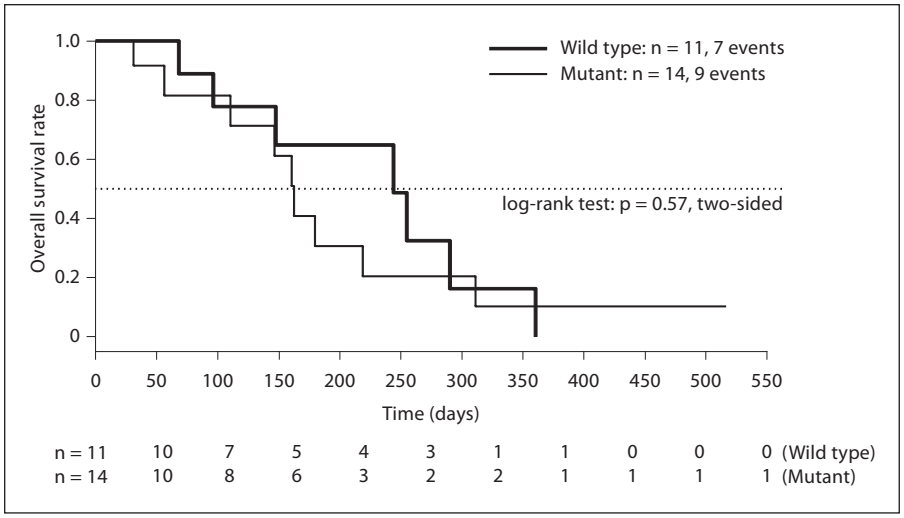

Fig. 2. Overall survival depending on KRAS mutation.

Table 1. Baseline characteristics

	Investigated subgroup of patients	Whole study population
Patients, n	25	62
Mean age (range), years	61.4 (37–72)	64.5 (31–78)
Male/female, n	19/6	41/21
Performance status, n		
70	2 (10%)	6 (12%)
80	9 (43%)	13 (25%)
90–100	10 (28%)	33 (63%)
Distant metastasis, n		
Lymph nodes (abdominal/pelvic)	1 (4%)	8 (1%)
Liver	23 (92%)	58 (93%)
Lung	4 (16%)	8 (1%)
Other	9 (36%)	22 (3%)
Adjuvant treatment (before start of trial), n	0	1

Table 2. Correlation of KRAS status with response (%)

	KRAS wild type (n = 11)	KRAS mutation (n = 13)	Total (n = 24)
Complete response	–	–	–
Partial response	2 (18)	5 (38)	7 (28)
Stable disease	4 (36)	1 (8)	5 (20)
Progressive disease	5 (45)	7 (54)	12 (50)

The presence of KRAS point mutations at codon 12 adversely influenced median survival time [162 vs. 263 days, KRAS mutation (+ vs. –), fig. 2], but the difference did not reach statistical significance (p = 0.57; HR 1.33; 95% CI 0.49–3.61). The overall survival rate after 6 months was 65% (95% CI 39–100%) in the wild-type group versus 31% (95% CI 12–78%) in the group with KRAS codon 12 mutations.

Median progression-free survival was 104 days in the wild-type group compared to 118 days within the group with KRAS mutation (p = 0.63; HR 0.79). In the wild-type group, progression-free survival after 6 months was 20% (95% CI 6–69%) and 22% (95% CI 7–75%) in the group with KRAS codon 12 mutation. Correlation of the KRAS status with response is shown in table 2. A further analysis of our initial clinical phase II trial showed that the presence of a rash (≥grade 2) was significantly correlated with overall survival (fig. 3). Sixty-one patients could be included in this analysis. Skin toxicity was usually mild to moderate (grade 1 or 2) in 43 (70%) patients; only 6 (10%) developed a grade 3 acne-like rash. Patients developing any grade of rash had a significantly longer median overall survival of 237 days compared to 148 days for patients with no rash (p = 0.014; HR 2.37; 95% CI 1.16–4.85).

Discussion

Systemic chemotherapy has so far failed to demonstrate sufficient impact on the survival in patients with advanced pancreatic cancer. The EGFR pathway is a rational molecular target for an alternative therapeutic approach. After promising preclinical and clinical trials [7, 8] findings of further phase II and III studies suggest that

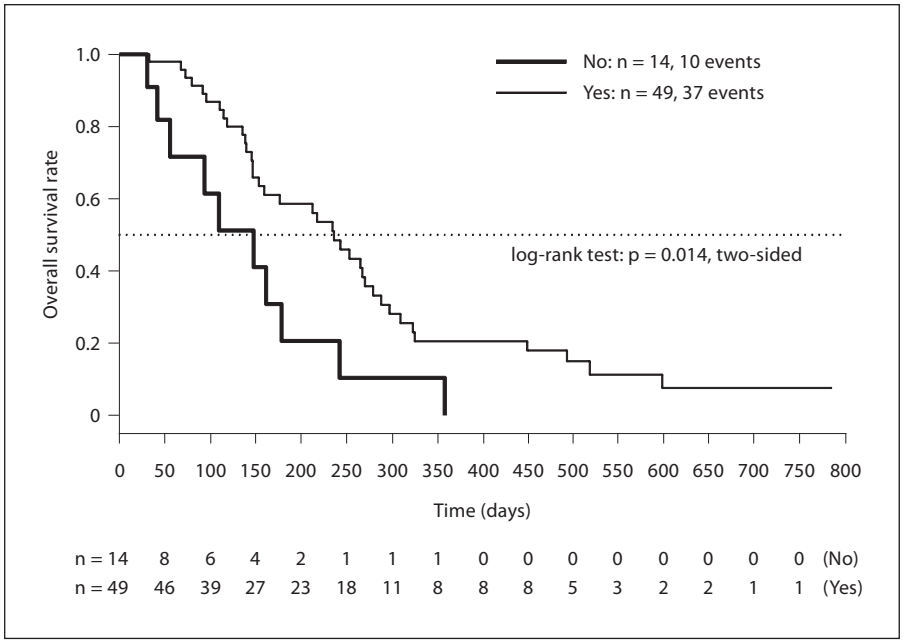

Fig. 3. Overall survival depending on rash.

cetuximab does not add any valuable activity to chemotherapy with gemcitabine or a combination of gemcitabine and cisplatin/oxaliplatin [9, 11, 12].

Cascinu et al. [11] reported data of a phase II trial in which patients with advanced pancreatic cancer were randomly assigned to treatment with cetuximab plus gemcitabine and cisplatin or chemotherapy alone. Sixty-one of 84 (73%) patients had metastatic disease. Seven of 40 (17.5%) patients had an objective response rate in the cetuximab group and 5/41 (12.2%) in the noncetuximab arm. No significant differences between the groups were noted in median progression-free survival or in median overall survival. Median progression-free survival was 3.4 months in the cetuximab group and 4.2 months in the noncetuximab group. Median overall survival was 7.5 and 7.8 months, respectively. Interestingly, toxic effects were not increased by cetuximab and at least 33/61 (54%) patients with metastastic disease received a second-line fluorouracil-based chemotherapy. The findings of Cascinu et al. [11] are in agreement with a phase III study evaluating cetuximab in combination with gemcitabine compared to gemcitabine alone in advanced pancreatic cancer. Seven hundred and thirty-five patients were randomly enrolled in this latter trial and 78% had metastatic disease. The median survival was 6 months in the gemcitabine arm and 6.5 months in the gemcitabine plus cetuximab arm, which fails to demonstrate a clinically significant advantage of the addition of cetuximab to gemcitabine.

In our study on 64 patients with metastatic pancreatic carcinoma, the addition of cetuximab to the combination of gemcitabine and oxaliplatin was well tolerated and exhibited a high response rate (33%). The median time to progression-free survival was 3.9 months and overall survival was 7.1 months. Again, these findings do not seem to be superior to the results achieved in the previous studies of gemcitabine and oxaliplatin alone.

What are the reasons for inactivity of cetuximab in pancreatic cancer and are there possibly predictive factors for response? No clinical studies evaluating efficacy of cetuximab molecular characterization including EGFR mutation have been reported so far.

Seventy to 100% of ductal adenocarcinoma exhibit KRAS mutations and all mutations were located in codon 12 [13, 14]. Differences were found between the type of mutations [14, 15]. Immervoll et al. [16] screened a series of 43 formalin-fixed paraffin-embedded ductal adenocarcinoma of the pancreas. When DNA was extracted from whole tissue sections, KRAS codon 12 mutations were detected in 67% of the tumors. When cancerous ducts were isolated by laser-assisted microdissection, 91% were positive for KRAS mutations. In the present study we performed manual microdissections and obtained a tumor cell population of 70% in every case. However, in many cases mutation analyses were done on a limited number of cells. The mutation rate in the present study was on a lower limit compared to previous studies.

There was a trend that survival after diagnosis varied according to the KRAS mutation subtype. Patients with the Gly12Val mutation survived much longer (23.5 months) than patients with Gly12Asp mutations (9.5 months). Lee et al. [17] analyzed EGFR mutation by DNA sequencing of exon 18–21 in the tyrosine kinase domain in 65 pancreatic cancer patients. Thirty-two (49%) of the 65 pancreatic adenocarcinomas examined harbored a point mutation in the KRAS gene. All KRAS mutations occurred at codon 12. The most frequently observed point mutation was Gly12Val. The presence of KRAS point mutations at codon 12 adversely influenced median survival time (9.1 vs. 13.4 months, KRAS mutation (+ vs. –), $p = 0.03$).

To our knowledge, this is the first study assessing the relationship between KRAS mutations and response or survival in patients with metastatic pancreatic cancer treated with cetuximab plus chemotherapy. Overall survival was longer in wild-type patients compared to patients with KRAS mutations (263 vs. 162 days). The difference did not reach statistical significance probably due to small sample size.

This is supported by recently reported preliminary data of the German AIO group on the role of KRAS mutation in erlotinib-treated patients with advanced pancreatic cancer [18]. Within this prospective multicenter phase III trial 281 patients were randomly assigned to first-line treatment with either capecitabine plus erlotinib or gemcitabine plus erlotinib. Tissue samples were available from 204 patients. 123 tumors (70%) harbored a somatic KRAS mutation. Patients with KRAS wild type had a longer overall survival (wild type: 8.0 months vs. mutation: 6.6 months, HR 1.62, $p = 0.011$). On the other hand, in a molecular subset analysis of patients from the NCIC CTG PA.3, the EGFR gene copy number and KRAS mutation status were not identified as markers predictive of a survival benefit from the combination of erlotinib with gemcitabine for the first-line treatment of advanced pancreatic cancer [19].

Taken together, our results and others reported recently in the literature provide evidence that KRAS mutation in codon 12 is possibly associated with reduced survival compared to KRAS wild type. KRAS mutation could be a predictive marker in patients with advanced pancreatic cancer analogous to colorectal cancer patients treated with cetuximab. As our study was a single-arm trial with no cetuximab-free control arm, it could be possible that KRAS is just a prognostic marker and not predictive of cetuximab efficacy in pancreatic cancer. The primary limitation of the current study was as already mentioned above the low number of samples suitable for molecular analysis. Small sample size and potential selection bias make it difficult to draw firm conclusions.

Therefore, the role of EGFR targeting therapy in patients with KRAS wild-type pancreatic cancer warrants further investigation in larger trials to exclude an epiphenomenon and confirm efficacy.

A further analysis of our clinical phase II trial showed that the presence of rash was significantly correlated with overall survival, consistent with the results of Xiong et al. [8]. A similar relationship between rash and clinical outcome could be demonstrated in a large phase III study for patients with advanced pancreatic cancer treated with erlotinib [6]. The reasons for the development of a rash are not fully understood. Possible explanations include differences in drug exposure, integrity of the immune system or EGFR polymorphisms [20].

These findings support the vision of a more individualized cancer treatment.

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