

Review Article

Optimizing EGFR Targeted Therapy in Pancreatic Cancer

Baxevanos P¹ and Fountzilias C^{2*}¹Athens Naval Hospital and Veterans Medical Center, Greece²Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, USA

*Corresponding author: Fountzilias C, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, USA

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Abstract

Pancreatic cancer is one of the major causes of cancer related death in the western hemisphere. Despite years of research effort, advanced disease remains incurable with median survival of less than a year. Systemic therapy targeting the epidermal growth factor receptor (small molecule tyrosine kinase inhibitors and monoclonal antibodies) has promising preclinical activity but clinical efficacy has been proven to be modest at best. In this paper, we review relevant clinical trials and discuss potential solutions for anti-EGFR therapy.

Keywords: Pancreatic cancer; EGFR; Therapy optimization; Drug exposure; Biomarkers

Abbreviations

EGF/EGFR: Epidermal Growth Factor/Epidermal Growth Factor Receptor; FcGR: Fc Gamma Receptors; FDA: Food and Drug Administration; FISH: Fluorescence In Situ Hybridization; HER2: Human Epidermal Growth Factor Receptor2; 5-FU: 5-Fluorouracil; IGFR: Insulin-like Growth Factor Receptor; IHC: Immunohistochemistry; K-RAS: Kirsten Rat Sarcoma; nab: Nanoparticle Albumin Bound; mAb: Monoclonal Antibody; NCCN: National Comprehensive Cancer Network; NCIC-CTG: The National Cancer Institute of Canada Clinical Trials Group; OS: Overall Survival; PC: Pancreatic Cancer; PCR: Polymerase Chain Reaction; PFS: Progression-Free Survival; RET: Rearranged During Transfection; TKI: Tyrosine Kinase Inhibitor; VEGF/VEGFR: Vascular Endothelial Growth Factor/Vascular Endothelial Growth Receptor

Introduction

Pancreatic cancer (PC) is the fourth most common cause of cancer-related death in the United States with an estimated 5-year survival rate of 8% [1]. Given the absence of early specific signs and symptoms and effective screening methods, PC is most frequently diagnosed at advanced stages (locally advanced or metastatic) [1]. In the late 1990s, gemcitabine was established as an active agent in advanced PC— mostly because of its palliative potential compared to 5-fluorouracil (5-FU)— and became the standard of care and basis for further systemic therapy development [2]. The culmination of research for advanced PC over next two decades was the combination of oxaliplatin/irinotecan/5-FU (FOLFIRINOX) and gemcitabine plus nanoparticle albumin bound (nab)-paclitaxel as the most appropriate first-line options only in patients with good performance status [3,4]. Both approaches significantly prolonged overall survival (OS) compared to Gem alone but still most patients with advanced PC succumb to their disease after a median of 11 months [3]. The epidermal growth factor receptor (EGFR/HER1) is another potential target in PC as it is over expressed in up to 60% of cases [5] and EGFR inhibition was shown to have antitumor activity in preclinical PC models [6]. Erlotinib, a small molecule tyrosine kinase inhibitor (TKI) that can selectively target EGFR, has also resulted in a statistically significant prolongation of OS in combination with gemcitabine compared to gemcitabine as single agent [7]. This combination is

approved by the National Comprehensive Cancer Network (NCCN) for the treatment of advanced PC but infrequently used by oncology providers given the modest prolongation of survival (less than 1 month) that was achieved [7,8].

Nevertheless, anti-EGFR targeted therapy with either TKIs or monoclonal antibodies remains a promising therapeutic approach, as long as we achieve better insight into the specific mechanisms that will help us use these agents in patients who are likely to benefit the most. In this manuscript, we will review major findings from phase 2 and 3 studies incorporating anti-EGFR therapy in advanced PC. Our purpose is to provide considerations regarding better patient selection and further development.

Summary of Clinical Findings

Erlotinib is a first generation EGFR TKI and presents the only Food and Drug Administration (FDA) approved targeted agent for advanced PC. The National Cancer Institute of Canada (NCIC) Clinical Trials Group (CTG) PA. 3 study was a randomized, placebo-controlled phase 3 trial (N=569) comparing the erlotinib/gemcitabine combination to gemcitabine alone [7]. Median OS and progression-free survival (PFS) were superior in the active drug group (6.24 vs. 5.91 months, P=0.038; and 3.75 months v 3.55 months, P=0.004 respectively). There was an 18% relative decrease in the risk of death and 23% relative decrease in the risk of progression or death with erlotinib therapy. Individuals assigned to erlotinib therapy had more episodes of diarrhea and rash even though mild to moderate in severity; diarrhea secondary to erlotinib did appear to affect patients' quality of life.

Gemcitabine with or without erlotinib has also been tested in patients with locally advanced disease. The LAP 07 study was a phase3 trial (N=449) randomizing patients with advanced, inoperable, non-metastatic PC to gemcitabine with or without erlotinib (first randomization); individuals who were progression-free after 4 months of therapy were randomized to continuation of the same regimen or concurrent chemo radiotherapy plus capecitabine [9]. This was a negative study for both the primary— the median OS from first randomization was 13.6 months for the gemcitabine/erlotinib combination vs. 11.9 months for gemcitabine monotherapy, P=0.09— and secondary outcomes – OS from second randomization and PFS

Table 1: Major clinical trials examining the role of EGFR TKIs in initial therapy of advanced PC.

| Trial | Phase | N | Population | Intervention | Primary outcome | Selected secondary outcomes |
|-------------------------|-------|-----|--------------------------------|------------------------------------|--|---|
| Moore et al, [7] | III | 569 | Locally advanced or metastatic | Gem+ E vs. Gem | OS (median): 6.24 months for Gem+ E vs. 5.91 months for Gem (S) | PFS (median): 3.75 months for Gem+ E vs. 3.55 months for E (S) |
| Hammelet al, [9] | III | 449 | Locally advanced | 1. Gem+ E vs. Gem 2. CT vs. CRT | OS (median): 13.6 months for Gem+ E vs. 11.9 months for Gem (NS) | OS (median): 16.5 months for CT vs. 15.2 months for CRT (NS) |
| Vaccaro et al, [58] | II | 46 | Locally advanced or metastatic | Gem+ E | PFS (median): 14 weeks | OS (median): 26 weeks ORR: 10.9% DCR: 56.5% |
| Fountzilias et al, [59] | II | 54 | Locally advanced or metastatic | Gem+gefitinib | 6-month PFS: 30% | ORR: 11% OS (median): 7.3 months |
| Lopez et al, [60] | II | 32 | Metastatic | Cape+E | ORR: 6% | PFS (median): 2.1 months and OS (median): 4.3 months |
| Hwang et al, [61] | II | 22 | Locally advanced or metastatic | Cisplatin+ Gem+ E | ORR: 26% | OS (median): 6.8 months |
| Oh et al, [62] | II | 47 | Metastatic | Gem+ Cape+ E | ORR: 32.6% DCR: 83.7% | PFS (median): 6.5 months OS (median): 12 months |
| Feliu et al, [63] | II | 42 | Locally advanced or metastatic | Gem+ E | ORR: 28% | Safety established PFS (median): 5 months OS (median): 8 months |
| Wang et al, [22] | II | 88 | Metastatic | Gem+ E vs. Gem | DCR: 64% for Gem+E vs. 25% for Gem | PFS (median): 3.8 months for Gem+ E vs. 2.4 months for Gem OS (median): 7.2 months for Gem+ E vs. 4.4 months for Gem Significance of EGFR mutations |
| Katopodis et al, [64] | II | 71 | Locally advanced or metastatic | Gem+ Oxali+ E | ORR: 21% | PFS (median): 5.2 months OS (median): 10.5 months |

Cape: Capecitabine; CRT: Chemoradiotherapy; CT: Chemotherapy; DCR: Disease Control Rate; E: Erlotinib; EGFR: Epidermal Growth Factor Receptor; Gem: Gemcitabine; NS: Non-Significant; ORR: Overall Response Rate; OS: Overall Survival; Oxali: Oxaliplatin; PFS: Progression-Free Survival; S: Significant; TKI: Tyrosine Kinase Inhibitor

from first and second randomization were not different between groups. Moreover, EGFR TKIs have been tested in several phase II trials as a partner of variable chemotherapeutic drugs. A summary of the results of clinical trials with EGFR TKIs in advanced PC as first-line therapy is presented in (Table 1).

Cetuximab is a chimeric IgG₁ monoclonal antibody (mAb) targeting the EGFR (the extracellular domain III) and has been extensively evaluated in advanced PC. In a phase II trial (N=41) patients with advanced PC expressing EGFR by immunohistochemistry (IHC) were treated with cetuximab and gemcitabine at the standard doses [10]. The overall response rate was 12%; 63% of the patients had stable disease. The median OS was 7 months. The encouraging results of this study led to a phase III study conducted by the Southwest Oncology Group (S0205) [11]. S0205 randomized 745 patients with advanced PC to gemcitabine plus cetuximab vs. gemcitabine alone; EGFR positivity by IHC was not a prerequisite for eligibility but was determined in the majority of enrolled patients. The study was negative for the primary outcome for OS (median OS 6.3 months with the combination vs. 5.9 months with gemcitabinemonotherapy, hazard ratio: 1.06; P=0.19). Similarly PFS appeared similar between groups (3.4 vs. 3 months in the monotherapy group). EGFR positivity by IHC did not appear to affect outcomes.

Nimotuzumab is a humanized IgG₂ mAb that targets the extracellular domain III of EGFR. In a phase 2 trial, 54 patients with advanced PC were treated with nimotuzumab after progression on first-line therapy [12]. In this heavily pretreated population (approximately 50% of the patients had already received 2 or more lines of systemic therapy) OS was almost 4.5 months; best response was stable disease in 6 patients. Mild rash (grade 1) and constitutional symptoms (grade 1or2) were the most common adverse events related to nimotuzumab. Nimotuzumab was combined with gemcitabine as

first-line therapy in a small (N=18) phase 2 trial; the combination was safe and well tolerated [13]. The median OS was 9.3 months. These trials led to a larger, randomized, placebo controlled, phase 2 trial evaluating gemcitabine with or without nimotuzumab [14]. One hundred ninety-two patients were randomized; the 1-year OS was 19.5% in the placebo and 34.4% with active drug arm (HR=0.69; P = 0.034). PFS also favored the nimotuzumab group. Older patients (older than 62 year) appeared to obtain significant benefit from therapy.

The reason for differential cetuximab and n-inotuzumab antitumoral activity observed to date is still unclear. Both antibodies bind the extracellular domain III of EGFR but nimotuzumab binds within an area overlapping with both cetuximab and EGF binding site and in contrast to cetuximab allows EGFR to adopt its active conformation [15]. In addition, the bivalent binding required for stable nimotuzumab attachment to EGFR may preferentially direct the antibody to cells with the highest EGFR expression, such as the tumor cells [16]. The results of clinical trials evaluating anti-EGFR mAbs in advanced PC are summarized in (Table 2).

Discussion

Clinical efficacy of EGFR-targeted therapy in PC with either TKIs or mAbs is disputable and many questions regarding the disappointing or modest results of clinical trials remain unanswered. First, it must be noted that this approach has been studied mostly in molecularly unselected patients, while the exploratory analyses of many trials for discovery of predictive biomarkers did not allow firm conclusions. The PA. 3 trial included EGFR analysis by IHC in 162 patients, defining EGFR positivity as at least 10% tumor cell membranous staining; S0205 defined EGFR positivity as any positive tumor cell membrane staining [7,11]. Both trials failed to show an

Table 2: Major clinical trials examining the role of EGFR mAbs in initial therapy of advanced PC.

| Trial | Phase | N | Population | Intervention | Primary outcome | Selected secondary outcomes |
|-----------------------|-------|-----|---|---|---|---|
| Philip et al, [11] | III | 745 | Locally advanced or metastatic | Gem+/- Cet | OS (median): 6.3 months for Cet vs. 5.9 months for control (NS) | PFS (median): 3.4 months for Cet vs. 3.0 months for control arm (NS) ORR: 12% for Cet vs. 14% for control arm (NS) |
| Strumberg et al, [14] | II | 192 | Locally advanced or metastatic | Gem+/-Nimo | 1-year OS: 34.4 for Nimo vs. 19.5% for control (S) | 1-year PFS: 21.5% for Nimo vs. 9.5% for control (NS) |
| Xiong et al, [10] | II | 61 | Locally advanced or metastatic | Gem+Cet | ORR:12.2% | PFS (median): 3.8 months OS (median): 7.1 months |
| Cascinu et al, [65] | II | 84 | Locally advanced or metastatic | Cisplatin +Gem +/- Cet | ORR: 17.5% vs. 12.2% for the control | DCR: 55% for Cet vs 58.5% for control (NS) PFS (median): 3.4 months for Cet vs. 4.2 months for control (NS) OS (median): 7.5 months for Cet vs. 7.8 months for control (NS) |
| Kullmann et al, [31] | II | 64 | Metastatic | Gem-Oxali-Cet | ORR: 33% | OS (median): 213 days |
| Merchan et al, [66] | II | 41 | Locally advanced or metastatic | Gem-Oxali-Cet | PFS (median): 6.9 months | ORR: 24% OS (median): 11.3 months |
| Fiore et al, [67] | II | 21 | Locally advanced or borderline resectable | Gem+ Cet+ RT | Feasibility established | ORR=24% OS (median): 15.3 months |
| Esnaola et al, [68] | II | 37 | Locally advanced or borderline resectable | Gem-Oxali-Cet followed by CRT | PFS (median): 10.4 months | OS (median): 11.8 months |
| Crane et al, [69] | II | 69 | Locally advanced | Gem-Oxali-Cet followed by CRT incorporating Cet | OS (median): 19.2 months | ORR: 18% DCR: 76% |

Cet: Cetuximab; CRT: Chemoradiotherapy; CT: Chemotherapy; DCR: Disease Control Rate; EGFR: Epidermal Growth Factor Receptor; Gem: Gemcitabine; Mab: Monoclonal Antibody; Nimo: Nimotuzumab; NS: Non-Significant; ORR: Overall Response Rate; OS: Overall Survival; Oxali: Oxaliplatin; PFS: Progression-Free Survival; S: Significant

obvious role of EGFR positivity by IHC as predictive biomarker and this may be related to the EGFR positivity definition and assays used. Higher IHC cutoffs (e.g. 3+) and/or other more standardized assays such as polymerase chain reaction (PCR) or fluorescent in situ hybridization (FISH) could have been more appropriate methods. FISH-positivity (defined as high polysomy and/or gene amplification) was evaluated as a prognostic/predictive marker in the PA. 3 study [17]. FISH-positivity was determined retrospectively in 107 patients; almost half of the patients were EGFR-positive. EGFR-positivity by FISH was not predictive of erlotinib efficacy in terms of OS (P for interaction = 0.32).

Activating EGFR mutations (e.g. mutations affecting the tyrosine kinase domain) in exons 19 and 21 are predictive of TKI benefit in lung cancer [18]. The presence of activating EGFR mutations in PC is rare; exon 20 mutations have been reported [19-21]. In a prospective randomized phase 2 (N=88) trial of erlotinib/gemcitabine vs. single-agent gemcitabine, 56% of the enrolled patients had tumors harboring EGFR mutations; presence of an EGFR mutation appeared predictive for OS (8.7 months vs. 6 months for monotherapy, P = 0.044) [22]. It's noteworthy that EGFR mutations were detected and confirmed by next generation sequencing in an unprecedented high percentage of patients; half of those mutations were in exon 20. This high frequency of EGFR mutations can be an effect of ethnic variations and environmental background. MARK was a randomized, phase 2 trial evaluating erlotinib vs. placebo in patients with advanced, pretreated PC or as first-line therapy in patients deemed unsuitable for chemotherapy [23]. No patients had tumors with EGFR activating mutations. The short EGFR CA-SSR1 polymorphism was suggested as a potential biomarker for erlotinib efficacy; high serum amphiregulin—an EGFR ligand—also appeared to be predictive of superior OS and PFS with erlotinib therapy. EGFR polymorphism A61G (rs4444903) is

another potential biomarker as suggested by a subgroup analysis of S0205 even though evidence is weak [24].

Kirsten rat sarcoma (K-RAS) is a major downstream effector molecule for many different surface growth factor receptors including EGFR. K-RAS mutations in exons 2, 3 and 4 are negative predictive markers of anti-EGFR mAbs in colorectal cancer [25]. Mutational activation of the K-RAS oncogene appears in approximately 95% of patients with PC [26]. Thus, the infrequency of wild type K-RAS (wt-K-RAS) in PC renders its predictive role in EGFR targeted therapy extremely difficult to be investigated. However, the presence of specific K-RAS mutations (in codon 12) as predictive markers of responsiveness to cetuximab has been suggested [27].

Apart from EGFR inhibition, anti-EGFR mAbs can exert their antitumor efficacy through antibody dependent cellular cytotoxicity (ADCC) that is mediated through the binding of fragment c (Fc) portion of IgG₁ to Fc gamma receptors (FcGR) in the surface of innate immune cells such as natural killer (NK) cells [28]. NK cells are dysfunctional and have impaired cytotoxic activity in PC [29]. NK stimulating cytokines such as IL-2, IL-12, and IL-21 can enhance cetuximab efficacy against EGFR positive malignant cells including pancreatic cancer [30]. Further, specific polymorphisms of FcGR gene (H131R and V158F) have been associated with improved PFS with cetuximab therapy in patients with metastatic colorectal cancer. This emphasizes the complexities of molecular selection, as both tumor and patient genome should be taken into account. To the best of our knowledge, there are no relevant published studies regarding the predictive value of FcGR polymorphisms in PC. Further research in this field of antitumor immunity is warranted for optimization of anti-EGFR mAb treatment.

Second, insufficient drug dose has been implicated as a potential

contributing factor for the observed modest efficacy of anti-EGFR therapies. Skin toxicity is a pharmacodynamic surrogate for optimal EGFR inhibition (on-target/off-tumor effect); development of rash has been associated with improved outcomes in PC [7,31,32]. In a phase 2 study, 49 gemcitabine-pretreated patients were treated with erlotinib; planned dose escalation to rash was feasible in 10 patients (20%) [33]. The highest dose achieved was 300mg daily. Grade 3 or worse rash and diarrhea occurred in 4% and 4% of the patients respectively. The best response was stable disease in 32% of the patients; median OS was 3.8 months. Erlotinib dose escalation has been also prospectively evaluated in combination with gemcitabine in a randomized phase 2 study (RACHEL) in patients with advanced, treatment-naïve PC [34]. After a 4-week run in period, patients with rash grade 0/1 were randomized to continuation of standard dose erlotinib vs. dose escalation up to development of rash grade 2 or higher. Four hundred sixty-seven patients were enrolled, of those 146 patients were randomized. OS from was not statistically different between the 2 groups (median OS: 8.4 vs. 7.0 months for standard and dose-escalation groups respectively, $P = 0.2$).

Further, inadequate erlotinib dosing may be related to differential metabolism in smokers compared to non-smokers, with smokers achieving lower erlotinib concentrations [35]. As 60-70% of patients with PC are active or current smokers [36,37], the effect of smoking on erlotinib metabolism is of potential significance. Indeed, never smokers have higher concentrations of erlotinib and its metabolites compared to active or past smokers [33,38]. It also appears that never smokers may have improved outcomes compared to past or current smoker [38]. Moreover, erlotinib bioavailability is affected by gastric pH, with acidic pH being necessary for absorption [39,40]. Use of acid suppressive therapy has been implicated in decreased erlotinib absorption [41]. In addition, gastric hypochlorhydria after removal of the gastric antrum with removal of gastrin-secreting cells is a potential consideration [42,43]. It remains to be determined if limited erlotinib absorption is clinically relevant in PC.

In an effort to optimize EGFR targeted therapy, attempts have been made to simultaneously target downstream mediators or parallel signaling pathways. MEK is a protein kinase downstream of RAS; dual EGFR/MEK inhibition is more effective than EGFR inhibition alone in preclinical models, an effect that is more pronounced in wt-K-RAS cells [44]. Selumetinib is an oral MEK 1/2 inhibitor that in combination with erlotinib as second-line therapy in patients with advanced PC resulted in a disease control rate of 41% with almost 25% of the patients having disease stability for at least 3 months [45]. OS at 6 and 12 months was 58% and 23% respectively. An epithelial phenotype as documented by preserved E-cadherin expression by IHC was associated with a biochemical response (e.g. decrease in CA19-9 levels). Eighty-five percent of the patients had K-RAS mutations detected in plasma cell-free DNA analysis with an apparent improved biochemical response in patients where mutant K-RAS was not detected in plasma.

Other combination strategies have been largely unsuccessful to date. HER2, the second member of the HER receptor family, is over expressed in 20% of PC cases [46]. Trastuzumab, a mAb against HER2, in combination with gemcitabine was shown to be active in preclinical PC models expressing HER 2+3 by IHC compared to

+1/+2. Further, dual HER1/HER2 inhibition with mAbs has been proven more efficacious to monotherapy *in vivo* [47]. A trial of lapatinib, a reversible oral dual HER 1/2 inhibitor, plus gemcitabine in previously untreated patients with advanced PC was terminated after enrollment of 29 out of the planned 125 patients for futility [48]. In another small (N=17) phase 2 trial, lapatinib plus capecitabine in previously treated patients with advanced disease, 6 patients had stable disease for at least 3 months; there were no objective responses [49]. Similarly, cetuximab/trastuzumab combination therapy in a phase 1/2 trial (THERAPY) in pretreated patients did not appear to significantly improve outcomes compared to historical controls as the median PFS and OS was 1.8 and 4.6 months respectively [50].

Simultaneous inhibition of angiogenesis was also expected to improve outcomes based on the results of dual EGFR/vascular endothelial growth factor receptor (VEGFR) inhibition *in vivo* [51]. A multicenter, randomized, double blind, placebo-controlled phase 3 trial (N=607) assessed the potential benefit of bevacizumab– a mAb that targets VEGF-A– when added to erlotinib plus gemcitabine in treatment-naïve patients with advanced PC [52]. The study was negative for the primary outcome of OS; the median was 7.1 months in the bevacizumab group and 6 months in the placebo group, respectively ($P = 0.2087$). Similarly, the addition of vandetanib– a TKI with dual activity against EGFR, VEGFR-2 and rearranged during transfection (RET)– to gemcitabine failed to demonstrate a survival benefit in a randomized, double blind, placebo-controlled phase II randomized trial (ViP trial) [53]. One hundred forty-two patients were enrolled, the median OS was 8.83 and 8.95 months in the vandetanib and placebo arm respectively ($P = 0.303$). Presence of RET polymorphisms and expression by IHC were evaluated as potential biomarkers for response but both did not appear to affect outcomes in multivariable analysis.

The insulin growth factor receptor (IGFR)-1 is over expressed in the majority of PC cases [54] and IGFR-1 inhibition can effectively inhibit tumor growth in PC xenografts [55]. IGFR-1 activation has been implicated in EGFR-targeted therapy resistance in a variety of tumors, mostly breast cancer [56]. S0727 was a randomized phase 2 trial with a run in phase evaluating the anti-IGFR-1 antibody cixutumumab in combination with erlotinib plus gemcitabine [57]. The control group was erlotinib plus gemcitabine. The study did not meet the primary outcome of PFS. The PFS in both groups was 3.6 months ($P = 0.97$); OS was also similar in between groups (7 months versus 6.7 months in the control arm). The absence of strong preclinical data specifically for PC may have been responsible for this failure of this study.

Conclusions

Despite promising preclinical data, EGFR inhibition with small molecule EGFR TKI inhibitors or mAbs has been a largely unsuccessful treatment strategy in advanced PC. Inadequate drug dosing as well as patient selection criteria may have contributed to the modest improvement in outcomes with the addition of erlotinib to gemcitabine in the first-line setting. Attempts to simultaneously inhibit multiple pathways and/or downstream molecules have demonstrated no obvious improvement to date with the exclusion of dual EGFR/MEK inhibition. Given the fact that EGFR TKIs are better tolerated compared to cytotoxic agents and multi-agent cytotoxic

chemotherapy is warranted only for patients with a good performance status, further development of these agents in PC is warranted taking into account lessons learned from previous unsuccessful attempts. Furthermore, novel and potentially more potent anti-EGFR therapies such as nimotuzumab should be evaluated in combination with modern, life-extending combination regimens such as gemcitabine with nab-paclitaxel.

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