**Phase II, randomized, biomarker identification trial (MARK) for erlotinib in patients with advanced pancreatic carcinoma**

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**Background:** A prospective, randomized phase II study, with mandatory tumor sampling at current disease stage, aimed to identify biomarkers predictive of improved progression-free survival (PFS) in patients with pancreatic cancer treated with erlotinib.

**Patients and methods:** Patients with histologically/cytologically confirmed, unresectable, locally advanced/metastatic pancreatic cancer, who had failed on or were unsuitable for first-line chemotherapy, underwent a tumor biopsy and were then randomized to receive once-daily erlotinib 150 mg or placebo. The primary end point was identification of biomarkers predicting improved PFS with erlotinib. Secondary end points included PFS, overall survival, response and toxicity.

**Results:** At data cut-off, 207 patients were enrolled and analyzed. Prespecified biomarker analyses of EGFR protein expression, EGFR gene copy number/mutations/polymorphisms and KRAS mutations did not identify any subgroups with a detrimental effect or a strong benefit for PFS with erlotinib. In the primary analysis, the median PFS was 6.1 versus 5.9 weeks in the erlotinib and placebo arms, respectively [hazard ratio (HR) 0.83; 95% confidence interval (CI) 0.63–1.10; P = 0.1909]. However, observed baseline imbalances indicated worse prognosis in the erlotinib arm. After adjustment for baseline characteristics, a significant PFS benefit for erlotinib was observed (HR 0.68; 95% CI 0.50–0.91; P = 0.0102). Exploratory biomarker analyses showed patients with high baseline serum amphiregulin levels might benefit from erlotinib.

**Conclusion:** This study in patients with inoperable pancreatic cancer did not identify any prespecified biomarkers predictive of PFS benefit with erlotinib. Exploratory analyses suggested high amphiregulin might predict PFS benefit from erlotinib.

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**Key words:** pancreatic cancer, biomarkers, erlotinib, predictive markers, personalized therapy

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changes to oncogenes and tumor suppressor genes, as well as activation of pathways involved in cell-cycle control, are considered to have a role in the development of pancreatic cancer [1]. Molecularly targeted therapies may therefore have potential for improving outcomes in advanced pancreatic cancer [2]. Erlotinib (Tarceva®, F. Hoffmann-La Roche Ltd, Basel, Switzerland) is an epidermal growth factor receptor (EGFR) tyrosine-kinase inhibitor that was approved in combination with gemcitabine as first-line therapy for locally advanced or metastatic pancreatic cancer based on the results of the pivotal phase III PA.3 trial [3].

The survival prolongation from erlotinib in PA.3 was modest. An exploratory analysis of outcomes to identify predictive biomarkers, specifically KRAS mutation status and EGFR gene copy number, in the 26% of patients in PA.3 for whom tissue was available, showed longer survival for patients whose tumors had a low EGFR gene copy number [assessed by fluorescence in situ hybridization (FISH)], although this did not reach statistical significance [4]. An exploratory study in the second-line setting found that erlotinib was associated with disease stabilization in 3 out of 10 patients, which persisted for up to 12 months [5]. These observations suggested that single-agent erlotinib might have benefit in selected patients and there may be predictive biomarkers that could identify such patients.

This ongoing randomized, phase II, double-blind, placebo-controlled, biomarker identification, post-approval commitment trial for erlotinib in patients with advanced pancreatic carcinoma (BO21129; bio Marker trial; MARK) was designed to identify biomarkers that might predict improved progression-free survival (PFS) in response to erlotinib. In order to gage the true predictive value of the biomarkers being assessed, it was necessary to administer erlotinib as monotherapy in a placebo-controlled setting; therefore, the trial was designed to administer erlotinib predominantly as a second-line treatment and included only those patients who had either already received first-line gemcitabine-based therapy for advanced pancreatic cancer or were unsuitable to receive such therapy.

patients and methods

patients

Eligible patients were aged ≥18 years with histologically or cytologically confirmed, unresectable, locally advanced or metastatic pancreatic cancer who had measurable disease (by Response Evaluation Criteria in Solid Tumors (RECIST)) that was accessible for biopsy, and who had failed on prior chemotherapy or were deemed unsuitable for first-line chemotherapy. All patients had Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2, a life expectancy of ≥6 weeks and adequate hematologic, renal and hepatic function. Key exclusion criteria included resectable pancreatic cancer, other malignancies within the last 5 years (except adequately treated cervical cancer, or basal or squamous-cell skin cancer) or major surgery <2 weeks before randomization. Spinal cord compression or central nervous system metastases were excluded by mandatory computed tomography scanning or magnetic resonance imaging in the 4 weeks before randomization.

study design

The MARK study was an international, phase II, multicenter, randomized, placebo-controlled, double-blind study in patients with advanced pancreatic cancer. Mandatory tumor biopsies were obtained after screening to establish baseline biomarker status. Serum samples were collected from patients at baseline, 3 weeks after commencing erlotinib and after treatment discontinuation. Patients were randomly assigned to receive erlotinib 150 mg/day or placebo. Dose reductions were permitted for toxicity that could not be controlled by optimal supportive care. Treatment continued until disease progression, unacceptable toxicity, withdrawal or death. At disease progression, investigators could request unblinding of the randomized treatment; those receiving erlotinib were treated at the investigator’s discretion and those on placebo were eligible to receive open-label erlotinib 150 mg/day or other active treatment. The study was conducted in accordance with the principles of the Declaration of Helsinki and Guidelines for Good Clinical Practice. All patients provided written informed consent before trial entry and the study was approved by the ethics committee or institutional review board at each site.

efficacy, biomarker and safety analyses

The primary objective of the study was to identify biomarkers predictive of improved PFS in patients treated with erlotinib. Secondary efficacy end points were PFS in the overall population, overall survival (OS), objective response rate (as measured by RECIST) and disease control rate (DCR). Clinical assessments were conducted every 3 weeks during treatment until week 24, and every 12 weeks thereafter. Response assessments were carried out after 6 weeks of treatment, then every 6 weeks until week 24. After week 24, response assessments were carried out every 12 weeks. The same target lesions were to be assessed at each time point using the same imaging technique.

EGFR protein expression by immunohistochemistry (IHC), EGFR gene copy number by FISH, EGFR and KRAS mutations (both using commercially available cobas® tests and carried out at Roche Molecular Systems, Pleasanton, CA) were analyzed using tissue from the tumor samples taken at baseline (see supplementary material, available at Annals of Oncology online).

Safety was assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 (NCI-CTCAE) [6].

statistical analyses

In this study, the hypothesis was that the survival distribution function for PFS was the same for erlotinib compared with placebo. The null hypothesis was tested using an unstratified log-rank test at the one-sided 5% level with no adjustment of P-values for multiple testing. However, P-values given within this paper are two-sided. A sample size of 200 patients was chosen to provide an adequate sample, taking into account that the study was conducted in a population in which it is clinically challenging to obtain tumor biopsy samples and to treat with placebo. Patients were assigned 1:1 to erlotinib or placebo using an adaptive randomization method [7] to ensure a balanced stratification of smoking status (current smoker versus former smoker versus never smoker), ECOG PS (0–1 versus 2) and geographic region (Asian versus non-Asian).

The primary analysis for efficacy was the Full Analysis Set (FAS), which was defined as all randomized patients. The safety population was defined as all patients who received at least one dose of study medication and had a safety follow-up, whether withdrawn prematurely or not.

The efficacy results were evaluated by prespecified biomarker status including EGFR protein expression [IHC positive (>10% of cells with membrane staining) versus negative], EGFR gene copy number [FISH positive (probe clusters in >10% of cells) versus negative], EGFR mutation status (activating mutation positive versus wild-type), KRAS mutation status (classical mutation positive versus wild-type) and EGFR polymorphisms (long versus short). Additional post hoc exploratory analyses were conducted (see supplementary material, available at Annals of Oncology online, for methodology).

Rash analysis included patients alive at day 21, thus excluding those who progressed too quickly to develop rash. The designation ‘early rash’ was used to limit the analysis to erlotinib-related rash only.
The cut-off date for the final analysis was 6 months after the last patient was randomized. The study is currently ongoing and will end when the last patient has stopped randomized treatment with erlotinib or placebo and completed their final visit before withdrawal or the start of the open-label extension phase.

**results**

**patients**

At the data cut-off on 22 December 2010, 207 patients had been enrolled and randomized (104 to erlotinib, 103 to placebo; Figure 1). The study was conducted in 49 centers across 15 countries. The first patient was randomized on 24 June 2008, and the last patient was randomized on 22 June 2010. Five patients remained on blinded treatment as of the data cut-off. All 207 patients were included in both the efficacy and safety analyses. Overall patient characteristics are listed in Table 1. Baseline characteristics, including stratification factors and line of therapy, were generally well matched between treatment arms. However, there were a number of imbalances in baseline characteristics that were small in themselves, but overall indicated a worse prognosis in the erlotinib arm, namely patients randomized to erlotinib were older (40% aged ≥65 years versus 30%), more likely to have ≥10% weight loss in the past 6 months (37% versus 26%), more frequently had poorly differentiated disease (32% versus 21%) and reported more pancreatic pain (75% versus 59%). Also, patients randomized to erlotinib had a shorter median time since first diagnosis than those randomized to placebo (6.95 versus 8.77 months, respectively). Biomarker status was generally similar between the erlotinib and placebo arms: 69% of the patients in both arms had EGFR IHC-positive tumors, and nearly half of the patients had a longer CA repeat of EGFR intron 1 polymorphism (48% versus 42% in the erlotinib and placebo arms, respectively) (supplementary Table S1, available at Annals of Oncology online).

No patients in the study had EGFR activating mutation-positive tumors. At disease progression, 53% of patients (57/102) in the placebo arm received open-label erlotinib, while only 18% of patients (19/104) in the erlotinib arm received further active therapies.

![Figure 1. CONSORT diagram. *Patients who had progressed on placebo were given the opportunity to switch to erlotinib in the open-label extension phase of the study, if deemed beneficial to the patient. AE, adverse event; PD, progressive disease; Tx, treatment.](image-url)

patients

- Placebo (n=103)
  - Completed (n=100)
  - AE (n=2)
  - Death (n=19)
  - PD (n=77)
  - Failure to return (n=1)
  - Refused Tx (n=1)
  - Open-label erlotinib* (n=57)
  - No open-label erlotinib (n=43)

- Erlotinib (n=104)
  - Completed (n=102)
  - AE (n=9)
  - Death (n=15)
  - PD (n=72)
  - Other protocol violation (n=1)
  - Refused Tx (n=5)

Patients randomized (n=207)

- Patients ongoing at data cutoff
  - Placebo (n=1)
  - Erlotinib (n=2)

- Patients ongoing at data cutoff
  - Placebo (n=3)
  - Erlotinib (n=5)

- Patients ongoing at data cutoff
  - Placebo (n=1)
  - Erlotinib (n=2)

Figure 1. CONSORT diagram. *Patients who had progressed on placebo were given the opportunity to switch to erlotinib in the open-label extension phase of the study, if deemed beneficial to the patient. AE, adverse event; PD, progressive disease; Tx, treatment.
The median PFS in the overall population was similar in the erlotinib and placebo arms [median 6.1 versus 5.9 weeks, respectively; hazard ratio (HR) 0.83 (95% confidence interval (CI), 0.63–1.10); log-rank P = 0.1909; Figure 2]; however, the primary model did not adjust for baseline imbalances. The adjusted post hoc analysis of PFS correcting for baseline prognostic differences, including smoking status, pain related to pancreatic cancer, location of the primary tumor in the tail, and lesions in the pancreas, liver and other sites, showed a statistically significant effect with erlotinib [adjusted HR 0.68 (95% CI 0.50–0.91); P = 0.0102 using Wald’s test]. No significant difference was observed between erlotinib and placebo in the pre-planned, unadjusted OS analysis [4.0 versus 3.1 months, respectively; HR 1.04 (95% CI 0.77–1.39); log-rank P = 0.8137; Figure 2], but a post hoc adjusted analysis showed a trend in favor of erlotinib [adjusted HR 0.83 (95% CI 0.60–1.16); P = 0.2867 using Wald’s test].

Efficacy analyses by skin rash (supplementary Figure S1, available at Annals of Oncology online) and biomarker subgroups are presented in full in the supplementary material, available at Annals of Oncology online. No statistically significant difference in the median PFS was observed between the study arms in the overall population or according to stratification factor or tumor biomarker status within the pre-planned unadjusted analysis (Figure 2; supplementary Figures S2–S5, available at Annals of Oncology online). However, an adjusted analysis of biomarker subgroups using the post hoc model showed HRs for the EGFR IHC-positive subgroup and the short EGFR CA-SSR1 polymorphism subgroup were 0.64 (95% CI 0.42–0.98) and 0.63 (95% CI 0.41–0.95), respectively (supplementary Table S4, available at Annals of Oncology online).

Pre-planned and post hoc analyses of OS did not identify any of the pre-planned tumor biomarkers that predicted a detrimental effect or a strong benefit with erlotinib. The factors identified by the stepwise selection of the Cox regression for OS in the FAS were smoking status, weight loss, pancreatic pain, location of the primary tumor in the tail, weight and tumor characteristics at screening (tumor size, number of target lesions, presence of non-target lesions, presence of tumors in the pancreas, liver and other sites). Different factors for PFS and OS were identified, as PFS is more driven by speed of tumor growth, while OS is more dependent on general health indicators such as weight.

An adjusted post hoc analysis of additional serum biomarkers (Figure 3) showed that erlotinib provided a greater PFS and OS benefit than placebo for patients with high levels of amphiregulin [PFS-adjusted HR 0.51 (95% CI 0.33–0.80); OS-adjusted HR 0.55 (95% CI 0.34–0.92)], and likely no benefit for patients with low levels of amphiregulin [PFS-adjusted HR 0.94 (95% CI 0.60–1.47); OS-adjusted HR 1.26 (95% CI 0.74–2.16)]. In addition, erlotinib provided a greater PFS benefit but no significant difference in OS versus placebo in patients with low levels of EGF, or high levels of TGF-α (Figure 3).

No complete responses were reported in this study, although partial responses were observed in 1% and 4% of erlotinib- and placebo-treated patients, respectively. DCR was higher with erlotinib compared with placebo (28% versus 18%, P = 0.1077), although the difference did not reach statistical significance.

### Table 1. Baseline demographic and clinical characteristics in the overall population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Erlotinib (n = 104)</th>
<th>Placebo (n = 103)</th>
</tr>
</thead>
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<tr>
<td>Age, years</td>
<td>Median (range)</td>
<td>62 (33–90)</td>
</tr>
<tr>
<td>Age, n (%)</td>
<td>≥65 years</td>
<td>42 (40)</td>
</tr>
<tr>
<td></td>
<td>&lt;65 years</td>
<td>62 (60)</td>
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<tr>
<td>Gender, n (%)</td>
<td>Male</td>
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<tr>
<td></td>
<td>Female</td>
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<td>ECOG performance status, n (%)a</td>
<td>0/1</td>
<td>88 (85)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16 (15)</td>
</tr>
<tr>
<td>Weight loss in past 6 months, n (%)</td>
<td>&gt;10%</td>
<td>38 (37)</td>
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<tr>
<td></td>
<td>≤10%</td>
<td>65 (63)</td>
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<td>Region, n (%)</td>
<td>Non-Asia</td>
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<td>Asia</td>
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<td>Smoking status, n (%)b</td>
<td>Current smoker</td>
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<tr>
<td></td>
<td>Never smoker</td>
<td>48 (46)</td>
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<tr>
<td></td>
<td>Former smoker</td>
<td>27 (26)</td>
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<tr>
<td>First-line treatment, n (%)c</td>
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<td>36 (35)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>68 (65)</td>
</tr>
<tr>
<td>Months since first diagnosis</td>
<td>Median (range)</td>
<td>6.95 (0.3–53.9)</td>
</tr>
<tr>
<td>Months since progressiona</td>
<td>Median (range)</td>
<td>0.97 (0.2–9.8)</td>
</tr>
<tr>
<td>Stage at screening, n (%)</td>
<td>Local</td>
<td>20 (19)</td>
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<tr>
<td></td>
<td>Metastatic</td>
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<tr>
<td>Histologic grade, n (%)</td>
<td>Well differentiated</td>
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<tr>
<td></td>
<td>Moderately differentiated</td>
<td>32 (31)</td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated</td>
<td>33 (32)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>28 (27)</td>
</tr>
<tr>
<td>Pain related to pancreatic cancer</td>
<td>Yes</td>
<td>78 (75)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>26 (25)</td>
</tr>
</tbody>
</table>

**Table 1.** Baseline demographic and clinical characteristics in the overall population

*Total n = 173.*

*aSmoking status: patients who had stopped smoking <1 year ago were counted as current smokers.

*bFirst-line treatment was defined as any patient who did not report taking palliative chemotherapy.

**efficacy**

The median PFS in the overall population was similar in the erlotinib and placebo arms [median 6.1 versus 5.9 weeks, respectively; hazard ratio (HR) 0.83 (95% confidence interval (CI), 0.63–1.10); log-rank P = 0.1909; Figure 2]; however, the primary model did not adjust for baseline imbalances. The adjusted

**safety and tolerability**

Safety data were available for all 207 patients in the study (supplementary Table S2, available at Annals of Oncology online). The AE profile for erlotinib was comparable with previous experience with the drug. In this study, there were no cases of interstitial lung disease (ILD) with erlotinib; however, one case
(1.0%) of ILD was observed in the placebo arm. No new safety signals were observed.

**Discussion**

This randomized, phase II, biomarker study in patients with advanced, pre-treated pancreatic cancer did not identify a population subset with a strong benefit or detrimental effect after treatment with erlotinib in this population with poor prognosis. The pre-planned, unadjusted analysis for PFS did not demonstrate a significant benefit for erlotinib. However, the study was not powered to detect a significant difference and a post hoc, adjusted analysis, taking into account imbalances in baseline characteristics, showed a statistically significant effect of erlotinib on PFS [adjusted HR 0.68 (95% CI 0.50–0.91); P = 0.0102].

**KRAS** wild-type disease and **EGFR** activating mutations have been associated with improved response to EGFR-targeted therapies in other tumors [8–11]. In this study, the results in the subgroups with KRAS mutations or EGFR wild-type disease were similar to the overall population (HR 0.79, 0.90 and 0.73, respectively). A post hoc adjusted analysis of serum biomarkers showed that patients with elevated baseline levels of amphiregulin, which is a potent mitogen acting at the EGFR in pancreatic cancer cells [12], derived a greater PFS and OS benefit from erlotinib versus placebo, while no difference was seen in those who had low levels of amphiregulin. In addition, a greater PFS benefit with erlotinib was observed in patients with low levels of EGF or high levels of TGF-α.

A definitive mechanism for the effect of these markers on clinical efficacy has not been established. High amphiregulin

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**Figure 2.** Progression-free survival (PFS) and overall survival (OS) by treatment arm. *The Kaplan–Meier curve shown is not adjusted for imbalances in baseline characteristics.
levels may indicate a poor prognosis, since it stimulates EGFR coupling to cell proliferation (as does TGF-α) [13]. Amphiregulin may also alter EGFR internalization and degradation, favoring accumulation of EGFR at the cell surface [14], thus making cells more susceptible to EGFR tyrosine-kinase inhibitors, while EGF competitively antagonizes amphiregulin and internalizes EGFR.

In this study, conducted in patients with a poor prognosis, there was no significant difference between the erlotinib and placebo arms in the unstratified analysis of OS. However, it should be noted that approximately half of the patients in the placebo arm received open-label erlotinib after progression and this cross-over may have influenced the results of the OS analysis.

The safety and tolerability profile of erlotinib in this study was as expected, and the incidence of AEs such as rash and gastrointestinal disturbances was consistent with previous experience with erlotinib in pancreatic cancer and other tumors [3, 9–11], with no new safety signals observed. In contrast with previous studies [15], skin rash was not predictive of improved

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**Figure 3.** Summary of exploratory serum biomarker analyses for progression-free survival (PFS) and overall survival (OS)-adjusted post hoc analysis.
PFS in this study, perhaps because of improved early intervention and effective prophylactic use of emollient creams.

To date, there is limited evidence to support the use of predictive biomarkers for patients with pancreatic cancer who could benefit from targeted therapies. CA19-9 is an adverse prognostic marker [16]. KRAS mutations may also adversely affect prognosis [17], as may Bcl-2-associated X protein (BAX), Bcl-2, survivin, Ki-67, cyclooxygenase-2, E-cadherin and S100 calcium-binding proteins, in particular S100A2 [18]. The observation based on the exploratory analyses from this study, that patients with elevated baseline levels of amphiregulin obtained a greater PFS and OS benefit with erlotinib, suggests some potential value for this biomarker in pancreatic cancer. Further research is still needed, however, to identify those patients with advanced pancreatic cancer who may benefit most from erlotinib.

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**disclosure**

DP, ID and LK have no conflicts of interest. JB has received honoraria from Merck and participated as an advisor for Roche. AF, CH and BK are paid employees of F. Hoffmann-La Roche Ltd. AF and BK also own stock in F. Hoffmann-La Roche Ltd. MD has received honoraria from Merck and participated as an advisor for Roche. AF, CH and BK also own stock in F. Hoffmann-La Roche Ltd. MB has received research funding from Roche and Chugai. Consultant/advisor for Roche, Merck Serono and Amgen; and has received research funding from Roche and Chugai.

**references**