A Multicenter, Open-Label Phase II Clinical Trial of Combined MEK plus EGFR Inhibition for Chemotherapy-Refractory Advanced Pancreatic Adenocarcinoma

Andrew H. Ko1, Tanios Bekaii-Saab2, Jessica Van Zifflle3, Olga M. Mirzoeva4, Nancy M. Joseph5, AmirAli Talasaz4, Peter Kuhn5, Margaret A. Tempero1, Eric A. Colisson1, R. Kate Kelley1, Alan P. Venook1, Elizabeth Dito1, Anna Ong1, Sharvina Ziyeh1, Ryan Courtin1, Regina Linetskaya1, Sanaa Tahiri2, and W. Michael Korn1

Abstract

Purpose: On the basis of preclinical evidence of synergistic activity between MEK and EGFR inhibitors in pancreatic ductal adenocarcinoma (PDAC), we evaluated the safety and efficacy of selumetinib, a MEK1/2 inhibitor, plus erlotinib in patients with previously treated advanced PDAC.

Experimental Design: In this single-arm phase II trial, eligible patients received the combination of erlotinib 100 mg plus selumetinib 100 mg daily in 3-week cycles. Study assessments included measurement of clinical outcomes, with a primary endpoint of overall survival, and exploration of potential molecular predictors of treatment benefit.

Results: Forty-six patients were enrolled and received a median of two cycles (range, 1–7). Although no objective responses were observed, 19 patients (41%) showed evidence of stable disease for ≥6 weeks, and 13 of 34 patients (38%) had a CA19-9 decline ≥50%. Median progression-free survival was 1.9 months [95% confidence interval (CI), 1.4–3.3 months], with a median overall survival of 7.3 months (95% CI, 5.2–8.0 months). Common adverse events included rash, diarrhea, and nausea/vomiting. Patients with tumors exhibiting an epithelial phenotype (demonstrated by a high level of E-cadherin expression) were more likely to be sensitive to study treatment. Tumor-derived DNA was detectable in plasma from the majority of patients using next-generation digital DNA sequencing, and its relative abundance correlated with tumor burden.

Conclusions: A therapeutic strategy of dual targeted inhibition of the MEK and EGFR pathways shows modest antitumor activity in pancreatic cancer. Specific molecular subtypes may derive greatest benefit from this combination. Further exploration, both with more potent MEK inhibitors and in molecularly enriched patient subsets, is warranted.

Cancer Therapy: Clinical

Introduction

Genetic alterations in the KRAS signaling pathway are found in approximately 90% of pancreatic ductal adenocarcinomas (PDAC; refs. 1, 2). Although directly targeting KRAS itself has proved elusive as a therapeutic strategy, two of the effector pathways downstream of KRAS, the MAPK (RAF–MEK–ERK) and PI3K (PI3K–AKT) signaling cascades, are each independently amenable to pharmacologic inhibition. However, due to cross-talk and pathway convergence, targeting one or the other shows limited efficacy in PDAC as well as other solid tumors (3, 4). We have demonstrated a negative regulatory feedback loop whereby pharmacologically inhibiting MEK induces feedback activation of PI3K, with this feedback mechanism mediated by hyperactivation of the EGFR (5, 6). Recognition of this fact suggests the potential therapeutic benefit of using a dual inhibitor strategy, and synergistic activity has been demonstrated between EGFR and MEK inhibitors in a number of preclinical models, including PDAC (5, 7–9). Indeed, EGFR is essential for the initiation of PDAC by oncogenic KRAS (10, 11), underscoring the extensive interplay between these two molecules.

New chemotherapy combinations such as FOLFIRINOX (infusional 5-FU, leucovorin, irinotecan, and oxaliplatin; ref. 12) and gemcitabine plus nab-paclitaxel (13) have produced improved clinical outcomes in patients with metastatic PDAC. However, once patients progress on these regimens, therapeutic options become less certain, with no universally accepted standard of care in this salvage setting. Targeted therapies have been disappointing in this disease, with only erlotinib showing a statistically significant

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Corresponding Author: Andrew H. Ko, UCSF Comp. Cancer Center, 550 16th Street, 6th floor, Box 3211, San Francisco, CA 94143. Phone: 415-353-7286; Fax: 415-353-7779; E-mail: andrewko@medicine.ucsf.edu

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Translational Relevance
Recent improvements in combination chemotherapy for metastatic pancreatic cancer have not been mirrored by similar advances in molecularly targeted therapies. Because of feedback signaling mechanisms and cross-talk between signaling pathways, for example, those downstream of KRAS, pathway-targeting agents may need to be administered not as monotherapies, but in rational combinations balancing toxicity concerns with pharmacodynamically relevant dosing. In this report, we describe results from a nonrandomized phase II clinical trial combining EGFR and MEK inhibition in patients with chemotherapy-refractory pancreatic cancer, a clinical context in which there is still a great unmet need. In addition to the clinical findings, we present preliminary but provocative findings indicating certain molecular subtypes of pancreatic cancer that may be more sensitive to this dual-targeted therapeutic approach than others. Future studies should prioritize identification of molecular predictors of therapeutic response, either through tissue-based samples or, preferably, more accessible sources of surrogate material.

Patients and Methods

Study design
This trial was a nonrandomized single-arm phase II study conducted at the University of California San Francisco (San Francisco, CA) and Ohio State University Comprehensive Cancer Centers (Columbus, OH). Institutional Review Board approval was obtained at each site. All participants provided written informed consent.

Primary study objective was to assess overall survival (OS; median survival and proportion of patients alive at 24 weeks) in all patients receiving at least one dose of the combination of selumetinib and erlotinib. Secondary objectives included progression-free survival (median PFS and proportion of patients with PFS at 12 and 24 weeks); CA19-9 biomarker response (defined as ≥50% decline in serum CA19-9 level if elevated at baseline); objective radiographic response by RECIST 1.0 criteria; and safety and toxicity profile of the study combination.

Main eligibility criteria
Patients were eligible for the study if they had histologically confirmed inoperable PDAC (either locally advanced or metastatic) for which they had received exactly one prior line of systemic therapy (excluding adjuvant chemotherapy completed >6 months previously). Prior radiation therapy for resectable or locally advanced disease was permitted. Other key eligibility criteria included evidence of either, or both, RECIST-defined measurable disease or an elevated serum CA19-9 at baseline (≥2X the upper limits of normal; ULN); an Eastern Cooperative Oncology Group performance score of 0-1; life expectancy greater than 8 weeks; and adequate bone marrow and organ function, as defined by ANC ≥1,500/μL, platelet count ≥100,000/μL, total bilirubin ≤2.0 mg/dL, AST and ALT ≤2.5 X ULN, INR ≤1.5, and creatinine ≤2.0 mg/dL. Exclusion criteria included prior therapy with either a MEK or EGFR inhibitor; mandatory use of a QT-prolonging medication; or any of the following: central nervous system metastases; HIV infection; a QTc interval >450 milliseconds or other factors known to increase the risk of QT prolongation or arrhythmic events; refractory nausea/vomiting, malabsorptive or inflammatory bowel conditions; or any other comorbidities that would increase risk for treatment-related complications.

Correlative analyses
IHC for E-cadherin was performed on 5 μm formalin-fixed, paraffin-embedded (FFPE) sections. Following antigen retrieval, sections were incubated with an anti E-cadherin mouse monoclonal antibody (Invitrogen #13-1700; Clone #HECD-1; 1: 150 dilution), followed by the secondary antibody incubation (Envision + Dual Link System- HRP Dako #K4063). The Dako # K3468 Liquid DAB+ substrate-chromogen system was used as peroxidase substrate. Slides were counterstained with hematoxylin. E-cadherin staining was scored on a scale of 0–2 for intensity (0 = absent staining, 1 = weak staining, 2 = strong staining) and further characterized by the percentage of tumor cells demonstrating positive staining by an experienced pathologist (N.M. Joseph).

KRAS mutation analysis was performed on FFPE tissue samples. Following macrodissection, KRAS hotspot (codons 12,13,61) mutations were detected using the SNuPshot Assay (Life Technologies), which PCR-amplifies KRAS (NM_004985.4) exons 2 and 3 from genomic DNA (adapted from ref. 15). The PCR product was subjected to a fluorescent nucleotide extension step of oligonucleotides specific for single nucleotide changes in KRAS codons 12 (c.34, c.35), 13 (c.37, c.38), and 61 (c.181, c.182, c.183). The lower limit of detection is 10% (16).

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For detection and quantification of circulating tumor cells (CTCs), we used a non-EpCAM-based, immunofluorescent, morphologic approach to quantify CTCs as described previously (17). CTCs were identified by immunofluorescence microscopy for cytokeratins, DAPI, and CD45 with automated morphometric analysis followed by manual validation by a pathologist-trained technician (Madelyn S. Lutgten). The technologist, microscopes, and automated imaging system were constant throughout the study.

The study protocol was amended partway through enrollment to include analysis of cell-free DNA (cfDNA) on pre- and posttherapeutic plasma samples using Guardant360 Digital Sequencing technology (Guardant Health), which allows sequencing of all exonic basis of 18 genes and sequencing of cancer-relevant exons of an additional 36 genes. The Guardant360 panel is an advanced laboratory diagnostic test (ADLT) that converts cell-free DNA molecules into digital sequences presequencing, then decodes/reconstructs the postsequencing signal such that over 50 genes (78,000 base pairs) can be sequenced without the false-positive results that accompany such long read lengths at very low concentrations of circulating tumor DNA (ctDNA) in plasma. Summarizing the method, cell-free DNA is extracted from plasma and genomic alterations are analyzed by massively parallel sequencing of amplified target genes utilizing an Illumina Hi-Seq platform complemented by systematic end-to-end process optimization including conversion of cell-free DNA fragments into digital sequences, improvements in the Illumina next-generation sequencing process itself, followed by bioinformatics algorithms that enable ctDNA to be measured as a quantitative percentage of total cell-free DNA. The lower limit of detection is a 0.1% frequency of single nucleotide variant (SNV) mutant alleles in a wild-type cfDNA background (18).

Statistical considerations and analysis

Using a minimax design, 46 evaluable patients were required to provide 80% power to detect a true 24-week survival rate of at least 51% with at least 95% probability of a negative result if the true 24-week survival rate was no more than 33%. This 24-week survival rate difference is equivalent to a 67% increase in the median OS (25 vs. 15 weeks). If at least 20 patients (at least 43.5%) were observed to survive 24 weeks among the 46 evaluable patients, this regimen would be considered worthy of further evaluation. A two-stage design was used, with plans to terminate the study early if no more than 8 of the first 25 patients enrolled were alive at 24 weeks.

Data were summarized with respect to demographic and baseline characteristics, safety observations and measurements, and efficacy observations and measurements. Overall and progression-free survival, defined from the time of starting study treatment, was summarized according to the method of Kaplan and Meier. Any patients lost to follow-up before 24 weeks were censored at the date of last contact. The frequencies of patients with adverse events were summarized by body system and by major adverse event codes (system/organ/class). Fisher exact test was used to assess statistical significance of differences in biomarker frequencies. Spearman Rho was calculated to assess a possible correlation between allele frequencies and changes in serum CA19-9 levels. Allele frequencies of alleles undetectable in on-treatment biopsies were set to the smallest number in the dataset (−1).

Results

Patients

Enrollment to this study took place between January 2011 and January 2013, with follow-up data collected through April 2014. Almost all patients had metastatic disease at the time of study entry, and the majority had an ECOG performance status of zero. Baseline characteristics are shown in Table 1.

The threshold for moving on to the second stage of the study, according to Simon's two-stage design, was met once the 15th patient enrolled on the study became the 9th patient to remain alive at the 24-week mark. Overall, 15 of the first 25 enrolled patients were alive at 24 weeks. Therefore, the study continued accrual to reach its final planned sample size of 46 patients.

Treatment administration

Patients received a median of two cycles of selumetinib plus erlotinib (range, 1–11 cycles). Eighteen patients (39%) required dose reduction of one or both agents during the course of their study treatment. Reasons for study discontinuation included disease progression (39 patients), disease-related complications (3 patients), treatment-related toxicity (2 patients), decrease in quality of life (1 patient), and development of a second primary tumor (1 patient).

Adverse events

The most common adverse events (any grade) occurring during study treatment are highlighted in Table 2. Cutaneous (maculopapular and acneiform rash, dry skin, pruritus) and gastrointestinal toxicities (nausea, vomiting, and diarrhea) were the most common categories of adverse events probably or definitely related to study medication. Although a variety of ocular disorders were reported by study patients, ophthalmologic evaluation did not reveal any cases of central serous retinopathy. In total, 36 patients (78.2%) experienced at least one grade 3 or higher adverse event, although in many instances (abdominal pain, fatigue, thromboembolic events), it was difficult to assign clear attribution to study treatment versus underlying disease. Of the two patients who discontinued study treatment due to drug-related toxicity, one experienced grade 3 rash and refractory hypertension necessitating a prolonged treatment delay and, ultimately, study discontinuation after cycle #5. The other patient had grade 3 nausea/vomiting/diarrhea, leading to dehydration and hypotension, during cycle #1; she was rechallenged at reduced doses, but due to persistent gastrointestinal side effects, had to discontinue study treatment. Four patients died while on or within 30 days of completing study treatment, but these were all

Table 1. Patient characteristics at baseline

<table>
<thead>
<tr>
<th>N</th>
<th>Dates of enrollment</th>
<th>Age (median); y</th>
<th>ECOG performance status (0/1)</th>
<th>Stage (metastatic/locally advanced)</th>
<th>Prior chemotherapy</th>
<th>Gemcitabine based</th>
<th>FOLFIRINOX</th>
<th>Other</th>
<th>Prior surgery</th>
<th>Prior radiation</th>
<th>Elevated baseline CA19–9 &gt; 2× ULN</th>
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<tr>
<td>46</td>
<td>Jan 2011–Jan 2013</td>
<td>67 (range, 40–84)</td>
<td>32/14 (70%/30%)</td>
<td>44/2 (96%/4%)</td>
<td></td>
<td>34 (74%)</td>
<td>10 (22%)</td>
<td>2 (4%)</td>
<td>14 (30%)</td>
<td>7 (15%)</td>
<td>34 (74%)</td>
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attributed to disease progression rather than direct toxic effects from therapy.

Efficacy

Nineteen patients (41%) demonstrated stable disease for greater than 6 weeks, including 12 (26%) with stable disease for at least 12 weeks. There were no objective responses by formal RECIST criteria, although 12 (26%) did demonstrate minor radiographic response (Supplementary Fig. S1). A waterfall plot of best response to study treatment is shown in Fig. 1. Thirteen of 34 patients (38%) with an elevated baseline CA19-9 level had a response to study treatment (Supplementary Fig. S1). A waterfall plot of best response to treatment as determined by RECIST criteria, although 12 (26%) did demonstrate minor radiographic response (Fig. 1).

Correlative studies

Molecular analyses of tumor samples. Point mutations in KRAS, a well-established and central pathogenic feature in this disease (2, 19), were identified in 24 of 26 (92%) tumor samples, including both FFPE tissue samples and fine-needle aspirates. A wide spectrum of KRAS mutations was observed, the most common being G12D (42%), followed by G12V (25%).

We also assessed E-cadherin expression in 23 patients in whom sufficient archived tumor material was available for IHC analysis. Most tumors demonstrated a heterogeneous staining pattern with varying fractions of E-cadherin–expressing cells (Supplementary Fig. S2). On the basis of our preclinical studies, we expected pancreatic cancers containing a higher proportion of E-cadherin–expressing cells, reflecting an epithelial phenotype, to be more sensitive to treatment with dual MEK plus EGFR inhibition, than those with more mesenchymal-type cells (lacking E-cadherin expression). Indeed, 7 of 11 patients whose tumors contained a higher percentage of E-cadherin–expressing cells experienced a 50% or greater CA19-9 decline, compared with 0 of 9 patients whose tumors had a lower proportion of E-cadherin–expressing cells ($P = 0.0047$; Fig. 3).

Circulating tumor cells. Enumeration of CTCs was assessed in 33 patients before and on treatment. Mean CTC concentrations were 2.71 and 2.94 cells/mL in pre- versus on-treatment samples, respectively, while the number of samples with CTC concentrations greater than 1 cell/mL were 10 and 7, respectively (data not shown). No clear association between CTC concentration and treatment effect was observed.

Cell-free DNA analyses. Plasma samples were collected from 32 patients for digital sequencing of cfDNA using the Guardant360 assay. DNA sequence germline SNPs were detected at 100% or near-to 100% allele frequency (homozygous variants), or 50% or

<table>
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<th>Table 2. Treatment-related adverse events</th>
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<td><strong>Adverse event</strong></td>
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<td>Rash</td>
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<td>Diarrhea</td>
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<td>Nausea/vomiting</td>
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<td>Fatigue</td>
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<td>AST/ALT elevation</td>
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<td>Anorexia</td>
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<td>Anemia</td>
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<tr>
<td>Dysgeusia</td>
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<td>Eye disorders</td>
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<td>Hypertension</td>
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<td>Leukopenia/neutropenia</td>
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<td>Thrombocytopenia</td>
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<td>Elevated creatinine</td>
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<td>AST/ALT elevation</td>
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<td>Diarrhea</td>
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<tr>
<td>Rash</td>
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*Including blurry vision, eye pain, floaters, and dry or watery eyes.

**Including cerebrovascular event (1).
near to 50% allele frequency (heterozygous variants), in all samples. In addition, sequence variants likely originating from the tumor (based on allele frequency and absence in the above-mentioned germline variants) were found in 27 (84%) pretreatment and 25 (78%) on-treatment samples. Average relative frequencies of tumor-derived mutant alleles were 4.59% (range, 0.1%–57.2%) and 3.95% (range, 0.1%–78.4%) pre- and on-treatment, respectively. Supplementary Table S1 lists all mutations found for the entire study cohort. After filtering results for mutations with known biologic function and those likely to impact on the functionality of the affected protein, 17 genes were found to be mutated at least once in pre- and/or on-treatment plasma samples (Supplementary Fig. S3). As expected, the most frequently mutated gene in pretherapeutic plasma samples, in which circulating tumor fraction is >0.4%, was KRAS (85%), followed by TP53 (60%), ATM (30%), and CDKN2A (15%). KRAS mutations in plasma were uniformly concordant with those found in tumor in all 11 cases for which paired samples were available. Interestingly, a decline of CA19-9 of 50% or greater was observed in 7 of 10 patients (70%) in whom pretreatment mutant KRAS was not detected in plasma, compared with 4 of 15 patients (26%) with detectable KRAS mutations at baseline ($P = 0.0486$).

The majority of mutations (66%) identified in pretreatment plasma samples were also present in on-treatment samples. A decrease in relative frequency for those alleles across these two time points showed a statistical trend toward positive correlation with CA19-9 decline ($R^2 = 0.1369, P = 0.08$; Fig. 4).

**Discussion**

Activated KRAS is an important driving force promoting and maintaining the malignant phenotype in PDAC (1, 20). Among the potential targets for novel therapeutic development for PDAC are effector pathways of KRAS signaling, including members of the
MAPK pathway (RAF–MEK–ERK). However, to date, MEK inhibitors have shown limited single-agent antitumor activity in PDAC (21, 22). For example, selumetinib (AZD6244; ARRY-142886), a selective, allosteric inhibitor of MEK1/2, has previously been evaluated in a randomized phase II study of selumetinib versus capecitabine monotherapy in advanced PDAC in which similar overall and progression-free survival was seen between these two agents (22).

The motivation for this trial emanated from work performed by our group identifying a novel negative regulatory feedback loop that helps explain the limitations of a MEK inhibitor administered as monotherapy. In both breast and pancreatic cancer cell lines, pharmacologic inhibition of MEK results in markedly enhanced phosphorylation of EGFR and activation of the PI3K–AKT cascade (6, 20). This feedback signal can be fully abolished by concomitant inhibition of EGFR kinase activity. The combination of MEK and EGFR inhibitors shows synergistic effects on cell growth in PDAC cell lines (irrespective of KRAS mutational status), and additive or synergistic antitumor activity in pancreatic tumor xenografts (20). Other groups have similarly reported synergistic activity in various preclinical models (9).

In the current study evaluating dual MEK/EGFR inhibition in patients with advanced chemotherapy-refractory PDAC, this combination conferred modest evidence of antitumor activity in a subset of patients, including prolonged disease control, minor radiographic responses, and significant declines in serum CA19-9 levels. Although the study did meet its primary survival endpoint suggesting promising activity of this combination, the limitations of a nonrandomized, single-arm trial need to be recognized in terms of interpreting the results. Certainly, an inherent patient selection bias was likely in this study in terms of enrollment of patients with more favorable disease biology, including a relatively high proportion of patients (30%) who had undergone prior resection; patients with metachronous metastatic disease trend to fare better overall when compared with those who present with stage IV disease at original diagnosis. Further indication of this selection bias was reflected by the fact that median overall survival for the study cohort (from the time of original pancreatic cancer diagnosis to death) was 18.9 months (range, 5.2–86.4 months), which far exceeds what one would normally expect for this patient population. We also note that more than half of patients did receive additional chemotherapy after discontinuing study treatment, although the benefit of third-line therapy in this disease context is unclear in terms of its impact on survival results.

The second-line setting for advanced pancreatic cancer, the clinical context in which this study was performed, is one in which there currently remains no accepted standard of care, with conventional chemotherapy regimens producing median survival times in the range of 6 months or less (reviewed in ref. 23). The current de facto approach for patients who have progressed following first-line gemcitabine-based chemotherapy has been to use a combination of oxaliplatin plus a fluoropyrimidine, based on results from a phase III German study [Charité Onkologie (CONKO) 003; ref. 24], although a subsequent Canadian trial of similar sample size did not suggest any benefit of FOLFOLX (5-FU, leucovorin, and oxaliplatin) when compared with 5-fluorouracil/leucovorin alone (25). A more recent international phase III trial showed that the addition of a nanoliposomal formulation of irinotecan to fluoropyrimidine-based therapy in gemcitabine-pretreated patients conferred a significant survival benefit (26), and may offer a new reference standard for future studies of second-line treatment in which randomized design is desirable to avoid the aforementioned selection biases.

Although it may now be possible to offer patients two or more lines of chemotherapy, a noncytotoxic, completely orally bioavailable regimen holds conceptual appeal in a patient population often characterized by inanition and declining performance status, for whom the side effects of chemotherapy may be considerable and potentially life-threatening. However, to date, “targeted therapy only” strategies have been met with fairly dismal outcomes in phase II trials (27, 28). A recently reported Southwest Oncology Group phase II trial (SWOG S1115) randomizing patients who had progressed on gemcitabine-based therapy to either selumetinib plus the AKT inhibitor MK-2206 or traditional chemotherapy (FOLFOX) found that survival was shorter in the targeted-therapy group, albeit in a molecularly unselected patient population (29). Moreover, although patients may wish to avoid the side effects associated with classical cytotoxic therapy, it is important to recognize the unique toxicities associated with molecularly targeted agents that may limit treatment administration and affect patients’ quality of life. For example, in the current study a relatively high proportion of patients required dose modifications of one or both agents, most commonly due to cutaneous or gastrointestinal adverse events. These observations highlight the challenges of administering multiple targeted therapeutic agents in pharmacologically relevant doses; furthermore, they raise important questions regarding the feasibility of merging this combinatorial approach with traditional cytotoxic agents in earlier lines of therapy, where the additive toxicities would likely be prohibitive.

A key component of our study was to identify molecular features of PDAC that might predict clinical benefit to combined EGFR/MEK inhibition. Our group previously defined three molecular subtypes of PDAC (classical/epithelial, quasimesenchymal, and exocrine-like) based on distinct transcriptional profiles derived from primary tissues and represented in preclinical models (30). These subtypes show differential responses to both cytotoxic and targeted therapies in vitro. Specific to dual MEK/EGFR inhibition, genes representative of the epithelial-like subtype (in particular E-cadherin) were highly expressed in sensitive cell lines, whereas each of the cell lines most resistant to this combination fell into the mesenchymal subtype (5). For this clinical study, we analyzed archived tumor samples to assess for subtype-specific differential responses. In agreement with published data (31, 32), the majority of tumors showed substantial intratumoral heterogeneity, harboring both epithelial- and mesenchymal subtypes at various ratios (as reflected by E-cadherin expression). Acknowledging sample size limitations, it is noteworthy that epithelial-type tumor cell fraction was significantly associated with treatment sensitivity, reflected by CA19-9 decline. It has been previously reported that cancer cells maintaining epithelial differentiation remain addicted to K-Ras (whereas epithelial to mesenchymal transition leads to reduced K-ras dependency), with a K-Ras dependency gene expression signature associated with greater sensitivity to EGFR kinase inhibitors (33). A separate study showed, similarly, that restoring E-cadherin expression in lung cancer cell lines increased sensitivity to this same class of drugs (34). Our study supports these findings and is the first to suggest an association between an epithelial-predominant subtype (as characterized by E-cadherin expression) and drug sensitivity in patients with pancreatic cancer.
Although it may be premature to propose routine E-cadherin immunohistochemistry testing on all tumors before initiation with erlotinib or other EGFR inhibitors, such testing could be embedded as part of correlative analyses within future clinical trial design where these agents are being tested.

We also explored the utility of blood-based biomarkers to monitor molecular events over the course of treatment. Measurements of CTC concentration using an advanced high-content image analysis system revealed significantly lower levels than observed in a prior pilot study (17), with no significant association with clinical response parameters observed. More promise was observed from digital sequencing of solid tumor cfDNA in plasma using a commercial assay that enabled the sequencing of 54 tumor-associated genes. We were able to detect tumor DNA in plasma in a remarkably high fraction (84%) of patients, suggesting this method could provide valuable molecular information for both predictive and monitoring purposes in this patient population. Although our results do not necessarily demonstrate on-target treatment effects specific to the particular EGFR/MEK inhibitor combination used for this study, we posit that shifts in frequency and type of mutations over the treatment course may reflect dynamic alterations in the composition of molecularly defined subclones within the tumor. Further preclinical and clinical studies will be necessary to elucidate such clonal dynamics and to understand the mechanisms behind treatment failure and tumor progression. Intriguingly, we found an association between changes in allele frequencies of the most frequent mutations identified in cfDNA and indicators of disease burden (notably CA19-9 levels) that almost reached statistical significance. It is conceivable that such quantitative changes in allele abundance could provide early information on therapeutic benefit or, conversely, development of resistance. Incorporation of both baseline and serial measurements of cfDNA throughout the course of treatment has potential utility for patient selection and treatment decision making in future clinical studies of this or related combination therapies, although establishing the concordance between mutations identified in cfDNA and tumor tissue will be important to ensure that this “liquid biopsy” is a reliable surrogate. Indeed, several groups have already shown high concordance rates between the two platforms in small series of patients (35, 36). Our group, for example, recently reported high levels of sensitivity and specificity of blood-based sequencing results across five genes examined (KRAS, TP53, APC, FBXW7, and SMAD4; ref. 36). Reassuringly, in the current study, 100% concordance in KRAS mutations was observed between tumor and plasma where paired samples were available, with the performing clinical laboratory blinded to the tissue-based SNaPshot sequencing results.

In summary, the combination of MEK and EGFR inhibitors evaluated in this clinical trial showed evidence of modest antitumor activity and offers proof of principle regarding both the feasibility and promise of this strategy. Successor studies could be developed using more potent MEK inhibitors and/or combining these agents with inhibitors of alternative signaling nodes, and ideally be evaluated in the context of a randomized trial design comparing a “targeted-only” approach to cytotoxic therapy, especially as second-line chemotherapy becomes more widely accepted as standard of care. Such studies would also ideally incorporate quality of life endpoints given the unique and often substantial toxicities associated with each of these therapeutic approaches.

Perhaps the most critical issue of all to address is whether the findings in our study convincingly define a particular subset of individuals most likely to benefit from this specific combination of targeted agents. One might reasonably ask whether a future study using a similar treatment strategy, for example, should limit patient enrollment to those with epithelial-type tumors. Although our clinical findings certainly have to be considered preliminary, they do corroborate our preclinical observations suggesting a subtype-specific sensitivity to this combination of agents. As such, we submit that it would be entirely appropriate to develop a study in which E-cadherin expression, clearly predefined and measured under CLIA-certified laboratory conditions, could be used as an integral biomarker for determining patient eligibility. This would represent an important next step in bringing the treatment of pancreatic cancer more squarely into the era of precision medicine.

Disclosure of Potential Conflicts of Interest

T. Bekaii-Saab is a consultant/advisory board member for Genentech. A.A. Talasaz has ownership interest (including patents) in Guardian Health Inc. P. Kuhn has ownership interest (including patents) in and is a consultant/advisory board member for Epic Sciences. E.A. Collisson is a consultant/advisory board member for Guardant Health. W.M. Korn has ownership interest (including patents) in Genentech. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: A.H. Ko, M.A. Tempero, W.M. Korn

Development of methodology: A.H. Ko, A.A. Talasaz, E.A. Collisson, W.M. Korn

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.H. Ko, T. Bekaii-Saab, J. Van Zifflle, O.M. Mirzoeva, A.A. Talasaz, P. Kuhn, R.K. Kelley, A.P. Venook, E. Dito, S. Ziyeh, R. Courtin, R. Linetskaya, S. Tahiri, W.M. Korn

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.H. Ko, T. Bekaii-Saab, N.M. Joseph, P. Kuhn, M.A. Tempero, E. Collisson, R.K. Kelley, W.M. Korn

Writing, review, and/or revision of the manuscript: A.H. Ko, T. Bekaii-Saab, E. Collisson, R.K. Kelley, A.P. Venook, W.M. Korn

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.H. Ko, T. Bekaii-Saab, O.M. Mirzoeva, S. Ziyeh, R. Courtin, R. Linetskaya, S. Tahiri


Other (nurse management and documentation of symptoms with pt on study): A. Ong

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