Retrospective Case Series Analysis of RAF Family Alterations in Pancreatic Cancer: Real-World Outcomes From Targeted and Standard Therapies

Andrew Hendifar, MD, MPH1; Edik M. Blais, PhD2; Brian Wolpin, MD, MPH3; Vivek Subbiah, MD4; Eric Collisson, MD5; Isha Singh, MBBS6; Timothy Cannon, MD7; Kenna Shaw, PhD8; Emanuel F. Petricoin III, PhD8; Samuel Klempner, MD9; Emily Lyons, MA10; Andrea Wang-Gillam, MD, PhD11; Michael J. Pishvaian, MD, PhD12; and Eileen M. O'Reilly, MD6

abstract

PURPOSE In pancreatic cancer (PC), the RAF family alterations define a rare subset of patients that may predict response to inhibition of the BRAF/MEK/ERK signaling pathway. A comprehensive understanding of the molecular and clinical characteristics of RAF-mutated PC may support future development of RAF-directed strategies.

METHODS Clinical outcomes were assessed across a multi-institutional case series of 81 patients with RAF family-mutated PC. Mutational subgroups were defined on the basis of RAF alteration hotspots and therapeutic implications.

RESULTS The frequency of RAF alterations in PC was 2.2% (84 of 3,781) within a prevalence cohort derived from large molecular databases where BRAF V600E (Exon 15), BRAF ΔNVTAP (Exon 11), and SND1-BRAF fusions were the most common variants. In our retrospective case series, we identified 17 of 81 (21.0%) molecular profiles with a BRAF V600/Exon 15 mutation without any confounding drivers, 25 of 81 (30.9%) with BRAF or RAF1 fusions, and 18 of 81 (22.2%) with Exon 11 mutations. The remaining 21 of 81 (25.9%) profiles had atypical RAF variants and/or multiple oncogenic drivers. Clinical benefit from BRAF/MEK/ERK inhibitors was observed in 3 of 3 subjects within the V600 subgroup (two partial responses), 4 of 6 with fusions (two partial responses), 2 of 6 with Exon 11 mutations (one partial response), and 0 of 3 with confounding drivers. Outcomes analyses also suggested a trend favoring fluorouracil-based regimens over gemcitabine/nab-paclitaxel within the fusion subgroup (P = .027).

CONCLUSION Prospective evaluation of RAF-directed therapies is warranted in RAF-mutated PC; however, differential responses to targeted agents or standard regimens for each mutational subgroup should be a consideration when designing clinical trials.

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INTRODUCTION Pancreatic cancer (PC) has a 5-year survival of 9% and is projected to be the second leading cause of cancer-related mortality in the United States before 2030.1,2 Despite the widespread availability of genomic profiling, US Food and Drug Administration (FDA)-approved therapies specifically for PC are mostly limited to combinatorial cytotoxic regimens including FOLFIRINOX,3 gemcitabine with nab-paclitaxel (Gem/nab-P),4 and nal-irinotecan with fluorouracil (5-FU).5 Beyond tumor-agnostic markers for PD-1 inhibitors and Trk inhibitors, each of which is rare in PC (< 1%),6-11 olaparib remains the only targeted therapy for a molecularly defined subset of PC.12 The molecular landscape of PC is dominated by a preponderance of KRAS mutations (92%-93%),6,13-22 limiting the scope of molecularly targeted strategies in PC. In KRAS wild-type PC, activating alterations in oncogenic drivers such as BRAF have been reported as potentially actionable,6,13,16,23-26; however, clinical outcomes on standard therapies and targeted therapies are difficult to capture for these rare molecularly defined PC subgroups.

Beyond PC, recurrent RAF family alterations are enriched in solid tumors including lung, colon, thyroid, and melanoma.27,28 Multiple BRAF inhibitors (eg, dabrafenib/encorafenib/vemurafenib) have been approved for use as a single agent or in combination with a MEK inhibitor (eg, trametinib/binimetinib/cobimetinib) across multiple disease types including metastatic
melanoma, non–small-cell lung cancer and anaplastic thyroid cancer.29–32 Recently, a BRAF inhibitor combined with an anti-EGFR antibody was also approved by the FDA for use in BRAF V600E–mutated colorectal cancer.33

Although BRAF inhibitor combinations have shown promising activity across a broad range of BRAF V600E–mutated tumor types, the feasibility of targeting RAF in PC has not yet been established given the relative rarity of KRAS wild-type tumors, the diversity of RAF alterations seen across PC subtypes, and limited outcomes available from those who have received BRAF/MEK/ERK inhibitors.17,18,34,35

Here, we provide an overview of RAF family alterations in epithelial pancreatic malignancies. By aggregating real-world molecular, clinical, treatment data from multiple institutions and a national registry, we describe the largest case series of RAF-altered PC. By establishing PC-specific RAF mutational subgroups (BRAF Exon 15, BRAF Exon 11, Fusions, and Other) on the basis of potential therapeutic implications, we summarize preliminary outcomes and responses to RAF-directed and standard therapies.

METHODS

RAF Family Alteration Frequency in a Real-World Cohort With Genomic Testing Results

To assess the frequency of RAF alterations in PC, real-world data were obtained via the Perthera Platform, which includes 1,802 patients who underwent molecular profiling as part of the Know Your Tumor Program35 and other hospital initiatives.36 Additional public data were obtained from 1,979 patients with genomic testing results available via the AACR GENIE project37 (release 6.1.0). Genomic profiles from this aggregated cohort of 3,781 patients with PC were analyzed to assess the prevalence of BRAF alterations (see Prevalence Cohort described in Table 1). Molecular profiles with fewer than three genomic variants detected were removed from the aggregated prevalence cohort to exclude low-quality profiles. Tumors with predominantly neuroendocrine features were excluded, whereas any epithelial histologies were allowed including ductal adenocarcinoma, acinar cell carcinoma, solid pseudopapillary neoplasm, and pancreaticoblastoma.

Case Series of RAF-Driven PC From Know Your Tumor Program and Academic Collaborators

Deidentified patient and genomic information was collected by collaborators from Dana-Farber, MD Anderson Cancer Center, Memorial Sloan Kettering (MSK), PanCAN, Inova Schar Cancer Institute, and Cedars-Sinai Medical Center. Individual patient charts were retrospectively reviewed, and clinical information was extracted.

PanCAN and Perthera initiated an institutional review board–approved observational registry trial to capture real-world outcomes across all lines of therapies and NGS testing results from Clinical Laboratory Improvement Amendments–certified commercial laboratories in addition to proteomics and/or phosphoproteomics data, as previously described.38 Additional subjects with MSK-IMPACT Assay (MSK-Integrated Mutation Profiling of Actionable Cancer Targets) results were identified at MSK.27 MD Anderson Cancer Center subjects were identified using the Molecular and Clinical Data Integration Platform of the Khalifa Institute for Personalized Cancer Therapy. At Dana-Farber, an institutionaly supported clinical assay (OncoPanel) was used.39 Additional genomic findings were abstracted from commercial laboratory reports.

Patient Outcomes Data

A total of 81 patients with RAF-mutated PC were identified. Demographic data, diagnosis date, staging information, treatment history, and response to therapy were collected under institution-specific institutional review board–approved protocols for each individual site. All data from sites were deidentified before analysis. Overall survival (OS) represents the time of the patient’s diagnosis of advanced PC until death (survival event) or last follow-up (censored event) for those received at least one therapy in the
TABLE 1. Overview of Four RAF Subgroups With Distinct Implications for Therapy That Are Defined on the Basis of Certain BRAF (or RAF1) Alterations Identified in Pancreatic Tumors via Genomic Profiling as well as the Presence or Absence of Confounding Drivers (eg, KRAS mutations) Which Might Otherwise Affect the Actionability of Therapies Targeting the MAPK Pathway

| RAF Subgroup | Molecular Definition | Potential Implications for Targeted Therapy | Estimated Prevalence* |
|--------------|----------------------|--------------------------------------------|-----------------------|
| BRAF Exon 15 | BRAF V600E (or similar nearby variants) AND no confounding drivers | Canonical BRAF inhibitors (eg, vemurafenib, dabrafenib, encorafenib) and/or MEK/ERK inhibitors | 26/3,781 (0.7%) |
| BRAF Exon 11 | BRAF N486,P490del (or similar variants) AND no confounding drivers | Select BRAF inhibitors (dabrafenib-sensitive, vemurafenib-insensitive) and/or MEK/ERK inhibitors | 21/3,781 (0.6%) |
| RAF Fusions  | BRAF/RAF1 fusions/rearrangements (likely activating/pathogenic only) AND no confounding drivers | Pan-RAF inhibitors (eg, regorafenib, sorafenib) and/or MEK/ERK inhibitors | 20/3,781 (0.5%) |
| Other or multiple drivers | Confounding driver present (overrides assignment to subgroups above) OR other BRAF alterations (eg, kinase-dead) reported as pathogenic by NGS testing labs | Not actionable (limited evidence to support the use of RAF/MEK/ERK inhibitors in these contexts because of dependence on RAS or upstream signaling) | 17/3,781 (0.4%) |

Abbreviations: MAPK, mitogen-activated protein kinase; NGS, next-generation sequencing.

*This Prevalence Cohort includes 3,781 patients with epithelial pancreatic cancers and molecular profiling data either from Perthera (a real-world database) or AACR GENIE (a public data set, v6.0.1).

advanced setting. Progression-free survival (PFS) was calculated from treatment initiation until discontinuation because of disease progression (survival event), cessation because of tolerability issues (censored event), or the last follow-up (censored event).

All analyses were implemented in an R/Bioconductor programming environment. Survival was assessed using Cox proportional hazards regression models with survival and survminer packages. Multivariate Cox regression models were used to account for line of therapy and histology as potentially confounding factors. Differences in frequencies were assessed using Fisher’s exact test.

RESULTS

RAF Alterations Are Recurrent Events in PC

In a real-world cohort of 3,781 patients with PC having genomic testing results available,97 we identified 84 patients (2.2% of 3,781 with PC) whose tumors harbored RAF family alterations within this Prevalence Cohort (Table 1). We categorized each patient’s molecular profile with an RAF alteration into one of four subgroups intended to distinguish the actionability for therapies targeting the MAPK pathway: BRAF Exon 15 mutations, BRAF Exon 11 mutations, BRAF/RAF1 fusions/rearrangements, or Other (Table 1). This Other subgroup includes nonactionable molecular profiles where the actionability of the RAF alteration is confounded by the presence of another oncogenic driver or the RAF alteration did not align to any of the other three subgroups.

For those without any confounding drivers, the most common BRAF alterations identified within the Prevalence Cohort included the canonical BRAF V600E mutation in Exon 15 (17 of 3,781, 0.45%), a recurring five-amino-acid in-frame deletion in the BRAF β3-αC loop within Exon 11 commonly referred to as ΔNVTAP or N486,P490del (16 of 3,781, 0.42%), and SND1-BRAF fusions (9 of 3,781, 0.24%). These three specific BRAF alterations have distinct implications for targeted therapy and form the basis of the Exon 15, Exon 11, and Fusion subgroups considered throughout this study.

Within the Prevalence Cohort, the proportion of RAF alterations was higher (P = .000000691, Fisher’s exact test) in pancreatic acinar cell carcinoma (9 of 49, 18.4%) relative to pancreatic adenocarcinoma (64 of 3,298, 1.9%), and they frequently harbored RAF fusion events (6 of 49, 12.2%). BRAF Exon 15 mutations were also observed in rare PC histologies, with one in a pancreatoblastoma and in a solid pseudopapillary neoplasm (Data Supplement).

Patient Outcomes in RAF-Altered PC

In this retrospective case series of patients with PC with clinically annotated outcomes data, we identified 81 patients with genomic alterations in RAF family genes (Fig 1). In this Clinical Cohort, the median age at diagnosis was 64 (42-86) years and 40 of 81 were women (Table 3). The majority of patients presented with advanced disease: 61 of 81 at initial diagnosis. The histologies were 62 of 81 adenocarcinoma, 14 of 81 acinar cell carcinoma, 4 of 81 IPMN (excluded from the analysis cohort), and one pancreatoblastoma (Table 3). The distributions of BRAF alterations were concentrated within Exons 11 and 15 (Fig 1A), similar to the Prevalence Cohort (Table 1). Notably, 69 of the 81 tumor genomic profiles were KRAS wild-type. Additional genomic testing results were available for other commonly mutated genes in PC (Fig 1B). The median overall survival of the analysis cohort (excluding IPMNs and cases with missing information) who presented with advanced disease (n = 54) was 1.51 years (95% CI = 1.11 to
TABLE 2. Overview of Recurring RAF Variants and Histological Subtypes Within Each RAF Subgroup Within the Clinical Cohorta

| RAF Subgroup | No. Represented in Clinical Cohort (%)a | Top RAF Variants Identified in Two or More Subjects (No.) | Pancreatic Cancer Histological Subtypes (No.) |
|--------------|----------------------------------------|---------------------------------------------------------|--------------------------------------------|
| BRAF Exon 15 | 17/81 (21.0) | V600E (13); T599_V600insT (3) | Adenocarcinoma (13); acinar cell (2); IPMN (1) pancreatoblastoma (1) |
| BRAF Exon 11 | 18/81 (22.2) | N486_P490del (16) | Adenocarcinoma (17); IPMN (1) |
| BRAF/RAF1 fusion | 25/81 (30.9) | SND1-BRAF fusion (12) | Adenocarcinoma (15); acinar cell (10) |
| Other or multiple drivers | 21/81 (25.9) | BRAF V600E and confounding driver (3) | Adenocarcinoma (18); acinar cell (1); IPMN (2) |

Abbreviation: IPMN, intraductal papillary mucinous neoplasms.

The Clinical Cohort includes a case series of 81 patients analyzed in this study from multiple institutions.

Within each actionable subgroup, the most common variants were BRAF V600E (Exon 15), BRAF N486_P490del (Exon 11), and SND1-BRAF fusion (Fusions; Fig 1A). Before subgroup assignments, we identified confounding drivers in three of 17 tumor profiles with BRAF V600E mutations (KRAS G12V, NTRK fusion, and SND1-BRAF fusion; Fig 1B). Activating KRAS mutations were notably mutually exclusive with BRAF N486_P490del (0 of 17) and BRAF fusion events (0 of 25).

Despite the many nuances to the biology of RAF variants (see the Data Supplement for variant-specific details related to each case), the presence of a confounding driver was the key defining feature of the RAF Other subgroup (17 of 21). One notable exception to these subgroup definitions is for the class 3 kinase-dead BRAF D594G variant, which does not confer similar actionability as the class 1 BRAF V600E despite its position nearby within Exon 15.39,40 This distinction is important because these BRAF variants are considered RAS-dependent and enriched for co-occurrence with KRAS mutations. In the Clinical Cohort, all but two of the BRAF short variants identified in protein-coding regions outside of Exons 11 and 15 were found alongside a confounding driver (Fig 1A). In contrast to RAS-dependent BRAF variants, RAS-independent variants (ie, class 1 or 2) are expected to be mutually exclusive of KRAS mutations.

MEK and RAF Inhibitors Have Activity in Patients With KRAS Wild-Type and RAF Family-Mutated PC

Response to molecularly targeted agents against the MAPK pathway was evaluated in 18 subjects who received targeted therapies (Fig 3). The most commonly implemented agents included: canonical BRAF inhibitors (n = 7), pan-RAF inhibitors (n = 2), MEK inhibitors (n = 13), and/or ERK inhibitors (n = 2). The most common combination and single agent regimens were dabrafenib plus trametinib (n = 5) and trametinib (n = 7), respectively.

The clinical benefit rate was highest at 100% in the BRAF Exon 15 subgroup in which three patients received dual BRAF/MEK-targeted therapy (Fig 3) including 1 with noncanonical BRAF T599_V600insT. In the BRAF/RAF1 fusions subgroup, 80% (4 of 5) of evaluable patients had clinical benefit including 2 with partial responses with single-agent MEK inhibitors. Within the BRAF Exon 11 subgroup, 40% (2 of 5) had clinical benefit on single-agent MEK inhibitors with one partial response. Patients with confounding drivers or other RAF alterations did not appear to derive any significant clinical benefit from targeted therapy consisting of MEK inhibitors given in combination with either a BRAF inhibitor (2/3) or immunotherapy (1/3) in this cohort (Fig 3).

**RAF Alterations May Predict Response to Standard Chemotherapy**

As an exploratory analysis, we evaluated median PFS across the clinical cohort and within each RAF subgroup on standard chemotherapy consisting of either 5FU-based regimens (n = 46) or Gem/nab-P (n = 40; Fig 4). In the first-line setting, patients with RAF-mutated PC receiving FOLFIRINOX (Fig 4A) or Gem/nab-P (Fig 4C) had a median PFS of 6.5 months (95% CI, 4.4 to not reached [NR]; n = 28) or 4.7 months (95% CI, 2.3 to NR; n = 19), respectively. In subsequent lines of therapy (limit 1 per patient), 5FU-based therapies or Gem/nab-P had a median PFS of 4.7 months (95% CI, 3.5 to NR; Fig 4B) or 4.0 months (95% CI, 2.8 to 6.5; Fig 4D). In patients receiving gemcitabine plus nab-paclitaxel, PFS did not significantly differ across subgroups. We observed a modest trend in favor of 5FU-based therapies that appeared to be specific to the Fusion subgroup (Appendix Fig A1).
FIG 1. Genomic profiling results from pancreatic tumors harboring RAF pathway alterations. (A) Lollipop plot highlighting amino acid positions along the BRAF gene where alterations were most commonly found in this case series (n = 81). Each stemmed circle represents the numbers of patients with a BRAF alteration at each position (or type for structural variants), counted separately on the basis of either the presence (downward lollipop) or absence (upward lollipop) of a confounding alteration in another oncogenic driver (eg, KRAS mutation). RAF-altered molecular profiles were categorized into four subgroups that have been associated with distinct implications for therapy: Exon 15 (blue; V600 mutations that have been associated with responsiveness to canonical BRAF inhibitors), Exon 11 (red; non-V600 mutations that confer RAS-independent activity but are likely vemurafenib-insensitive), Fusions (teal; intergenic structural variants), and Other (orange; structural and/or short variants, either uncharacterized, characterized as RAS-dependent mutations, or found alongside confounding driver mutations). The three most common variants (BRAF V600E, BRAF N486_P490del also known as ANVTAAl, and SND1-BRAF fusions) are highlighted at three hotspots that form the basis for the subgroups. (B) Molecular matrix organized by RAF subgroup shows genomic testing results for each patient including specific BRAF variants, RAF fusions, confounding drivers, and p53/CDKN2A/SMAD4 mutations.
In a follow-up analysis focusing on the Fusion subgroup, we identified a significant difference in PFS ($P = .0051$; hazard ratio [HR] $= 0.1$ [0.02 to 0.50]) between FOLFIRINOX (mPFS $= 8.9$ months [7.5 to NR], $n = 14$, first line or later) and Gem/nab-P (mPFS $= 2.8$ months [1.9 to NR], $n = 12$, first line or later) via univariate Cox regression (Appendix Fig A2A). This subgroup analysis included only the patients who received the entire FOLFIRINOX regimen, and these differences remained significant when applying a multivariate Cox model ($P = .027$; HR $= 0.08$ [0.01 to 0.75]).

### TABLE 3. Summary of Patients With RAF-Mutated Pancreatic Cancer in the Clinical Case Series Cohort and Overall Survival Analysis Cohorts

| Baseline Characteristic | Clinical Cohort (n = 81), No. (%) | OS Cohort (n = 52), No. (%) | OS Matched (n = 16), No. (%) | OS Unmatched (n = 36), No. (%) |
|-------------------------|----------------------------------|-----------------------------|-----------------------------|--------------------------------|
| Sex                     | Female 40/81 (49.4) 28/52 (54)    | 11/16 (69)                  | 17/36 (47)                  |                                |
|                         | Male 41/81 (50.6) 24/52 (46)     | 5/16 (31)                   | 19/36 (53)                  |                                |
| Age at diagnosis, years | ≥ 64 41/81 (50.6) 24/52 (46)     | 5/16 (31)                   | 19/36 (53)                  |                                |
|                         | < 64 40/81 (49.4) 28/52 (54)     | 11/16 (69)                  | 17/36 (47)                  |                                |
| Pancreatic subtype      | Adenocarcinoma 62/81 (76) 43/52 (83) | 11/16 (69) | 32/36 (89) |                                |
|                         | Acinar cell carcinoma 14/81 (18) 9/52 (17) | 5/16 (31) | 4/36 (11) |                                |
|                         | IPMN 4/81 (5) 0/52 (0)           | 0/16 (0)                    | 0/36 (0)                    |                                |
|                         | Pancreatoblastoma 1/81 (1)       | 0/52 (0)                    | 0/16 (0)                    | 0/36 (0)                       |
| Stage at diagnosis      | 0/I (IPMN) 4/81 (5)              | 0/52 (0)                    | 0/16 (0)                    | 0/36 (0)                       |
|                         | IIA/B 15/81 (19)                 | 0/52 (0)                    | 0/16 (0)                    | 0/36 (0)                       |
|                         | III 5/81 (6)                     | 0/52 (0)                    | 0/16 (0)                    | 0/36 (0)                       |
|                         | IV 57/81 (70) 52/52 (100)       | 16/16 (100)                 | 36/36 (100)                 |                                |
| Lines of therapy        | 3 lines or more 24/81 (30)       | 18/52 (35)                  | 10/16 (62)                  | 8/36 (22)                      |
|                         | 2 lines 17/81 (21) 15/52 (29)    | 1/16 (6)                    | 14/36 (39)                  |                                |
|                         | 1 line 23/81 (28) 19/52 (37)     | 5/16 (31)                   | 14/36 (39)                  |                                |
|                         | None (advanced) 17/81 (21)       | 0/52 (0)                    | 0/16 (0)                    | 0/36 (0)                       |

Abbreviations: IPMN, intraductal papillary mucinous neoplasm; OS, overall survival.

In a follow-up analysis focusing on the Fusion subgroup, we identified a significant difference in PFS ($P = .0051$; hazard ratio [HR] $= 0.1$ [0.02 to 0.50]) between FOLFIRINOX (mPFS $= 8.9$ months [7.5 to NR], $n = 14$, first line or later) and Gem/nab-P (mPFS $= 2.8$ months [1.9 to NR], $n = 12$, first line or later) via univariate Cox regression (Appendix Fig A2A). This subgroup analysis included only the patients who received the entire FOLFIRINOX regimen, and these differences remained significant when applying a multivariate Cox model ($P = .027$; HR $= 0.08$ [0.01 to 0.75]).
factoring in line of therapy (first line v later lines: \( P = .75; \) HR = 1.34 [0.22 to 8.26]; Appendix Fig A2B) with or without a third term accounting for differences in adeno-carcinoma versus acinar cell carcinoma histology (see Appendix Fig A2).

**DISCUSSION**

With this multi-institutional retrospective case series, we report the first comprehensive evaluation of patients with RAF-altered PC including clinical outcomes on MAPK pathway inhibitors as well as standard of care. Although rare, KRAS wild-type PC tumors are enriched for potentially actionable RAF alterations centered around three hotspot variants: BRAF V600E in Exon 15, BRAF N486_P490del in Exon 11, and SND1-BRAF fusions. We categorize RAF subgroups around these three hotspot mutations (plus a fourth subgroup for any nonactionable or RAS-dependent profiles), each of which is rare in PC (0.4%-0.7%) and has distinct implications for therapy. In this cohort, we confirm previous reports of clinical responses to MEK and BRAF inhibition in subjects with biologically significant RAF alterations. Benefit was well aligned with the classification system described by Yaeger et al, as many subjects within the BRAF Exon 15 and RAF Fusion subgroups responded to targeted therapies. All three subjects in the nonactionable BRAF Other subgroup had rapidly progressive disease on targeted combinations which is likely attributable to the presence of confounding drivers.

The proportion of BRAF ΔNVTAP deletions was unexpectedly high in this case series given limited reports on Exon 11 mutations in cancers enriched for BRAF V600E mutations (eg, melanoma, thyroid, lung, and colon). In this study, single-agent MEK inhibitors demonstrated limited activity within the Exon 11 subgroup; however, next-generation agents with selectivity against BRAF N486_P490del warrant further investigation. Importantly, BRAF ΔNVTAP does not confer sensitivity to the BRAF inhibitor
vemurafenib despite an increase in RAS-independent dimerization-dependent kinase activity for cells with this in-frame deletion. However, there are clinical case reports of significant activity with dabrafenib in patients with 

BRAFΔNVTAP deletions, which aligns with the observation that dabrafenib fits better than vemurafenib inside the BRAF pocket at the conformational binding-level. Interestingly, there is an important structural paralogy between BRAF and EGFR where BRAFV600E mutations in Exon 15 and BRAFΔNVTAP deletions in Exon 11 conceptually mirror EGFRL858R mutations in Exon 21 and various EGFR deletions in Exon 19, which have represented the core actionable subset of activating EGFR variants in non–small-cell lung cancer.

In our cohort, only individuals receiving approved MEK inhibitors or approved combinations of MEK and BRAF inhibitors had clinical benefit. In a recently published study, three patients with PC were enrolled in a 172-patient BRAF V600E basket trial and the results were consistent with our findings. In the NCI-MATCH subprotocol H arm (N = 31), two patients with PC were enrolled (n = 1 unevaluable with progressive disease, n = 1 stable disease). Notably, these RAF alterations occur across a spectrum of epithelial pancreatic tumors underscoring the importance of routine molecular profiling, irrespective of histology across PCs, particularly acinar cell carcinomas (which commonly harbor BRAF fusions) and other pancreaticobiliary tumors (eg, cholangiocarcinoma, ampullary, and duodenal carcinomas).

We examined RAF categorization as a prognostic or predictive factor. In our cohort, RAF categorization was not associated with differences in overall survival. Unlike previous reports in colon cancer and lung cancer, BRAFV600E alterations were not predictive of poor response to chemotherapy. However, we found that RAF fusion abnormalities may speculatively represent a predictive marker of improved response to FOLFIRINOX and poor response to gemcitabine and nab-paclitaxel. These findings are limited by sample sizes not sufficiently large to account for potentially confounding factors. We were

FIG 4. PFS analysis across RAF classes for two types of standard therapies commonly implemented in pancreatic cancer. PFS while receiving either (A) first-line FOLFIRINOX, (B) 5FU-based chemotherapy (10 on FOLFIRINOX; two on FOLFOX; two on FOLFIRI; four on 5FU/nal-irinotecan) in second-line or later, or gemcitabine/nab-paclitaxel given in (C) first line or (D) later lines were analyzed for each of the four categories of BRAF alterations with median PFS values (95% CIs) shown. FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFIRINOX, infusional fluorouracil, leucovorin, irinotecan, and oxaliplatin; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; FU, fluorouracil; NA, not applicable; NR, not reached; PFS, progression-free survival.
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There are selection biases for those who receive raf-directed therapy that cannot be accounted for in this design. Observational bias can occur when recording responses to therapy in select groups of patients. These case reports were collected from academic medical centers. Therefore, this case series may not adequately represent important population-level factors (eg, differences in insurance coverage, socioeconomic status, urban vs rural cohorts, and academic vs community settings) that can influence patient outcomes as well as access to targeted therapies either off label or on a clinical trial.

Nonetheless, because of the high unmet need in the PC patient population and the infrequency of BRAF alterations, a single-arm prospective trial confirming substantial response rates and durability of responses would likely be sufficient to pursue an application to expand fda-approved labels for BRAF inhibitor combinations with MEK inhibitors to include patients with BRAF-mutated PC within the exon 15 subgroup. Following the recent approval of a BRAF inhibitor plus an EGFR antibody (but not for the triple targeted approach that included a MEK inhibitor) in BRAF V600E-mutated colon cancer, 58 multipronged strategies targeting BRAF alongside other signaling components beyond the RAF/MEK/ERK signaling cascade may warrant further investigation. Profiling the activation of upstream receptors following MAPK pathway inhibition may provide clues into adaptive resistance mechanisms that could be exploited in a disease-specific manner. 59 As future generations of BRAF-directed therapies enter clinical trials, it will be imperative to understand the binding affinity of these novel agents for different RAF variant subgroups and to screen for potential mechanisms of acquired (MEK mutation) or intrinsic (KRAS mutation) resistance. 31

Herein, we have described a cohort of RAF-mutated PC that comprises 2% of PC cases. We report promising treatment responses and encouraging outcomes in patients with BRAF exon 15 and BRAF/RAF1 fusions receiving MAPK pathway-directed therapies. Prospective studies are warranted to confirm these hypothesis-generating results and establish the optimal treatment approaches for BRAF-mutated PC taking into account current standards of care.

affiliations
1 Cedars-Sinai Medical Center, Los Angeles, CA
2 Perthera, Inc, McLean, VA
3 Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
4 The University of Texas MD Anderson Cancer Center, Houston, TX
5 Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA
6 Memorial Sloan Kettering Cancer Center, New York, NY
7 Inova Schar Cancer Institute, Fairfax, VA
8 George Mason University, Perthera, Inc, Fairfax, VA
9 Massachusetts General Hospital, Harvard Medical School, Boston, MA
10 Pancreatic Cancer Action Network, Manhattan Beach, CA
11 Washington University School of Medicine, St Louis, MO
12 Johns Hopkins University School of Medicine, Perthera Inc, McLean, VA

CORRESPONDING AUTHOR
Andrew Hendifar, MD, MPH, Cedars-Sinai Medical Center, 8700 Beverly Blvd, Suite 1042 ac, Los Angeles, CA 90048; e-mail: Andrew.hendifar@csbs.org

EQUAl CONTRIBUTION
A.H. and E.M.B. are co-lead authors.
M.J.P. and E.M.O. are co-senior authors.

AUTHOR CONTRIBUTIONS
Conception and design: Andrew Hendifar, Edik M. Blais, Vivek Subbiah, Eric Collisson, Kenna Shaw, Emanuel F. Petricoin III, Andrea Wang-Gilliam, Michael J. Pishvaia, Eileen M. O’Reilly
Administrative support: Vivek Subbiah, Kenna Shaw, Emanuel F. Petricoin III, Emily Lyons

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Provision of study materials or patients: Brian Wolpin, Vivek Subbiah, Isha Singh, Timothy Cannon, Kenna Shaw, Emanuel F. Petricoin III, Samuel Klemper, Eileen M. O’Reilly
Collection and assembly of data: Andrew Hendifar, Edik M. Blais, Brian Wolpin, Vivek Subbiah, Isha Singh, Timothy Cannon, Kenna Shaw, Emanuel F. Petricoin III, Samuel Klemper, Emily Lyons, Andrea Wang-Gilliam, Michael J. Pishvaia, Eileen M. O’Reilly
Data analysis and interpretation: Andrew Hendifar, Edik M. Blais, Vivek Subbiah, Eric Collisson, Emanuel F. Petricoin III, Samuel Klemper, Andrea Wang-Gilliam, Michael J. Pishvaia, Eileen M. O’Reilly
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Andrew Hendifar
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Travel, Accommodations, Expenses: Halozyme

Edik M. Blais
Employment: AstraZeneca, Perthera, Emerald Cloud Labs

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FIG A1. OS analysis comparing patients who received a molecularly matched therapy targeting the MAPK signaling pathway (eg, BRAF/MEK/ERK inhibitors) versus those who only received unmatched therapies in the advanced treatment setting. OS differences between matched and unmatched subgroups were not considered statistically significant for either RAF subgroups (A) Exon 15, (B) Exon 11, (C) Fusions, or (D) Other alterations when analyzed individually ($P > .05$). For the broader subset of patients, mOS differences were trending toward benefit but not considered significant ($P = .07252$; HR = 0.48 [0.21 to 1.07]) when comparing matched (mOS = 1.92 years [1.37 to NA], n = 14) and unmatched (mOS = 1.51 years [0.95 to 2.89], n = 25) subgroups. Only patients who were initially diagnosed with metastatic disease were included in these analyses (see OS Matched and OS Unmatched subgroups in Table 3 for additional baseline characteristics across the combined cohort). HR, hazard ratio; MAPK, mitogen-activated protein kinase; mOS, median overall survival; NA, not applicable; OS, overall survival.
FIG A2. PFS analyses highlighting favorable trends for 5FU-based therapies versus gemcitabine/nab-paclitaxel in patients with RAF fusions or (B) separated for first line of therapy versus later lines. A significant difference in mPFS was observed for FOLFIRINOX versus gemcitabine/nab-paclitaxel within the BRAF fusion subgroup (A) using a univariate Cox regression model across all lines of therapy ($P = .0051; HR = 0.1$ [0.02 to 0.50]) or (B) using a multivariate model ($P = .027$) that factored in therapies given in first line of therapy versus later lines. Although these trends were considered significant, prospective evaluation is warranted when considering the imbalance between treatment choices for first line, an unexpected trend of longer PFS for later lines versus first line (note that this term was not significant in the multivariate model), the relatively small sample sizes, among other potentially confounding factors. Within this subset of the BRAF fusion analysis cohort, acinar cell carcinoma histology was seen in five (36% of 14) and six (50% of 12) for 5FU-based versus gemcitabine-based therapies, respectively (the rest were adenocarcinoma). This variable was not significantly enriched by Fisher’s exact test ($P = .69$), and its addition to the multivariate Cox regression model yielded similar results for the contrast between regimens ($P = .0405; HR = 0.1$ [0.01 to 0.9]). FOLFIRINOX, infusional fluorouracil, leucovorin, irinotecan, and oxaliplatin; FU, fluorouracil; HR, hazard ratio; PFS, progression-free survival.