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TARGETED DRUG THERAPY

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

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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

ASSOCIATED CONTENT

Appendix

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

To quantify the prevalence of *RAF* alterations in epithelial pancreatic cancer and report real-world outcomes to standard and targeted therapies from a multi-institution case series.

Knowledge Generated

The frequency of *RAF* alterations in pancreatic cancer 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. Clinical benefit from targeted therapies occurred in patients with *BRAF V600E* mutations, *RAF* fusion abnormalities, and Exon 11 mutations.

Relevance

In *KRAS* wild-type pancreatic cancer, certain *RAF* alterations predict benefit from map-kinase targeted therapy. Further prospective trials are warranted.

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 Program[15] and other hospital initiatives.[36] Additional public data were obtained from 1,979 patients with genomic testing results available via the AACR GENIE project[37] (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 pancreatoblastoma.

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 institutionally supported clinical assay (OncoPanel) was used.[29] 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

***BRAF* Subgroup**	**Molecular Definition**	**Potential Implications for Targeted Therapy**	**Estimated Prevalence[a]**
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 nearby 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.

[a]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,[37] 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 Cohort[a]

***BRAF* Subgroup**	**No. Represented in Clinical Cohort (%)[a]**	**Top *BRAF* 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 mucosal neoplasms.
[a]The Clinical Cohort includes a case series of 81 patients analyzed in this study from multiple institutions.

2.01] with a median follow-up of 1.22 years and 38 total events. The median number of lines of therapy was 2.

Actionability of *RAF* Variants and Confounding Drivers

To assess response to *BRAF*-directed therapy, genomic profiles from the Clinical Cohort were classified into one of four subgroupings (Table 2), as described above for the Prevalence Cohort: Exon 15 (17 of 81, 21.0%), Fusions (25 of 81, 30.9%), Exon 11 (18 of 81, 22.2%), or Other (21 of 81, 25.9%). The resulting distributions of potentially actionable *RAF* variants in PC were enriched in *BRAF* Exons 11 and 15 (Fig 1A).

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.

Survival Analysis by RAF Subgroup

We performed exploratory analyses to assess the prognostic impact of each *RAF* subgrouping (Fig 2) across the 54 patients who presented with metastatic disease (see OS Cohort in Table 3). No significant differences in median overall survival was observed (Fig 2).

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/RAF*1 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).

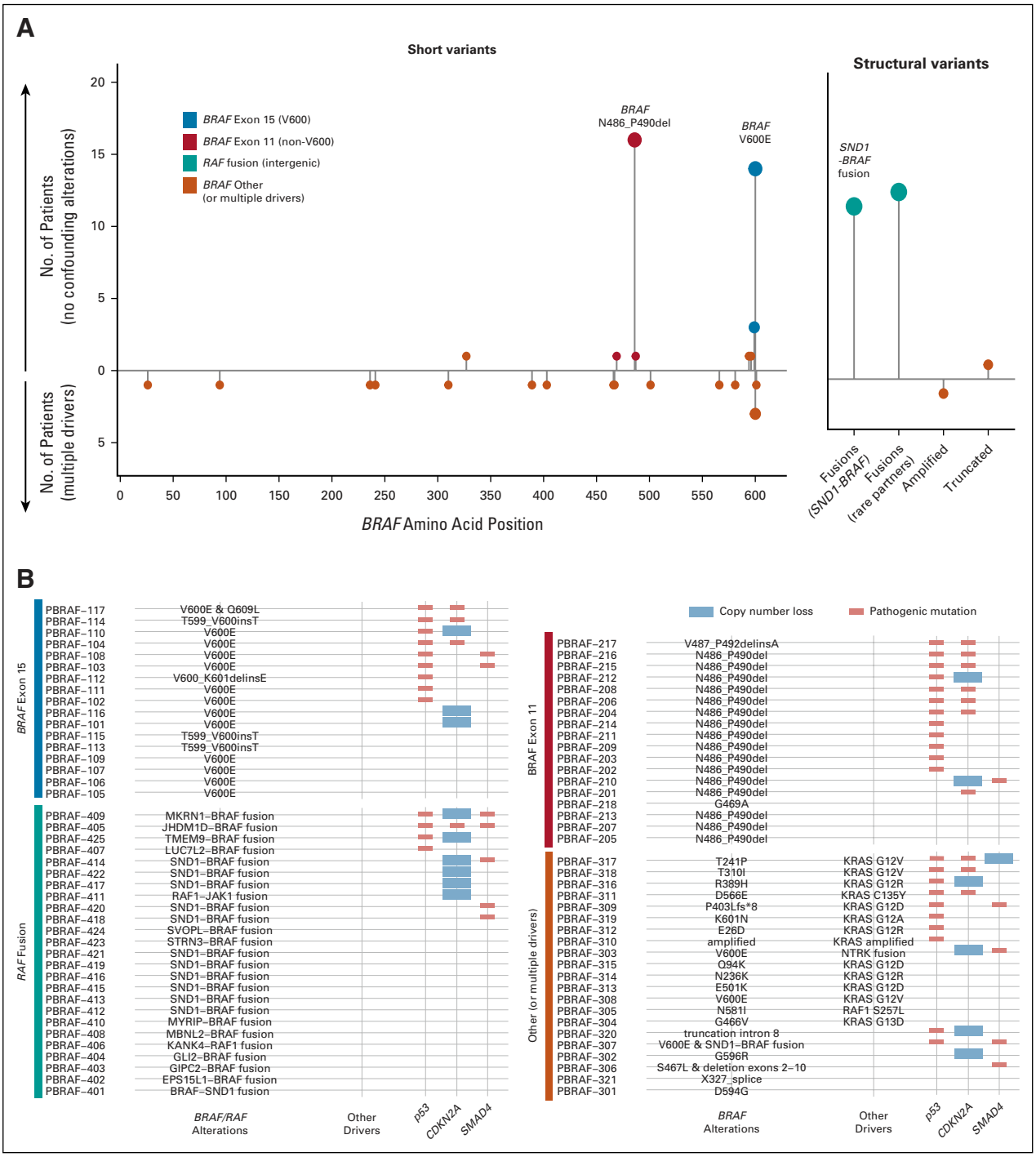

FIG 1. Genomic profiling results from pancreatic tumors harboring *RAF* pathway alterations. (A) Lollipop plot highlighting amino acid positions along the *BRAF* gene where alterations were most commonly found in this case series (n = 81). Each stemmed circle represents the numbers of patients with a *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 ΔNVTAP, 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.

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])

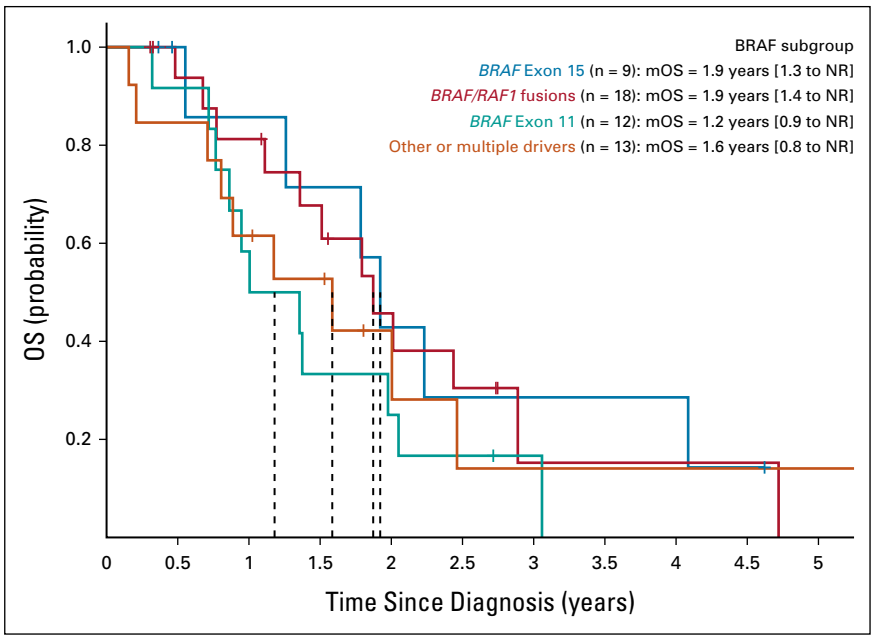

FIG 2. OS of advanced PC subjects by RAF subgroup. No significant differences in OS (from initial diagnosis) were observed across these four categories ($P > .05$, pairwise comparisons evaluated by Cox regression), suggesting that these functional classifications of RAF alterations are not likely prognostic. For this survival analysis, patients diagnosed with IPMN's and resected disease were excluded (see OS Cohort in Table 3 for additional baseline characteristics). IPMN, intraductal papillary mucinous neoplasm; NR, not reached; OS, overall survival.

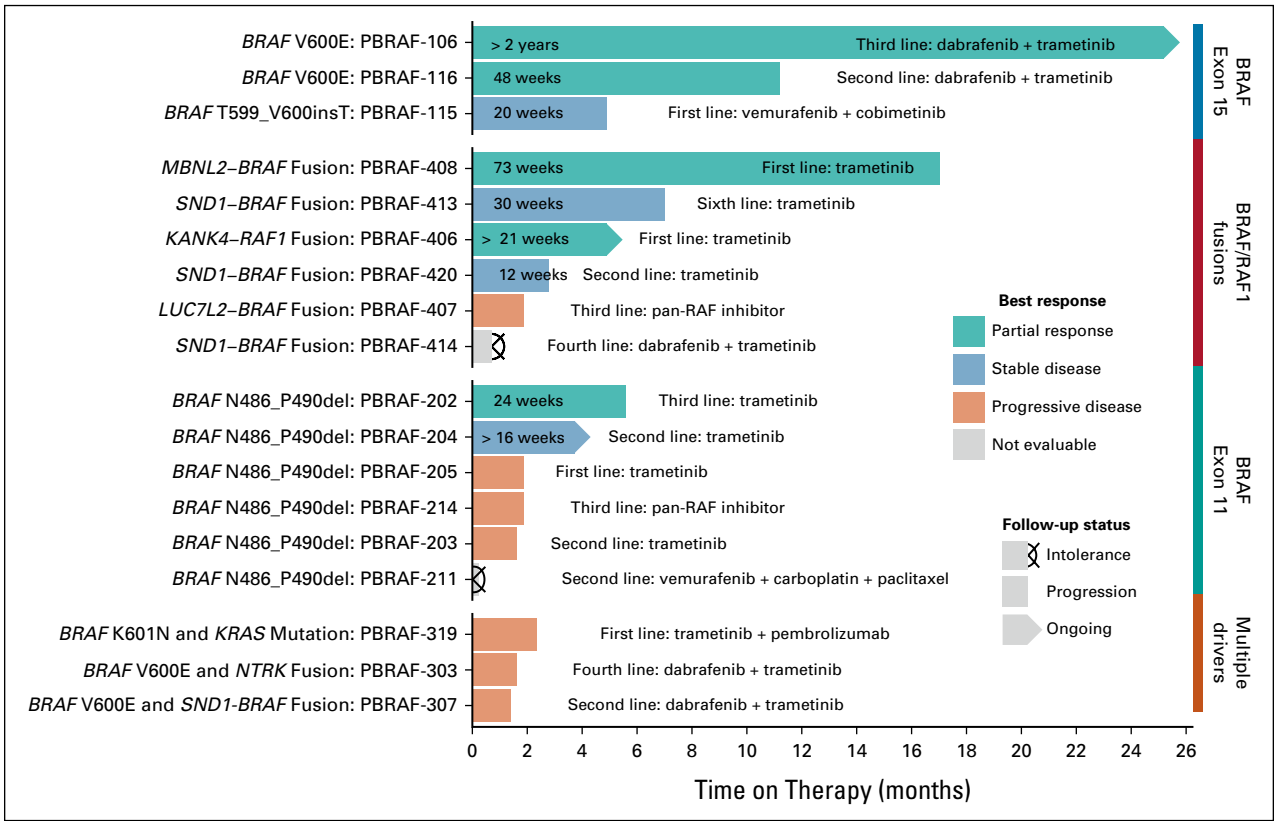

FIG 3. PFS data while on *BRAF/MEK/ERK* inhibitors for patients with *RAF*-mutated pancreatic cancer. Responses to 17 patients treated with *RAF*-directed therapy categorized by *RAF* subgroup, including six patients treated with combination *BRAF* and *MEK* and eight patients with *MEK* inhibitor therapy alone. Each horizontal bar represents the time on therapy without disease progression (ie, PFS). Teal represents treatment lines with partial response as best response, blue are patients who achieved stable disease, light red bars are patients with rapidly progressive disease (no response), and gray bars indicate unevaluable subjects who had discontinued due to tolerability issues (censored event). Bars capped with arrows indicate lines of therapy that were continuing as of the last available progress note (censored event). It is important to note that vemurafenib plus chemotherapy given to *PBRAF*-211 is considered an unmatched therapy (see Table 1 for actionability definitions), as is considered for all targeted therapies given to patients in the Other subgroup. PFS, progression-free survival.

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 adenocarcinoma 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[34,37,38,41-43] centered around three hotspot variants: *BRAF* V600E in Exon 15, *BRAF* N486_P490del in Exon 11,[44] 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.[40]

In this cohort, we confirm previous reports of clinical responses to *MEK* and *BRAF* inhibition in subjects with biologically significant *RAF* alterations.[43,45-49] Benefit was well aligned with the classification system described by Yaeger et al,[50] 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

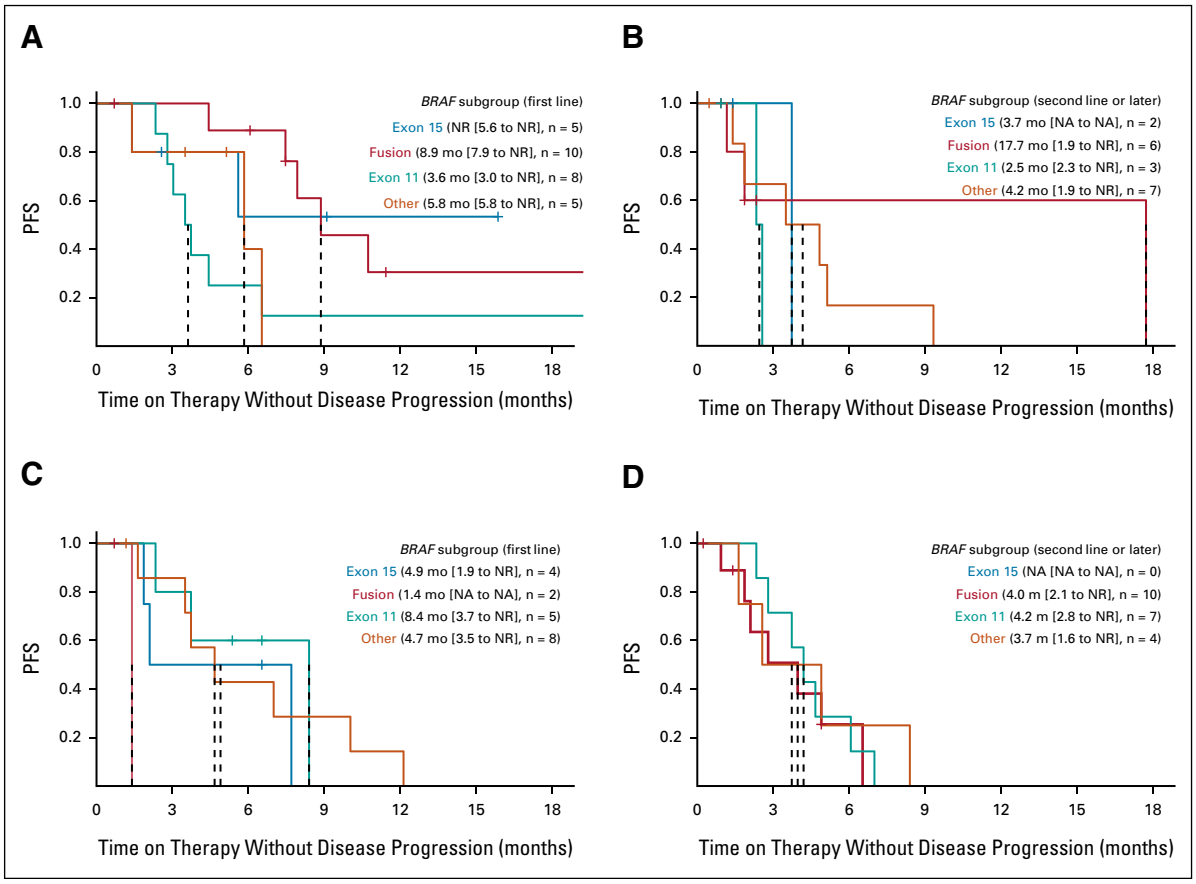

FIG 4. PFS analysis across *RAF* classes for two types of standard therapies commonly implemented in pancreatic cancer. PFS while receiving either (A) first-line FOLFIRINOX, (B) 5FU-based chemotherapy (10 on FOLFIRINOX; two on FOLFOX; two on FOLFIRI; four on 5FU/nal-irinotecan) in second-line or later, 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.

vemurafenib despite an increase in *RAS*-independent dimerization-dependent kinase activity for cells with this in-frame deletion.[13,51] However, there are clinical case reports of significant activity with dabrafenib in patients with *BRAF* ΔNVTAP deletions,[52] 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 *BRAF* V600E mutations in Exon 15 and *BRAF* ΔNVTAP deletions in Exon 11 conceptually mirror *EGFR* L858R 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.[13]

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* V600 basket trial and the results were consistent with our findings.[24] 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).[53] 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).[38,54,55]

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,[47,56] *BRAF* V600E 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

unable to find any evaluation of chemotherapeutic response to tumors harboring fusion abnormalities outside of pemetrexed therapy in lung cancers with ROS1 fusion abnormalities.[57]

Our data set is limited by its retrospective design and modest sized cohort as the abnormality of interest is rare. 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 *v* rural cohorts, and academic *v* 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 within *BRAF* Exon 15 and *BRAF/RAF*1 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.

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APPENDIX

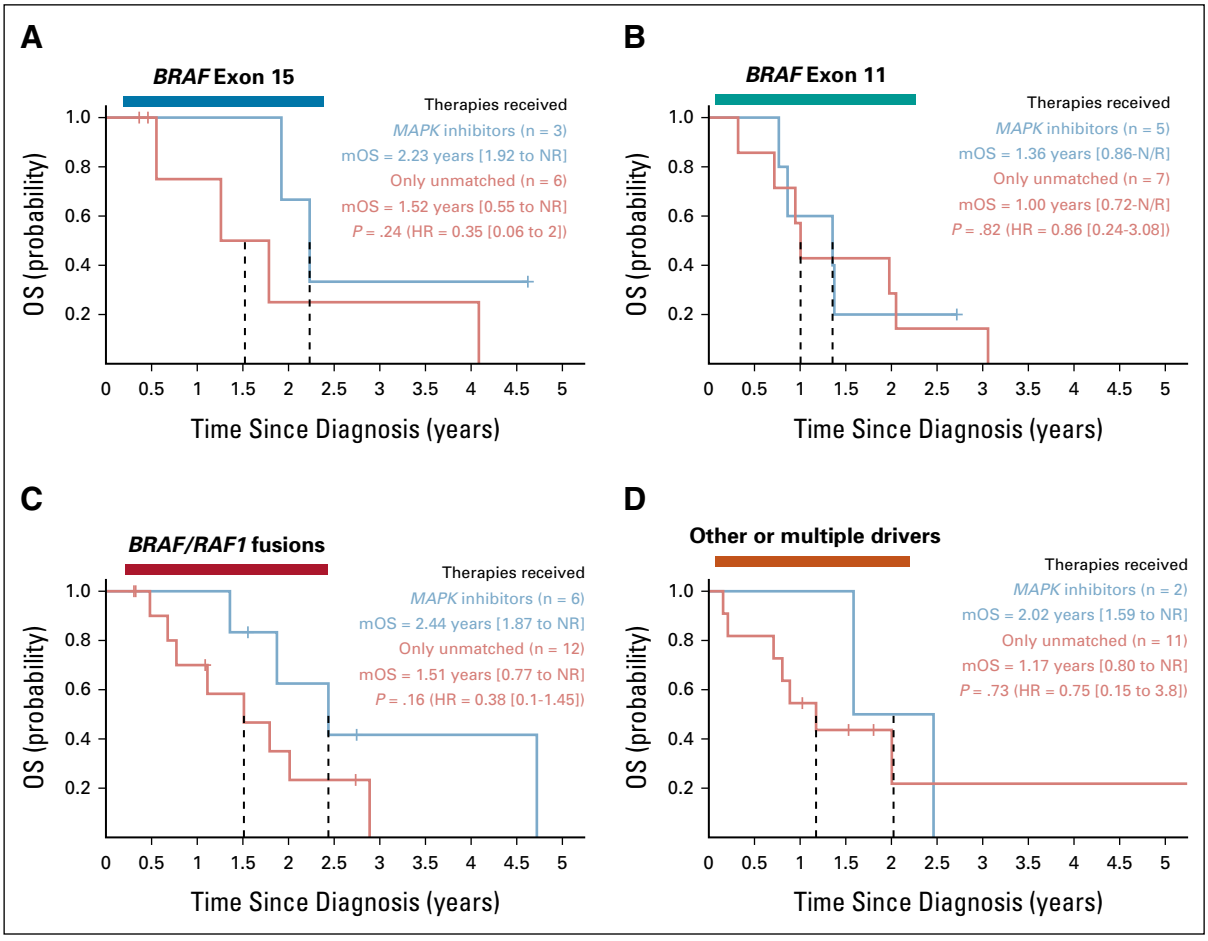

FIG A1. OS analysis comparing patients who received a molecularly matched therapy targeting the *MAPK* signaling pathway (eg, *BRAF/MEK/ERK* inhibitors) versus those who only received unmatched therapies in the advanced treatment setting. OS differences between matched and unmatched subgroups were not 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.

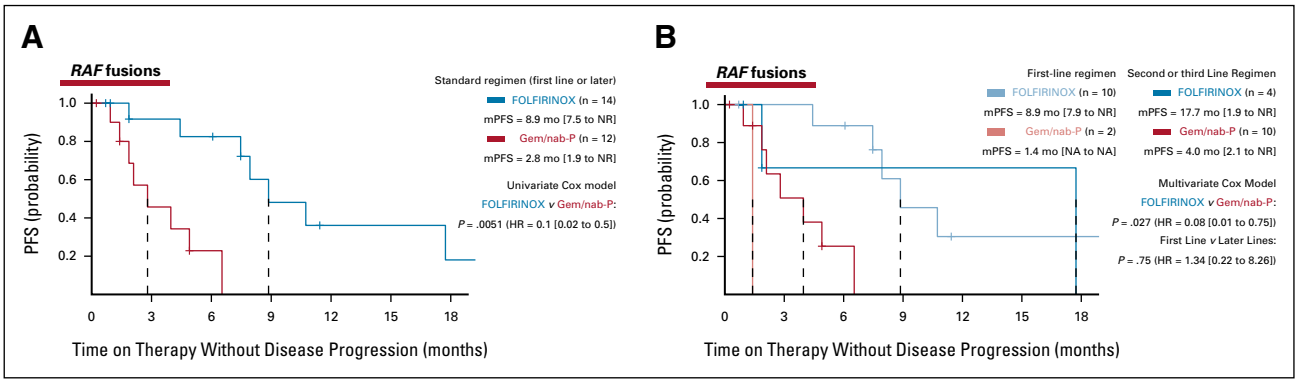

FIG A2. PFS analyses highlighting favorable trends for 5FU-based therapies versus gemcitabine/nab-paclitaxel in patients with *RAF* fusions or (B) separated for first line of therapy versus later lines. A significant difference in mPFS was observed for FOLFIRINOX versus gemcitabine/nab-paclitaxel within 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.

