JAMA Oncology | Brief Report

Association of Alterations in Main Driver Genes With Outcomes of Patients With Resected Pancreatic Ductal Adenocarcinoma

Zhi Rong Qian, MD, PhD; Douglas A. Rubinson, MD, PhD; Jonathan A. Nowak, MD, PhD; Vicente Morales-Oyarvide, MD, MPH; Richard F. Dunne, MD; Margaret M. Kozak, MD; Marisa W. Welch, BA; Lauren K. Brais, MPH; Annacarolina Da Silva, MD; Tingting Li, MD, PhD; Wanwan Li, MD; Atsuhiro Masuda, MD, PhD; Juhong Yang, MD, PhD; Yan Shi, MD, PhD; Mancang Gu, PhD; Yohei Masugi, MD, PhD; Justin Bui, BS; Caitlin L. Zellers, BA; Chen Yuan, MS; Ana Babic, PhD; Natalia Khalaf, MD; Andrew Aguirre, MD, PhD; Kimmie Ng, MD, MPH; Rebecca A. Miksad, MD, MPH; Andrea J. Bullock, MD; Daniel T. Chang, MD; Jennifer F. Tseng, MD, MPH; Thomas E. Clancy, MD; David C. Linehan, MD; Jennifer J. Findeis-Hosey, MD; Leona A. Doyle, MD; Aaron R. Thorner, PhD; Matthew Ducar, MS; Bruce Wollison; Angelica Laing, BA; William C. Hahn, MD, PhD; Matthew Meyerson, MD, PhD; Charles S. Fuchs, MD, MPH; Shuji Ogino, MD, PhD, MS; Jason L. Hornick, MD, PhD; Aram F. Hezel, MD; Albert C. Koong, MD, PhD; Brian M. Wolpin, MD, MPH

IMPORTANCE Although patients with resected pancreatic adenocarcinoma are at high risk for disease recurrence, few biomarkers are available to inform patient outcomes.

OBJECTIVE To evaluate the alterations of the 4 main driver genes in pancreatic adenocarcinoma and patient outcomes after cancer resection.

DESIGN, SETTING, AND PARTICIPANTS This study analyzed protein expression and DNA alterations for the *KRAS*, *CDKN2A*, *SMAD4*, and *TP53* genes by immunohistochemistry and next-generation sequencing in formalin-fixed, paraffin-embedded tumors in 356 patients with resected pancreatic adenocarcinoma who were treated at the Dana-Farber/Brigham and Women's Cancer Center (October 26, 2002, to May 21, 2012), University of Rochester Medical Center (March 1, 2006, to November 1, 2013), or Stanford Cancer Institute (September 26, 1995, to May 22, 2013). Associations of driver gene alterations with disease-free survival (DFS) and overall survival (OS) were evaluated using Cox proportional hazards regression with estimation of hazard ratios (HRs) and 95% CIs and adjustment for age, sex, tumor characteristics, institution, and perioperative treatment. Data were collected September 9, 2012, to June 28, 2016, and analyzed December 17, 2016, to March 14, 2017.

MAIN OUTCOMES AND MEASURES The DFS and OS among patients with resected pancreatic adenocarcinoma.

RESULTS Of the 356 patients studied, 191 (53.7%) were men and 165 (46.3%) were women, with a median (interquartile range [IQR]) age of 67 (59.0-73.5) years. Patients with *KRAS* mutant tumors had worse DFS (median [IQR], 12.3 [6.7 -27.2] months) and OS (20.3 [11.3-38.3] months) compared with patients with *KRAS* wild-type tumors (DFS, 16.2 [8.9-30.5] months; OS, 38.6 [16.6-63.1] months) and had 5-year OS of 13.0% vs 30.2%. Particularly poor outcomes were identified in patients with *KRAS* G12D-mutant tumors, who had a median (IQR) OS of 15.3 (9.8-32.7) months. Patients whose tumors lacked CDKN2A expression had worse DFS (median, 11.5 [IQR, 6.2-24.5] months) and OS (19.7 [10.9-37.1] months) compared with patients who had intact CDKN2A (DFS, 14.8 [8.2-30.5] months; OS, 24.6 [14.1-44.6] months). The molecular status of *SMAD4* was not associated with DFS or OS, whereas *TP53* status was associated only with shorter DFS (HR, 1.33; 95% CI, 1.02-1.75; $P = .04$). Patients had worse DFS and OS if they had a greater number of altered driver genes. Compared with patients with 0 to 2 altered genes, those with 4 altered genes had worse DFS (HR, 1.79 [95% CI, 1.24-2.59; $P = .002$]) and OS (HR, 1.38 [95% CI, 0.98-1.94; $P = .06$]). Five-year OS was 18.4% for patients with 0 to 2 gene alterations, 14.1% for those with 3 alterations, and 8.2% for those with 4 alterations.

CONCLUSIONS AND RELEVANCE Patient outcomes are associated with alterations of the 4 main driver genes in resected pancreatic adenocarcinoma.

JAMA Oncol. 2018;4(3):e173420. doi:10.1001/jamaoncol.2017.3420
Published online November 2, 2017. Corrected on March 7, 2019.

Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Brian M. Wolpin, MD, MPH, Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, 450 Brookline Ave, Boston, MA 02215 (bwolpin@partners.org).

Pancreatic cancer is the third leading cause of cancer-related death in the United States.[1] Large-scale genome sequencing studies have identified multiple molecular pathways involved in pancreatic adenocarcinoma initiation and progression.[2-4] Four main driver genes have been identified—*KRAS* (NCBI 3845), *CDKN2A* (NCBI 1029), *SMAD4* (NCBI 4089), and *TP53* (NCBI 7157)—that are critical for pancreatic cancer growth. The association of these driver gene alterations with patient outcomes has not been clearly established. Therefore, we characterized the status of these 4 driver genes using immunohistochemistry (IHC) and next-generation sequencing (NGS) of DNA in a large, highly annotated, multi-institutional patient population with resected pancreatic adenocarcinoma.

Key Points

Question Are alterations in the 4 main driver genes for pancreatic adenocarcinoma associated with patient outcomes after pancreatic cancer resection?

Findings In this study involving 356 patients with resected pancreatic adenocarcinoma, immunohistochemistry and next-generation DNA sequencing of formalin-fixed, paraffin-embedded pancreatic adenocarcinoma resection specimens identified alterations in the 4 main driver genes (*KRAS*, *CDKN2A*, *SMAD4*, and *TP53*). Disease-free survival and overall survival were associated with the presence and pattern of alterations in these 4 genes independent of previously identified prognostic factors.

Meaning Identifying the pathogenic alterations in the 4 main driver genes of pancreatic adenocarcinoma informs patient outcomes.

Methods

The study population consisted of 356 patients with resected pancreatic adenocarcinoma, of whom 126 were treated at the Dana-Farber/Brigham and Women's Cancer Center between October 26, 2002, and May 21, 2012; 90 were treated at the University of Rochester Medical Center between March 1, 2006, and November 1, 2013; and 140 were treated at the Stanford Cancer Institute between September 26, 1995, and May 22, 2013. The institutional review board at each institution granted approval for this study. Patients treated at the Dana-Farber/Brigham and Women's Cancer Center signed written informed consent for participation in this study. Informed consent was waived by the University of Rochester Medical Center and the Stanford Cancer Institute as patients were identified retrospectively, according to institutional review board exempt protocols. Data were collected from September 9, 2012, to June 28, 2016. Data analysis took place from December 17, 2016, to March 14, 2017.

Immunohistochemistry for CDKN2A, SMAD4, and TP53 was performed on formalin-fixed, paraffin-embedded whole-tissue sections (eAppendix and eFigure 1 in the Supplement). After macroscopic dissection, genomic DNA was extracted from tumor tissue and adjacent normal tissue. Pyrosequencing for *KRAS* hotspot mutations and NGS using a customized, massively parallel sequencing panel were performed to determine the molecular status of *KRAS*, *CDKN2A*, *SMAD4*, and *TP53*

Table 1. Disease-Free Survival and Overall Survival by *KRAS*, *CDKN2A*, *SMAD4*, and *TP53* Tumor Status

	Disease-Free Survival (n = 335)						Overall Survival (n = 338)					
			Rate						Rate			
Driver Gene	Patients, No. (%)	Median (IQR), mo	2-y Survival, %	5-y Survival, %	HR (95% CI)[a]	*P* Value[b]	Patients, No. (%)	Median (IQR), mo	2-y Survival, %	5-y Survival, %	HR (95% CI)[a]	*P* Value[b]
KRAS												
Wild-type	27 (8.1)	16.2 (8.9-30.5)	30.2	20.2	1 [Reference]		27 (8.0)	38.6 (16.6-63.1)	63.0	30.2	1 [Reference]	
Mutant	308 (91.9)	12.3 (6.7-27.2)	27.5	12.4	1.72 (1.04-2.84)	.03	311 (92.0)	20.3 (11.3-38.3)	44.5	13.0	1.55 (0.96-2.51)	.08
CDKN2A												
Intact	111 (33.1)	14.8 (8.2-30.5)	31.2	16.9	1 [Reference]		112 (33.1)	24.6 (14.1-44.6)	53.8	19.5	1 [Reference]	
Lost	224 (66.9)	11.5 (6.2-24.5)	26.0	11.5	1.62 (1.19-2.20)	.002	226 (66.9)	19.7 (10.9-37.1)	42.3	11.9	1.44 (1.08-1.91)	.01
SMAD4												
Intact	172 (51.3)	11.5 (6.6-30.1)	27.1	14.4	1 [Reference]		173 (51.2)	21.3 (18.2-26.7)	49.1	15.8	1 [Reference]	
Lost	163 (48.7)	13.6 (7.4-27.0)	28.4	12.3	1.18 (0.90-1.55)	.25	165 (48.8)	20.5 (11.3–39.3)	43.0	12.9	1.07 (0.83-1.38)	.62
TP53												
Wild-type	118 (35.2)	14.8 (8.1-30.5)	31.4	13.9	1 [Reference]		119 (35.2)	24.6 (13.5-44.6)	50.7	18.7	1 [Reference]	
Altered	217 (64.8)	10.8 (6.2-24.5)	25.7	12.6	1.33 (1.02-1.75)	.04	219 (64.8)	20.3 (11.1-37.8)	43.5	12.3	1.18 (0.91-1.53)	.23

Abbreviations: HR, hazard ratio; IQR, interquartile range.

[a] Cox proportional hazards regression model adjusted for age, sex, pathologic N stage, tumor grade, lymphovascular invasion, receipt of perioperative treatment, resection margin status, and institution.

[b] *P* value for multivariable-adjusted Cox proportional hazards regression.

(eAppendix in the Supplement). For *KRAS*, tumors were classified as mutant or wild-type on the basis of NGS or pyrosequencing if predefined NGS coverage metrics were not met (eFigure 2 in the Supplement). For *CDKN2A* and *SMAD4*, tumors were classified as intact or lost on the basis of IHC results. For *TP53,* IHC and sequencing data were combined to make an integrated call as wild-type or altered (eAppendix in the Supplement).

Disease-free survival (DFS) was defined as time between surgery and disease recurrence, and *overall survival* (OS) was defined as time between surgery and death. Disease recurrence was classified as "local" when it occurred in or adjacent to the pancreatic remnant or in the retroperitoneum. Disease recurrence outside these sites was classified as "distant." Follow-up continued through June 28, 2016, for the Dana-Farber/Brigham and Women's Cancer Center; March 17, 2016, for the University of Rochester Medical Center; and March 11, 2016, for the Stanford Cancer Institute. A flow diagram of the study population is presented in eFigure 3 in the Supplement.

Statistical Analysis

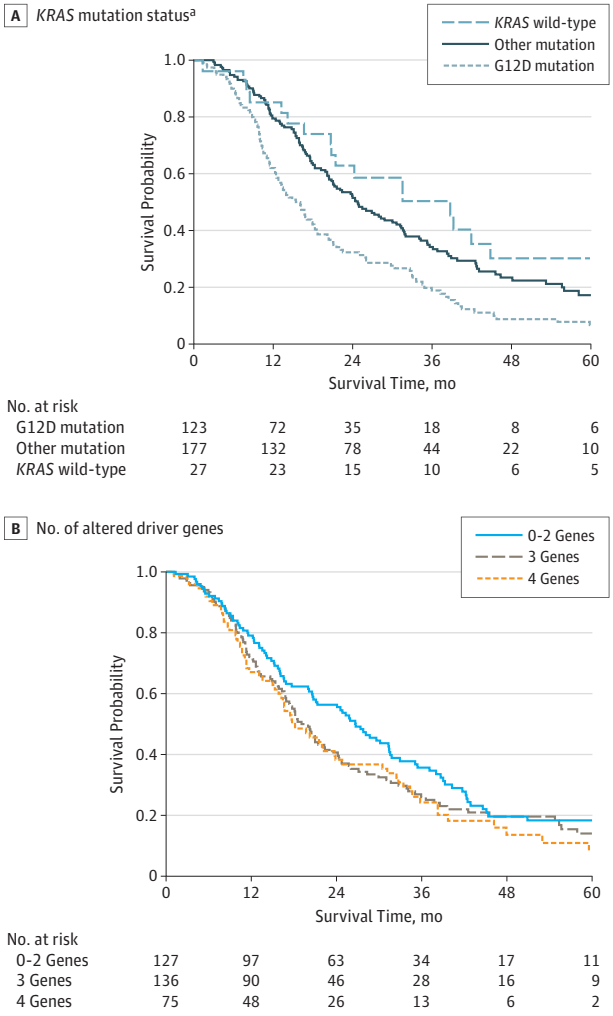
We evaluated the associations of driver gene alterations with DFS and OS using multivariable-adjusted Cox proportional hazards regression (eAppendix in the Supplement), calculating hazard ratios (HRs) and 95% CIs. We generated Kaplan-Meier curves, from which we calculated median survival, 2-year survival, and 5-year survival. In addition, we analyzed the association of gene alterations with pattern of first recurrence using multivariable-adjusted logistic regression, calculating odds ratios and 95% CIs. All hypothesis tests were 2-sided, and a 2-sided $P < .05$ indicated statistical significance.

Results

Baseline characteristics of the study population by institution are shown in eTable 1 in the Supplement and by the 4 main driver genes are presented in eTable 2 in the Supplement. Of the 356 patients studied, 191 (53.7%) were men and 165 (46.3%) were women, with a median (interquartile range [IQR]) age of 67 (59.0-73.5) years. The median (IQR) DFS was 13.1 (7.0-27.8) months and OS was 21.0 (11.4-39.6) months, which are comparable to those in randomized trials.[5,6]

Activating *KRAS* mutations were observed in 328 patients (92.1%) (eTable 3 in the Supplement); *KRAS* mutations affecting 2 separate codons were found in 11 tumors (3.4%). Patients who had *KRAS* mutant tumors had worse DFS (median [IQR], 12.3 [6.7-27.2] months) and OS (20.3 [11.3-38.3] months) compared with patients who had *KRAS* wild-type tumors (DFS, 16.2 [8.9-30.5] months; OS, 38.6 [16.6-63.1] months) (**Table 1**). Five-year OS rates were 13.0% for patients whose tumors were *KRAS* mutant and 30.2% for those with *KRAS* wild-type tumors. In addition, patients with *KRAS* G12D-mutant tumors had particularly poor outcomes, with worse DFS (median [IQR], 9.5 [4.7-17.6] months) and OS (15.3 [9.8-32.7] months) compared with patients with *KRAS* G12D wild-type tumors (DFS, 14.8 [7.9-32.8]; OS, 24.8 [15.0-46.2]) (**Figure** and **Table 2**; eFigure 4 in the Supplement).

Figure. Kaplan-Meier Survival Curves for Overall Survival (OS)



Overall survival was analyzed based on *KRAS* mutation status (A) and number of altered driver genes (*KRAS, CDKN2A, SMAD4,* and *TP53*) (B). The median (interquartile range [IQR]) OS for patients with *KRAS* G12D-mutant tumors was 15.3 (9.8-32.7) months, was 24.6 [15.0-45.4] months for patients with other *KRAS* mutations (hazard ratio [HR], 0.68; 95% CI, 0.52-0.91; $P = .008$), and was 38.6 [16.6-63.1] months for patients with *KRAS* wild-type tumors (HR, 0.49; 95% CI, 0.29-0.82; $P = .006$). Patients with 0 to 2 gene alterations had a median (IQR) OS of 26.7 (13.1-42.5) months. Those with 3 gene alterations had a median (IQR) OS of 19.1 (11.3-37.8) months (HR, 1.22; 95% CI, 0.91-1.64; $P = .18$), whereas those with 4 gene alterations had a median (IQR) OS of 17.8 (10.7-35.8) months (HR, 1.38; 95% CI, 0.98-1.94; $P = .06$). The Cox proportional hazards regression model was adjusted for age, sex, pathologic N stage, tumor grade, lymphovascular invasion, receipt of perioperative treatment, resection margin status, and institution.

[a] Excludes 11 patients with 2 distinct *KRAS* codon mutations within the same tumor.

By IHC, CDKN2A protein expression was lost in 240 patients (67.4%). Patients who had CDKN2A expression loss by IHC had worse DFS (median [IQR], 11.5 [6.2-24.5] months) and OS (19.7 [10.9-37.1] months) compared with patients with intact CDKN2A (DFS, 14.8 [8.2-30.5] months; OS, 24.6 [14.1-44.6] months) (Table 1). In sensitivity analyses, we classified *CDKN2A* status using IHC and sequencing data (eTable 4 in the

Table 2. Disease-Free Survival and Overall Survival by *KRAS* Codon Mutation and Combined *KRAS*, *CDKN2A*, *SMAD4*, and *TP53* Gene Alterations

Driver Gene Alteration	Disease-Free Survival (n = 335)			Overall Survival (n = 338)		
	Patients, No. (%)	HR (95% CI)[a]	*P* Value[b]	Patients, No. (%)	HR (95% CI)[a]	*P* Value[b]
KRAS mutation[c]						
G12D	122 (36.4)	1 [Reference]		123 (36.4)	1 [Reference]	
G12V	104 (31.0)	0.57 (0.41-0.79)	<.001	105 (31.1)	0.63 (0.46-0.87)	.005
G12R	44 (13.1)	0.67 (0.43-1.05)	.08	45 (13.3)	0.82 (0.54-1.25)	.35
Other codon	25 (7.5)	0.63 (0.37-1.10)	.10	25 (7.4)	0.83 (0.50-1.39)	.48
2 Codon mutations	11 (3.3)	0.27 (0.11-0.69)	.006	11 (3.3)	0.55 (0.26-1.15)	.11
Wild-type	27 (8.1)	0.38 (0.22-0.65)	<.001	27 (8.0)	0.50 (0.30-0.83)	.008
No. of altered genes						
0-2 Genes	126 (37.6)	1 [Reference]		127 (37.6)	1 [Reference]	
3 Genes	135 (40.3)	1.37 (1.01-1.86)	.05	136 (40.2)	1.22 (0.91-1.64)	.18
4 Genes	74 (22.1)	1.79 (1.24-2.59)	.002	75 (22.2)	1.38 (0.98-1.94)	.06
Gene combinations[d]						
0-2 Genes	126 (37.6)	1 [Reference]		127 (37.6)	1 [Reference]	
3 Genes						
KRAS, SMAD4, TP53	35 (10.4)	1.16 (0.74-1.82)	.53	35 (10.4)	1.08 (0.69-1.69)	.75
KRAS, CDKN2A, TP53	64 (19.1)	1.51 (1.04-2.20)	.03	64 (18.9)	1.38 (0.96-1.98)	.08
KRAS, CDKN2A, SMAD4	34 (10.1)	1.27 (0.79-2.05)	.32	35 (10.4)	1.28 (0.80-2.06)	.30

Abbreviation: HR, hazard ratio.

[a] Cox proportional hazards regression model adjusted for age, sex, pathologic N stage, tumor grade, lymphovascular invasion, receipt of perioperative treatment, resection margin status, and institution.

[b] *P* value for multivariable-adjusted Cox proportional hazards regression.

[c] Data not reported for patients with *KRAS* G12C mutations due to the small sample size (n = 2).

[d] Data not reported for patients with combination of *CDKN2A*, *SMAD4*, and *TP53* alterations due to the small sample size (n = 2). Percentages do not add up to 100% in *KRAS* mutation analysis because we do not present data for 2 patients with G12C mutations.

Supplement). Loss of CDKN2A expression by IHC was associated with worse DFS and OS regardless of *CDKN2A* molecular status (eTable 5 in the Supplement), which likely reflects the inability of NGS to detect the silencing of CDKN2A expression due to promoter methylation and reduced sensitivity for copy number loss in low-cellularity tumors.

By IHC, SMAD4 protein expression was lost in 175 patients (49.2%). Loss of SMAD4 expression was not significantly associated with DFS or OS in our patient population (Table 1). We used our sequencing data to predict whether SMAD4 expression would be present or lost (eTable 4 in the Supplement). The molecular status of *SMAD4* was not associated with DFS or OS when SMAD4 expression was lost by IHC (eTable 5 in the Supplement).

For *TP53*, we used IHC and molecular data to generate an integrated call of *TP53* as wild-type or altered (eTable 4 in the Supplement). By this approach, *TP53* was altered in 231 patients (64.9%). Altered *TP53* was associated with shorter DFS (HR, 1.33; 95% CI, 1.02-1.75; *P* = .04) but was not associated with OS (HR, 1.18; 95% CI, 0.91-1.53; *P* = .23) (Table 1).

Twenty-four patients (6.7%) received preoperative therapy, which was not associated with the pattern of driver gene alterations (eTable 6 in the Supplement). In sensitivity analyses excluding these 24 patients, associations between driver gene alterations and patient outcomes were largely unchanged (eTable 7 in the Supplement).

We analyzed the association of combinatorial gene alterations with DFS and OS (eTable 8 in the Supplement). Compared with patients with 0 to 2 gene alterations, patients with 3 or 4 gene alterations had worse DFS (3 alterations HR, 1.37 [95% CI, 1.01-1.86; *P* = .05]; 4 alterations HR, 1.79 [95% CI, 1.24-2.59; *P* = .002]) and OS (3 alterations HR, 1.22 [95% CI, 0.91-1.64; *P* = .18]; 4 alterations HR, 1.38 [95% CI, 0.98-1.94; *P* = .06]) (Table 2 and Figure; eFigure 4 in the Supplement). The worst outcomes were identified in patients with both *KRAS* mutant tumors and CDKN2A expression loss. Five-year OS rates were 18.4% for patients with 0 to 2 gene alterations, 14.1% for patients with 3 gene alterations, and 8.2% for patients with 4 gene alterations. In our patient population, alterations in the 4 driver genes were not significantly associated with local recurrence as the first site of disease recurrence (eTable 9 in the Supplement).

Discussion

In a large, multi-institutional population of patients with resected pancreatic adenocarcinoma, patient outcomes were associated with alterations of the 4 main driver genes. Previous studies have assessed these genes and patient outcomes individually using a variety of methods and patient populations and revealing inconsistent results.[7-10] One study assessed all 4 driver genes among 79 patients who underwent rapid autopsy after death from pancreatic adenocarcinoma.[11] Tumors were sequenced by polymerase chain reaction for *KRAS*, *CDKN2A*, and *TP53*, and IHC was performed for

CDKN2A, SMAD4, and TP53. Although the sample size was small and included all stages of disease, patients whose tumors had 3 or 4 altered genes had worse DFS and OS than did patients whose tumors had 1 or 2 altered genes in unadjusted analysis. The primary results of the current study are confirmatory in a large, multi-institutional patient population, but multiple-hypothesis testing should be acknowledged when interpreting data across several biomarkers.

Previous studies have suggested that loss of SMAD4 protein expression by IHC was associated with extensive metastatic spread, generating interest in SMAD4 staining as an informative biomarker to guide the use of radiotherapy.[12] However, a subsequent study of 127 patients with resected pancreatic cancer did not replicate these findings.[13] In our study population, SMAD4 staining was not associated with pattern of disease recurrence after surgical resection.

Adjuvant treatment following surgical resection of pancreatic cancer improves patient survival, but outcomes remain suboptimal.[5] With the intent of improving cure rates, novel and more aggressive multiagent treatment programs are currently being devised and evaluated in the adjuvant setting.[6] Furthermore, increasing numbers of patients are receiving chemotherapy and radiation before surgical resection to rapidly initiate therapy against micrometastatic disease and select out those patients with early disease progression unlikely to benefit from surgery.[14] In the future, molecular assessment of pancreatic cancer may help guide the use and components of perioperative treatment programs.[15]

Conclusions

This study demonstrates that alterations in the 4 main driver genes are associated with patient outcomes in a large, multi-institutional population of patients with resected pancreatic adenocarcinoma. Understanding the molecular events that determine patient outcomes has the potential to improve treatment approaches for patients with this aggressive malignancy.

ARTICLE INFORMATION

Published Online: November 2, 2017. doi:10.1001/jamaoncol.2017.3420

Correction: This article was corrected on March 7, 2019, to correct omissions in the Conflict of Interest Disclosures.

Accepted for Publication: August 1, 2017.

Author Affiliations: Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts (Qian, Rubinson, Morales-Oyarvide, Welch, Brais, Da Silva, Zellers, Yuan, Babic, Aguirre, Ng, Thorner, Hahn, Meyerson, Fuchs, Ogino, Wolpin); Department of Oncologic Pathology, Dana-Farber Cancer Institute, Boston, Massachusetts (Qian, Nowak, Da Silva, T. Li, W. Li, Masuda, Yang, Shi, Gu, Masugi, Ogino); Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Nowak, Doyle, Ducar, Meyerson, Hornick); Division of Hematology and Oncology, Department of Medicine, Wilmot Cancer Institute, University of Rochester Medical Center, Rochester, New York (Dunne, Hezel); Department of Radiation Oncology, Stanford Cancer Institute, Stanford, California (Kozak, Bui, Chang); Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, Massachusetts (Yuan, Ogino); Division of Gastroenterology, Hepatology, and Endoscopy, Brigham and Women's Hospital, Boston, Massachusetts (Khalaf); Department of Hematology and Oncology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts (Miksad, Bullock); Department of Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts (Tseng); Department of Surgery, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Clancy); Department of Surgery, University of Rochester Medical Center, Rochester, New York (Linehan); Department of Pathology, University of Rochester Medical Center, Rochester, New York (Findeis-Hosey); Center for Cancer Genome Discovery, Dana-Farber Cancer Institute, Boston, Massachusetts (Thorner, Ducar, Wollison, Laing, Hahn, Meyerson); Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts (Meyerson); Division of Molecular Pathological Epidemiology, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Ogino); Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston (Koong).

Author Contributions: Drs Qian, Rubinson, Nowak, and Morales-Oyarvide share first coauthorship. Drs Hezel, Koong, and Wolpin share last coauthorship. Drs Morales-Oyarvide and Wolpin had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Qian, Rubinson, Nowak, Hahn, Meyerson, Ogino, Koong, Wolpin.
Acquisition, analysis, or interpretation of data: Qian, Rubinson, Nowak, Morales-Oyarvide, Dunne, Kozak, Welch, Brais, Da Silva, T. Li, W. Li, Masuda, Yang, Shi, Gu, Masugi, Bui, Zellers, Yuan, Babic, Khalaf, Aguirre, Ng, Miksad, Bullock, Chang, Tseng, Clancy, Linehan, Findeis-Hosey, Doyle, Thorner, Ducar, Wollison, Laing, Fuchs, Ogino, Hornick, Hezel, Koong, Wolpin.
Drafting of the manuscript: Qian, Rubinson, Nowak, Morales-Oyarvide, Welch, T. Li, Shi, Gu, Bui, Zellers, Doyle, Thorner, Laing, Wolpin.
Critical revision of the manuscript for important intellectual content: Qian, Rubinson, Nowak, Morales-Oyarvide, Dunne, Kozak, Brais, Da Silva, W. Li, Masuda, Yang, Shi, Gu, Masugi, Yuan, Babic, Khalaf, Aguirre, Ng, Miksad, Bullock, Chang, Tseng, Clancy, Linehan, Findeis-Hosey, Doyle, Thorner, Ducar, Wollison, Hahn, Meyerson, Fuchs, Ogino, Hornick, Hezel, Koong, Wolpin.
Statistical analysis: Morales-Oyarvide, T. Li, Yuan, Ng, Wollison, Wolpin.
Obtained funding: Rubinson, Fuchs, Ogino, Koong, Wolpin.
Administrative, technical, or material support: Nowak, Morales-Oyarvide, Dunne, Welch, Brais, Da Silva, W. Li, Masuda, Yang, Gu, Masugi, Bui, Zellers, Babic, Miksad, Bullock, Chang, Clancy, Linehan, Doyle, Thorner, Ducar, Hahn, Fuchs, Hornick, Hezel, Koong.
Study supervision: Qian, Rubinson, Nowak, Tseng, Doyle, Thorner, Ducar, Hahn, Meyerson, Fuchs, Ogino, Hezel, Koong, Wolpin.

Conflict of Interest Disclosures: Dr Fuchs has been a consultant and/or a scientific advisor for Eli Lilly, Entrinsic Health, Pfizer, Merck, Sanofi, Roche, Genentech, Merrimack Pharmaceuticals, Dicerna, Bayer, Celgene, Agios, Gilead Sciences, Five Prime Therapeutics, Taiho, and KEW. No other disclosures were reported.

Funding/Support: This study was funded in part by a grant from the Department of Medical Oncology translational research grant program of the Dana-Farber Cancer Institute (Dr Qian); grant K07 CA148894 from the National Institutes of Health (Dr Ng); grant R35 CA197735 from the National Cancer Institute (Dr Ogino); grants from the Hale Center for Pancreatic Cancer Research, the Perry S. Levy Fund for Gastrointestinal Cancer Research, and the Pappas Family Research Fund for Pancreatic Cancer, as well as grants R01 CA124908 and P50 CA127003 from the National Institutes of Health (Dr Fuchs); funding from MyBlueDots (Dr Koong); and grants from the Hale Center for Pancreatic Cancer Research, the Lustgarten Foundation for Pancreatic Cancer Research, the Pancreatic Cancer Action Network, the Noble Effort Fund, the Peter R. Leavitt Family Fund, the Wexler Family Fund, and Promises for Purple, as well as grant CA130288 from the US Department of Defense and grant U01 CA210171 from the National Institutes of Health/National Cancer Institute (Dr Wolpin).

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: These individuals assisted with designing the targeted sequencing panel: Nabeel Bardeesy, PhD, Massachusetts General Hospital and Harvard Medical School; Alec Kimmelman, MD, PhD, NYU Langone Medical Center; R. Coleman Lindsley, MD, PhD, and Mandar Muzumdar, MD, Dana-Farber Cancer Institute and Harvard Medical School; Eliezer Van Allen, MD,

Dana-Farber Cancer Institute and Broad Institute of Massachusetts Institute of Technology and Harvard; and Matthew Vander Heiden, MD, PhD, Koch Institute for Integrative Cancer Research of the Massachusetts Institute of Technology. These individuals were not compensated for their contribution.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66(1):7-30.

2. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*. 2008;321(5897):1801-1806.

3. Witkiewicz AK, McMillan EA, Balaji U, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun*. 2015;6:6744.

4. Bailey P, Chang DK, Nones K, et al; Australian Pancreatic Cancer Genome Initiative. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature*. 2016;531(7592):47-52.

5. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA*. 2007;297(3):267-277.

6. Li D, O'Reilly EM. Adjuvant and neoadjuvant therapy for pancreatic cancer. *Surg Oncol Clin N Am*. 2016;25(2):311-326.

7. Gerdes B, Ramaswamy A, Ziegler A, et al. *p16^{INK4a}* Is a prognostic marker in resected ductal pancreatic cancer: an analysis of *p16^{INK4a}*, *p53*, *MDM2*, and *Rb*. *Ann Surg*. 2002;235(1):51-59.

8. Blackford A, Serrano OK, Wolfgang CL, et al. *SMAD4* gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res*. 2009;15(14):4674-4679.

9. Rachakonda PS, Bauer AS, Xie H, et al. Somatic mutations in exocrine pancreatic tumors: association with patient survival. *PLoS One*. 2013;8(4):e60870.

10. Oshima M, Okano K, Muraki S, et al. Immunohistochemically detected expression of 3 major genes (*CDKN2A/p16*, *TP53*, and *SMAD4/DPC4*) strongly predicts survival in patients with resectable pancreatic cancer. *Ann Surg*. 2013;258(2):336-346.

11. Yachida S, White CM, Naito Y, et al. Clinical significance of the genetic landscape of pancreatic cancer and implications for identification of potential long-term survivors. *Clin Cancer Res*. 2012;18(22):6339-6347.

12. Iacobuzio-Donahue CA, Fu B, Yachida S, et al. *DPC4* gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol*. 2009;27(11):1806-1813.

13. Winter JM, Tang LH, Klimstra DS, et al. Failure patterns in resected pancreas adenocarcinoma: lack of predicted benefit to SMAD4 expression. *Ann Surg*. 2013;258(2):331-335.

14. Perez K, Clancy TE, Mancias JD, Rosenthal MH, Wolpin BM. When, what, and why of perioperative treatment of potentially curable pancreatic adenocarcinoma [published online December 28, 2016]. *J Clin Oncol*. 2016;JCO2016702134.

15. Valero V III, Saunders TJ, He J, et al. Reliable detection of somatic mutations in fine needle aspirates of pancreatic cancer with next-generation sequencing: implications for surgical management. *Ann Surg*. 2016;263(1):153-161.