

# Genomic Predictors Associated With Exceptional Response to Systemic Therapy in Advanced Pancreatic Cancer

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## ABSTRACT

**Introduction:** Exceptional response to therapy is rare in patients with advanced pancreatic cancer. This study explored potential genomic differences between typical and exceptional responses that could confer more favorable biology.

**Methods:** We included exceptional responders and controls with advanced pancreatic cancer from Cleveland Clinic from April 2013 to August 2017. Exceptional responders were defined as patients with an overall survival of more than 18 months for metastatic disease and more than 24 months for locally advanced disease. Clinical data were obtained, and next-generation sequencing was performed. Statistical analyses comparing the 2 groups were performed using descriptive statistics, the Kaplan-Meier method, and the log-rank test.

**Results:** The study comprised 4 exceptional responders and 6 controls. Both groups were well balanced in age, sex, race, and treatment regimens. Exceptional responders had significantly fewer nonsynonymous mutations than controls (2.25 vs 5.17;  $P = .014$ ). A mutation count of less than 3 was associated with significantly better progression-free survival (17.2 vs 2.3 months;  $P = .002$ ) and overall survival (29.4 vs 4.6 months;  $P = .013$ ). Tumor mutational burden did not differ between exceptional responders and controls (4.88 vs 5.70 mut/Mb;  $P = .39$ ).

**Conclusion:** A lower number of nonsynonymous mutations may correlate with exceptional outcomes in patients with pancreatic cancer. These findings should encourage future studies into genomic signatures of exceptional response.

## Introduction

Pancreatic cancer is associated with poor outcomes at any stage. However, a very small number of patients—approximately 3% of those with metastatic disease—experience long-term survival through 5 years and durable responses to systemic therapy.<sup>1</sup> The biological mechanisms that underlie the benefits observed with these *exceptional responders* are not completely understood. It is possible that certain tumor molecular features, including somatic mutations or tumor mutation burden, affect the tumor microenvironment and lead to more indolent biology or increased sensitivity to specific therapies.

Because exceptional responders are rare, the molecular underpinnings of exceptional response also may be rare and thus difficult to study and characterize. In pancreatic cancer, this challenge is further compounded by a high proportion of small tissue specimens obtained via fine needle aspiration (FNA) that are often insufficient for comprehensive genomic profiling.

The ongoing National Cancer Institute (NCI) Exceptional Responders Initiative provides the most robust molecular data thus far; it includes 111 patients who are exceptional responders to date. For 26 patients (23.4%), likely genomic mechanisms for exceptional response

**TABLE 1. Baseline Characteristics of the Study Population Divided Into Exceptional Responders and Matched Controls**

	<b>Exceptional responders (n = 4)</b>	<b>Matched controls (n = 6)</b>
	<b>n (%)</b>	<b>n (%)</b>
<b>Median Age (years)</b>	<b>69 (range, 65–82)</b>	<b>67.5 (range, 57–71)</b>
<b>Sex</b>		
Female	1 (25%)	2 (33%)
Male	3 (75%)	4 (67%)
<b>Ethnicity</b>		
Latino or Hispanic	0 (0%)	1 (17%)
Not Latino or Hispanic	4 (100%)	5 (83%)
<b>Race</b>		
White	3 (75%)	4 (67%)
Black	1 (25%)	2 (33%)
Asian	0 (0%)	0 (0%)
<b>Stage at diagnosis</b>		
I	0 (0%)	0 (0%)
II	2 (50%)	1 (17%)
III	0 (0%)	0 (0%)
IV	2 (50%)	5 (83%)
<b>Primary pancreatic tumor site</b>		
Head	2 (50%)	4 (67%)
Neck	0 (0%)	0 (0%)
Body	0 (0%)	2 (33%)
Tail	2 (50%)	0 (0%)
<b>Treatment regimens</b>		
<b>Adjuvant</b>	2 (50%, gem [1], gem + nab-pac [1])	0 (0%)
<b>First line</b>	<b>(n = 4)</b>	<b>(n = 6)</b>
FOLFOX	2 (50%)	0 (0%)
FOLFIRINOX	2 (50%)	2 (33%)
Gem + nab-pac	0 (0%)	4 (67%)
<b>Second line</b>	<b>(n = 4)</b>	<b>(n = 3)</b>
5-FU	1 (25%)	0 (0%)
FOLFOX	1 (25%)	2 (67%)
Gem + nab-pac	2 (50%)	1 (33%)
<b>Third line</b>	<b>(n = 2)</b>	<b>(n = 0)</b>
5-FU + lipo irinotecan	1 (50%)	0 (0%)
FOLFOX	1 (50%)	0 (0%)
<b>Fourth line</b>	<b>(n = 1)</b>	<b>(n = 0)</b>
5-FU + lipo irinotecan	1 (100%)	0 (0%)

5-FU, 5-fluorouracil; FOLFIRINOX, 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; gem, gemcitabine; lipo, liposomal; nab-pac, nab-paclitaxel.

were found, predominantly in DNA damage response, intracellular signaling, and immunologic engagement pathways.<sup>2</sup> However, only 1 patient had pancreatic adenocarcinoma, and for 76.6% of patients, no discernible genomic cause for exceptional response was found. Another series that performed comprehensive genomic profiling of tumors from 16 exceptional responders showed similar alterations in DNA damage repair pathways and immune cell infiltration mechanisms.<sup>3</sup> However, none of these patients had pancreatic adenocarcinoma.

Given the relative lack of data on exceptional responders with pancreatic adenocarcinoma, this study aimed to identify potential molecular signatures of exceptional response, which could influence prognostic modeling or identify novel predictive biomarkers for future therapies.

**Methods**

The study population comprised consecutive exceptional responders and matched controls with locally advanced or metastatic pancreatic cancer from Cleveland Clinic Ohio and Cleveland Clinic Florida from April 2013 to August 2017. Exceptional responders were defined as patients with overall survival (OS) greater than 18 months for metastatic disease and greater than 24 months for locally advanced disease. Matched controls were defined as patients with OS less than 9 months for metastatic disease and less than 12 months for locally advanced disease. They were selected among the patients in our tumor registry who were diagnosed between April 2013 and August 2017 and were matched based on age, sex, disease stage, and type of chemotherapy. The study was approved by the Institutional Review Board of Cleveland Clinic Taussig Cancer Institute.

Clinical data including patient demographics, comorbidities, disease

**TABLE 2. Summary of Individual Patient-Level Data on Baseline Characteristics, Cancer Stage, Treatment History, and Outcomes**

Patient number	Age (years)	Sex	Stage at diagnosis	Treatments	Treatment duration (months)	Best response	Overall survival (months)
<b>Exceptional responders</b>							
1	65	Male	IIB	Surgery	NA	NA	51.5
				Adjuvant gemcitabine	6	NA	
				FOLFOX	6.5	SD	
				5-FU	30	SD	
				FOLFOX	2.5	PD	
				5-FU + liposomal irinotecan	0.25	PD	
2	71	Female	IV	FOLFIRINOX	11.5	PR	23
				Gemcitabine + nab-paclitaxel	4.5	PD	
				5-FU + liposomal irinotecan	0.5	PD	
3	67	Male	IV	FOLFIRINOX	16.5	SD	36
				Gemcitabine + nab-paclitaxel	9	SD	
4	82	Male	IIB	Surgery	NA	NA	29.5
				Gemcitabine + nab-paclitaxel	5	PD	
				FOLFOX	4.5	SD	
				5-FU + liposomal irinotecan	1	PD	
<b>Matched controls</b>							
5	70	Female	IV	FOLFIRINOX	1.5	PD	7
				Gemcitabine + nab-paclitaxel	2	PD	
6	61	Female	IV	Gemcitabine + nab-paclitaxel	4	PD	4.5
7	69	Male	IIA	Gemcitabine + nab-paclitaxel	3	SD	8
				FOLFOX	1	PD	
8	71	Male	IV	Gemcitabine + nab-paclitaxel	0.5	PD	1.5
9	57	Male	IV	FOLFIRINOX	2.5	PD	3.5
10	66	Male	IV	Gemcitabine + nab-paclitaxel	3	PD	7
				FOLFOX	0.5	PD	

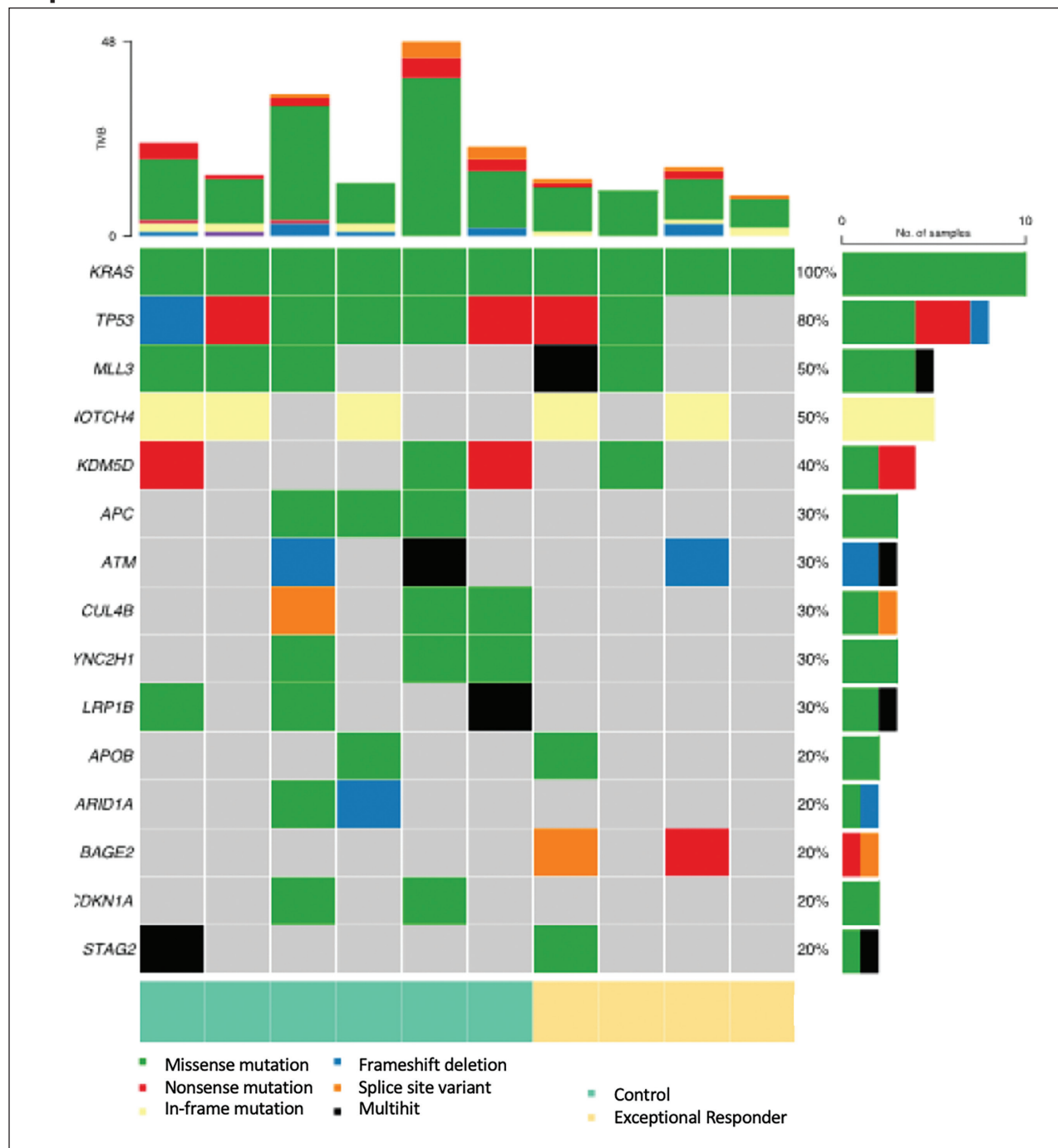
5-FU, 5-fluorouracil; FOLFIRINOX, 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; NA, not applicable; PD, progressive disease; PR, partial response; SD, stable disease.

characteristics, and treatment history were collected. In addition, DNA next-generation sequencing (NGS) was performed for 648 genes, microsatellite instability, and tumor mutation burden (TMB) using the Tempus xT platform.

Briefly, this panel uses formalin-fixed, paraffin-embedded tissue from the tumor and a matched normal specimen from peripheral blood or saliva to detect single nucleotide variants, insertions and deletions, and copy number

variants in 648 genes and for genomic rearrangements in 23 genes. Genes are sequenced to an average on-target depth of 500 times using the Illumina HiSeq 4000.<sup>4</sup> Due to insufficient tissue, additional comprehensive genomic profiling,

**FIGURE 1. Number of Functional, Nonsynonymous Genomic Alterations Divided by Exceptional Responders and Matched Controls**



Frequency and type of all functional, nonsynonymous variants detected in exceptional responders and matched controls.

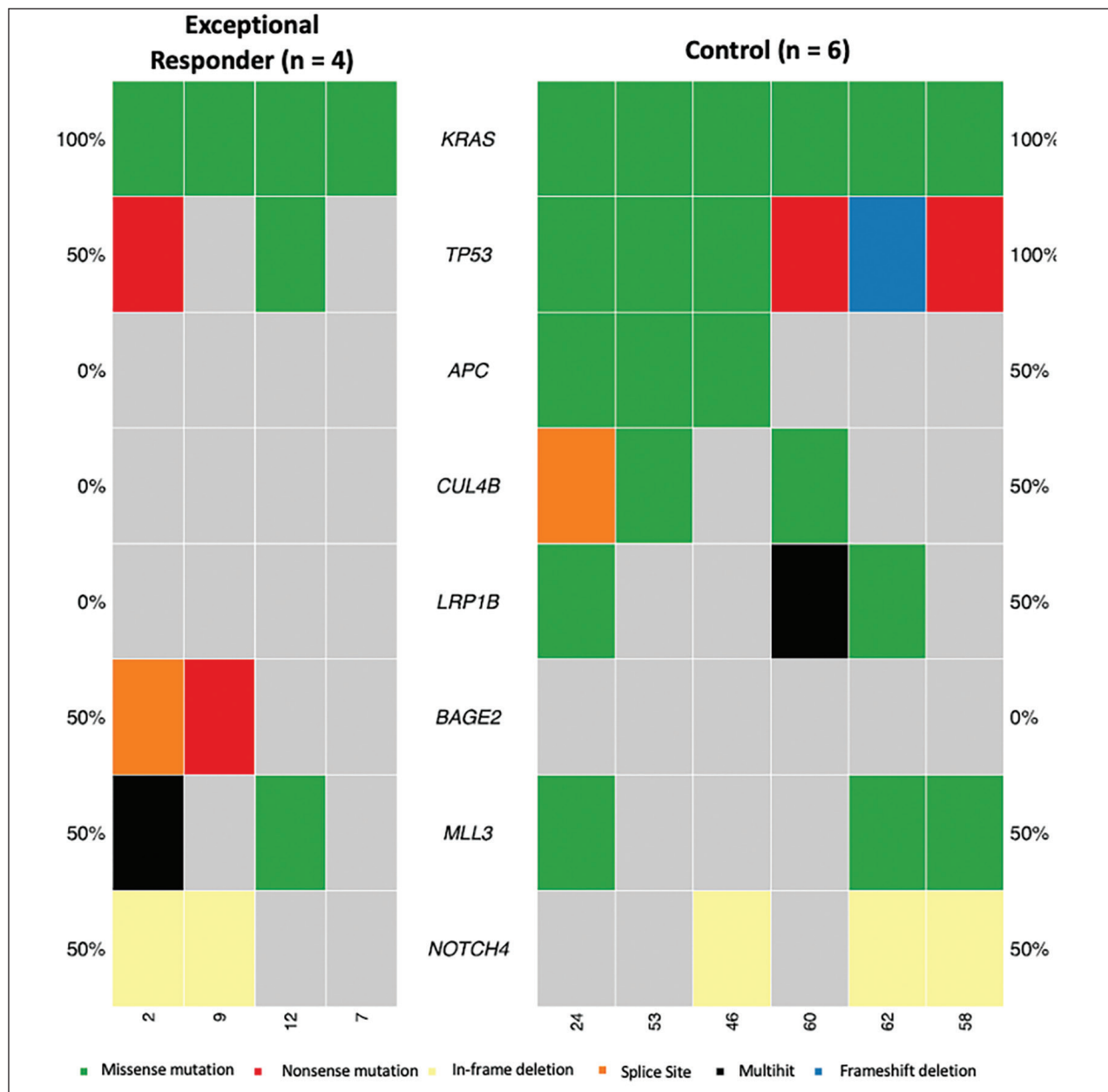
including whole exome sequencing and RNA sequencing, was not performed.

Statistical analyses comparing differences in clinical characteristics or genomic alterations between exceptional responders and matched controls

were performed using descriptive statistics. Differences in the total number of functional, nonsynonymous mutations or TMB between exceptional responders and matched controls were analyzed using the log-rank test. Progression-free

survival (PFS) and OS were estimated using the Kaplan-Meier method, and differences in survival outcomes based on the number of functional, nonsynonymous mutations or TMB were assessed using the log-rank test.

**FIGURE 2. Number of Somatic Actionable Mutations Divided by Exceptional Responders and Matched Controls**



Frequency and type of biologically relevant or somatic actionable mutations detected in exceptional responders and matched controls.

**Results**

The study population initially comprised 14 exceptional responders and 42 matched controls. However, due to insufficient tissue for comprehensive genomic profiling, only 4 exceptional responders and 6 matched controls were included for analysis.

Both groups were well balanced in terms of age, sex, race, and first-line chemotherapy regimen. The median ages for exceptional responders and matched controls were 69.0 and 67.5 years, respectively. Both exceptional responders and matched controls were predominantly men (75% vs 67%, respectively)

and predominantly White (75% vs 67%, respectively). Half of the exceptional responders had pancreatic tail primary tumors, compared with none of the matched controls. These data are summarized in Table 1.

Exceptional responders were labeled as patients 1 to 4, and matched controls

were labeled as patients 5 to 10. Patient 1 had the longest OS (51.5 months) and during one period experienced prolonged stable disease for 30 months on 5-fluorouracil (5-FU) alone. Patient 2 experienced a partial response to first-line FOLFIRINOX (leucovorin calcium, 5-FU, irinotecan hydrochloride, and oxaliplatin) and did not have progression of disease for 11.5 months. Patient 3 experienced relatively prolonged stable disease on first-line FOLFIRINOX and second-line gemcitabine plus nab-paclitaxel (16.5 months and 9.0 months, respectively). These data and others for each exceptional responder and matched control are summarized in Table 2.

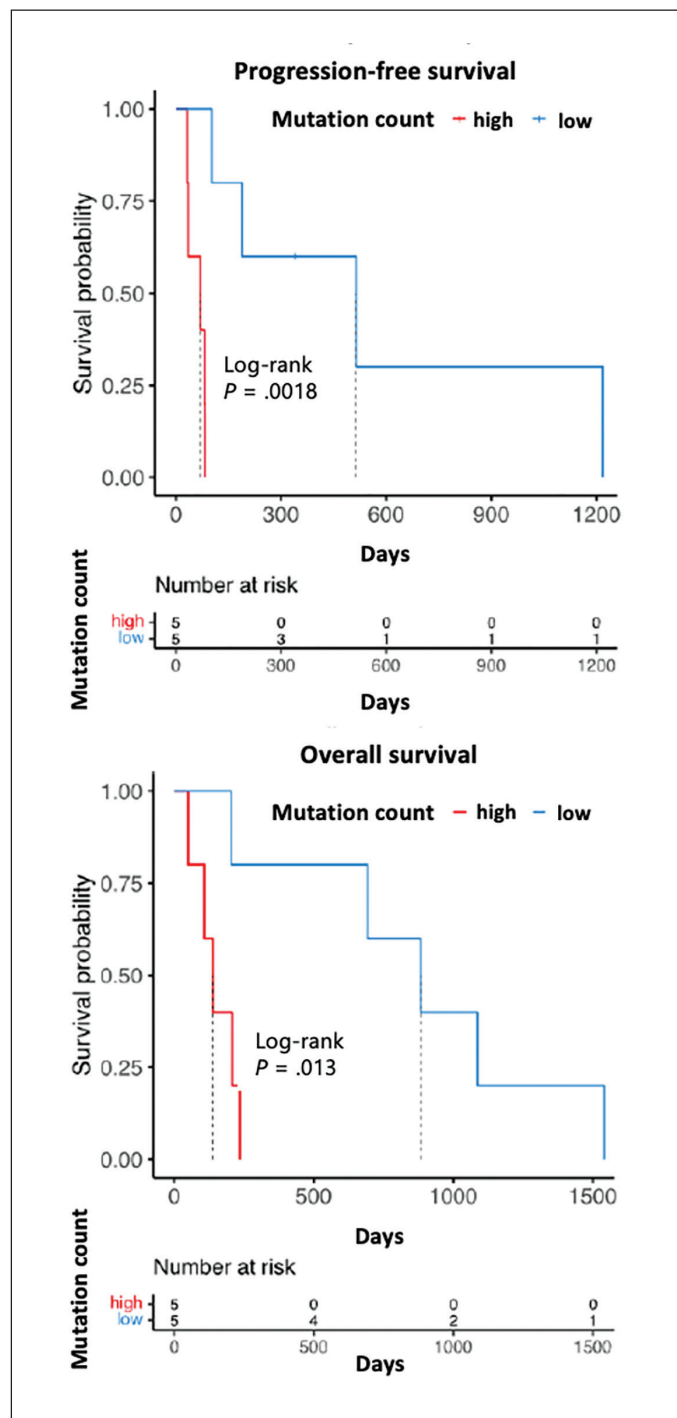
A total of 208 functional, nonsynonymous variants were detected in the study population, 52 in the exceptional responders and 156 in the matched controls. The genes with alterations and the types of variants detected are shown in Figure 1. Focusing on biologically relevant or somatic actionable mutations, 40 mutations were detected in the study population: 9 in the exceptional responders and 31 in the matched controls. Missense mutations in *KRAS* were present in 100% of exceptional responders and matched controls. Of the exceptional responders, 50% had alterations in *BAGE2* vs 0% of the matched controls. Conversely, 50% of the matched controls had alterations in *LRP1B*, *CUL4B*, or *APC* vs 0% of the exceptional responders. These data are summarized in Figure 2.

Exceptional responders had significantly fewer functional, nonsynonymous mutations compared with matched controls (2.25 vs 5.17,  $P = .014$ ). Mutation count of less than 3 was associated with significantly better PFS (17.2 vs 2.3 months,  $P = .002$ ) and OS (29.4 vs 4.6 months,  $P = .013$ ). Kaplan-Meier curves illustrating these data are shown in Figure 3. TMB did not differ between exceptional responders and matched controls (4.88 vs 5.70 Mut/Mb,  $P = .39$ ).

### Discussion

We found that patients with advanced pancreatic cancer with exceptional response to systemic therapy had tumors that were associated with fewer functional, nonsynonymous mutations. There are several possible explanations for the correlation between the number of functional, nonsynonymous mutations and exceptional response. One possibility

**FIGURE 3. Outcomes for Patients With High Vs Low Number of Genomic Mutations**



Progression-free survival and overall survival curves as estimated by the Kaplan-Meier method. The mutation count cut point used was less than or equal to 3 (low) vs greater than 3 (high). Time was measured in days.

is that exceptional responders are more driven by oncogenic addiction. Oncogenic addiction is defined as the presence of a genetic alteration within the tumor cell that causes it to be highly dependent on the downstream proteins and pathways of that individual gene. This could render these tumors more sensitive to standard cytotoxic chemotherapy, leading to exceptional responses. For example, the NCI Exceptional Responders Cohort included 1 patient with pancreatic adenocarcinoma and a germline *BRCA1* mutation who had a complete response to FOLFOX (5-FU, leucovorin, oxaliplatin), suggesting exquisite platinum sensitivity due to impaired DNA damage response (DDR) caused by the *BRCA1* mutation.<sup>2</sup> This cohort and multiple others have highlighted the importance of DDR pathway defects in exceptional responders.<sup>2,3,5</sup>

In another series of 18 patients with advanced pancreatic cancer who were treated with genomically targeted therapies, those who received therapies with higher matching scores based on tumor genomic profile experienced higher response and clinical benefit rates, including 1 patient with *KRAS* and *CDKN2A/CDKN2B*-mutated pancreatic cancer who had a durable response for 18 months with trametinib, palbociclib, and bevacizumab.<sup>6</sup> Because the matching score is derived from the number of genomic alterations with matched targeted therapies divided by the total of genomic alterations identified, it is possible that having fewer genomic alterations could lead to improved outcomes with targeted therapies.<sup>7</sup> Although our study did not identify specific genes that could engender oncogenic addiction, these genes may exist but remain undiscovered.

Our results contrast with a prior analysis that performed targeted

genomic sequencing on 16 exceptional responder patients and found high frequencies of *EPHA5* mutations and *NF1* splicing mutation (88% and 69%, respectively).<sup>3</sup> However, none of the patients in this prior study had pancreatic adenocarcinoma, and it is likely that molecular drivers of exceptional response will differ across tumor types. From a cancer immunology perspective, prior work examining neoantigen quality and editing in pancreatic adenocarcinoma has shown that exceptional responders have primary tumors with approximately 12 times as many activated CD8+ T cells predicted to target immunogenic neoantigens and more clonal T-cell expansion compared with average responders. These findings were associated with fewer genomic mutations and fewer neoantigens.<sup>8,9</sup> It is possible that having fewer genomic alterations in an immunologically *cold* tumor such as pancreatic adenocarcinoma allows for improved T-cell identification of the resultant neoantigens and clonal expansion. Because our study could not adequately investigate mechanisms by which a lower number of functional, nonsynonymous mutations could potentiate exceptional responses, more work is needed to understand the significance of this correlation.

We found that a numerically higher proportion of exceptional responders had *BAGE2* alterations compared with the matched controls. Given the small sample size, our study cannot establish a correlation between *BAGE2* alterations and exceptional response, but this could be an interesting area for future research. *BAGE2* is a member of the family of genes located in the juxtacentromeric regions of chromosomes 13 and 21 that encode tumor antigens that can be recognized by cytotoxic T cells.<sup>10</sup> *BAGE2* alterations are well described in melanoma, but little is known about their significance

in pancreatic adenocarcinoma.<sup>10,11</sup> One study evaluating distinct molecular subtypes in pancreatic cancer that included 178 patients from The Cancer Genome Atlas found only 2 patients with *BAGE2* deletions, which showed no correlation with exceptional response or favorable outcomes.<sup>12</sup>

Our study highlights the importance of searching for biomarkers of response to identify smaller subsets of patients who experience exceptional responses not observed in the broader population. For example, the original trials of EGFR inhibitors in lung adenocarcinoma with no biomarker selection showed only modest activity, but once EGFR was identified as a predictive biomarker in a subset of patients, the true activity of these drugs became apparent.<sup>13-15</sup> Similarly, EGFR inhibition in advanced colorectal cancer initially appeared ineffective, but improved biomarker prediction has shown this drug class is effective in those with *RAS/RAF* wild-type, HER2-negative, and left-sided primary tumors.<sup>16,17</sup>

Our study was limited by our inability to obtain complete RNA and DNA sequencing because of insufficient tissue for the majority of patients, which limited the strength of our results. Due to the anatomic location, pancreatic mass biopsies are often FNAs obtained via endoscopic ultrasound (EUS), which yield insufficient tissue for NGS. The hallmark features of pancreatic adenocarcinoma—low tumor cellularity and high stromal content—also diminish the yield for NGS for many patients. This highlights the importance of obtaining core biopsies from metastatic sites or referring to advanced endoscopists who are experienced in obtaining fine needle biopsies via EUS rather than FNA. NGS panels that can be performed successfully on more limited tissue specimens should be utilized in cases with insufficient tissue with the understanding

that they are less comprehensive for DNA and RNA sequencing and have reduced depth and breadth of coverage.<sup>18,19</sup> Because this is not dependent upon having sufficient tissue or tissue-based NGS testing, obtaining plasma circulating tumor DNA NGS at the time of diagnosis also can mitigate this issue.

Because of the limitations of this study from the small sample size and limited genomic sequencing due to insufficient tissue for most patients, these results should be viewed as hypothesis generating. Our study did not look at differences in tumor immune microenvironment, microbiome, metabolome, or other factors that may influence underlying tumor biology and response to therapy. We used a survival-based definition for exceptional response, whereas most prior studies in the literature have defined exceptional response by prolonged response duration, which may limit the generalizability of our findings. However, we chose our definition because survival outcomes are far more clinically meaningful than response outcomes and the previous definitions were not systematically validated enough to be considered accepted standards. Although outcomes for many malignancies have improved in the past decade, progress has been slower and more modest in pancreatic cancer. This suggests that survival

cutoffs of 18 months and 24 months for metastatic and locally advanced disease, respectively, are relevant, especially for patients who received a diagnosis in 2016 or earlier.

### Conclusion

Exceptional responders are rare in pancreatic cancer, but they do occur. Having fewer functional, nonsynonymous mutations may be associated with exceptional response and improved survival outcomes in patients with advanced pancreatic cancer receiving systemic therapy. Although these data are inadequate to inform clinical practice, if they were confirmed in a larger cohort, there may be an opportunity to use maintenance therapy or brief treatment suspension more frequently in select cases of exceptional response. More work is needed in a larger population to confirm these findings and to elucidate potential mechanisms mediating the relationship between the number of functional, nonsynonymous mutations and exceptional response. ■

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### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

SDK: Consulting/advisory role: Exelixis, Foundation Medicine, Guardant Health, Seagen, Tempus. Speaking: Merck, Seagen

DS: Consulting or speaking: Sanofi S.A.

AAK: Honoraria: Bayer; Halozyme; Janssen; Medscape; Nektar Therapeutics; Pfizer Consulting or advisory role: Bayer; Halozyme; Janssen; Pfizer; Pharmacoclytics; PharmaCyte Biotech; Seagen

JR, YN, MS PB, DA, AN, TN, BD, MS, MJM, BEN, KGN: No relevant conflicts of interest to disclose

### AUTHOR CONTRIBUTIONS

SDK: Conception/design, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript

JR: Conception/design, collection and/or assembly of data, data analysis and interpretation, final approval of manuscript

YN: Conception/design, collection and/or assembly of data, data analysis and interpretation, final approval of manuscript

MS, PB, DA, AN, TN, BD, DS, MS, MJM, BNE, KGN: Provision of study material or patients, final approval of manuscript

AAK: Conception/Design, Provision of study material or patients, data analysis and interpretation, final approval of manuscript

### DATA AVAILABILITY STATEMENT

Authors SDK and AAK had full access to all the data in the study. We take full responsibility for the integrity of the data. The data that support the findings of this study are available from the corresponding author on request.

 For references visit [cancerjournal.org/12.23\\_Pancreatic](https://cancerjournal.org/12.23_Pancreatic)