Tumor Mutational Burden in Real-World Patients With Pancreatic Cancer: Genomic Alterations and Predictive Value for Immune Checkpoint Inhibitor Effectiveness

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ABSTRACT

PURPOSE Pancreatic ductal adenocarcinoma (PDAC) is largely considered a nonimmunogenic malignancy; however, approximately 1%, of patients may have tumors with deficient mismatch repair, high microsatellite instability, or high tumor mutational burden (TMB ≥10 mutations/Mb), which may be predictive of response to immune checkpoint inhibitor (ICI) therapy. We sought to analyze outcomes of patients with high-TMB and pathogenic genomic alterations observed in this population.

METHODS This study included patients with PDAC who underwent comprehensive genomic profiling (CGP) at Foundation Medicine (Cambridge, MA). Clinical data were obtained from a US-wide real-world clinicogenomic pancreatic database. We report genomic alterations in those with high and low TMB, and compare outcomes on the basis of receipt of single-agent ICI or therapy regimens not containing ICI.

RESULTS We evaluated 21,932 patients with PDAC who had tissue CGP data available, including 21,639 (98.7%) with low-TMB and 293 (1.3%) with high-TMB. Among patients with high-TMB, a greater number of alterations were observed in *BRCA2*, *BRAF*, *PALB2*, and genes of the mismatch repair pathway, whereas fewer alterations were observed in *KRAS*. Among patients who received an ICI (n = 51), those with high-TMB had more favorable median overall survival when compared with the low-TMB subset (25.7 *v* 5.2 months; hazard ratio, 0.32; 95% CI, 0.11 to 0.91; $P = .034$).

CONCLUSION Longer survival was observed in patients with high-TMB receiving ICI compared with those with low-TMB. This supports the role of high-TMB as a predictive biomarker for efficacy of ICI therapy in PDAC. Additionally, we report higher rates of *BRAF* and *BRCA2* mutations and lower rates of *KRAS* mutation among patients with PDAC and high-TMB, which to our knowledge, is a novel finding.

ACCOMPANYING CONTENT

Visual Abstract

Appendix

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INTRODUCTION

Pancreatic cancer remains one of the leading causes of cancer-related death, with an estimated 64,050 new cases and 50,550 deaths in the United States in 2023.[1] Among various malignancies, pancreatic cancer is considered highly aggressive and exhibits one of the lowest 5-year survival rates at 10%.[1] Pancreatic ductal adenocarcinoma (PDAC) accounts for approximately 90% of cases of pancreatic cancer, and standard first-line therapy for metastatic PDAC in patients with good performance status typically consists of cytotoxic chemotherapy with oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) or gemcitabine plus nab-paclitaxel.[2,3] Because of its poor prognosis despite systemic chemotherapy and success in other tumor types, there has been increased interest in using immunotherapy in the treatment of PDAC.

PDAC has largely been considered a nonimmunogenic cancer, with poor response to immune checkpoint inhibitor (ICI) therapy.[4,5] One phase II study evaluating the combination of tremelimumab and durvalumab, and durvalumab monotherapy, in patients with metastatic PDAC who had progression on first-line chemotherapy found an objective response rate of 3.1% and 0%, respectively.[6] An additional phase II trial evaluating ipilimumab demonstrated response

CONTEXT

Key Objective

Pembrolizumab has been approved by US Food and Drug Administration (FDA) for patients with clinically advanced solid tumors with tumor mutational burden (TMB) ≥10 mutations/Mb who have progressed after previous treatment. However, the role of immune checkpoint inhibitors (ICIs) in patients with pancreatic ductal adenocarcinoma (PDAC) remains unclear.

Knowledge Generated

Alterations in *BRCA2*, *BRAF*, *PALB2*, and genes of the mismatch repair pathway were more frequently noted, while *KRAS* mutations were less common in patients with high-TMB compared with low-TMB. In patients with PDAC receiving single-agent ICI, high-TMB was associated with significantly longer median overall survival.

Relevance

This study validates the use of FDA-approved ICI in patients with PDAC harboring high-TMB.

in 0 of 20 patients.[7] These studies demonstrate a lack of efficacy in the use of ICI therapy for patients with PDAC who are not preselected by ICI biomarkers.

However, in a small subset of patients with PDAC, approximately 1%, tumors may exhibit high microsatellite instability (MSI-H), deficient mismatch repair (dMMR), and/or tumor mutational burden (TMB) ≥10 mutations/Mb, which may be predictive of response to ICI therapy.[8,9] The role of ICI therapy in management of tumors with dMMR or MSI-H was evaluated in the KEYNOTE-158 trial, which enrolled 223 patients with advanced noncolorectal solid malignancies who had progression on other lines of therapy, with confirmed dMMR/MSI-H tumors.[10] Patients enrolled in this trial received pembrolizumab 200 mg once every 3 weeks for 2 years or until disease progression, and exhibited an objective response rate of 34.3%, median progression-free survival (mPFS) of 4.1 months, and median overall survival (mOS) of 23.5 months. A separate planned cohort included 102 patients with tumors harboring high TMB, defined as TMB ≥10 mutations/Mb. In this subpopulation, the objective response rate was 29%, with 4% of patients achieving complete responses. Remarkably, almost half of the patients had durable responses lasting more than 2 years. This trial also included 22 patients with MSI-H/dMMR who demonstrated an overall response rate of 18.2%, with mPFS of 2.1 months and mOS of 4.0 months. The role of ICI therapy in PDAC is further supported by an additional retrospective analysis of 833 patients with pancreatic cancer, which identified dMMR in seven patients (0.8%), and with ICI therapy, one patient had complete response, two had partial response, and one had stable disease.[9]

On the basis of the results of KEYNOTE-158, the US Food and Drug Administration (FDA) granted approval of pembrolizumab for patients with clinically advanced solid tumors with TMB ≥10 mutations/Mb who have progressed after previous treatment. Currently, National Comprehensive Cancer Network guidelines recommend pembrolizumab for patients with metastatic PDAC whose tumors exhibit dMMR, MSI-H, or TMB ≥10 mutations/Mb. Because of the low prevalence of TMB ≥10 mutations/Mb in PDAC, few studies have evaluated the role of ICIs in this subpopulation. In the current study, we examined outcomes of patients with PDAC and high-TMB (TMB ≥10 mutations/Mb) who received ICI therapy and compared with patients with low-TMB (TMB <10 mutations/Mb) who also received ICI therapy. In addition, we compared outcomes in patients with high-TMB receiving ICI versus other therapies. Furthermore, we examined the genomic landscape of TMB-defined PDAC cohorts.

METHODS

Study Population and Analysis Overview

This study included patients with a confirmed diagnosis of PDAC who underwent genomic testing using comprehensive genomic profiling (CGP) at Foundation Medicine (Cambridge, MA) during routine clinical care between August 2012 and September 2022. All cases underwent a central review of the patient's pathology report and submitted single tissue block or unstained slide for histology review and confirmation of the PDAC diagnosis. Clinical data for a subset of patients with metastatic PDAC were available from the US-wide Flatiron Health and Foundation Medicine clinicogenomic pancreatic database (CGDB). In CGDB, retrospective, deidentified, longitudinal clinical data were derived from electronic health records (EHRs) from approximately 280 US cancer clinics (>800 sites of care) between March 2014 and September 2022 and include patient-level structured and unstructured data, curated via technology-enabled abstraction of clinical notes and radiology/pathology reports. Clinical data include demographics, clinical and laboratory features, time of therapy exposure, and survival. These were linked to genomic data derived from Foundation Medicine testing by deidentified, deterministic matching.[11]

Pathogenic genomic alterations were compared between patients with high-TMB versus low-TMB in the study population. The CGDB study population was divided into two main cohorts. The first cohort included patients with PDAC who received an ICI (pembrolizumab or nivolumab monotherapy) to compare outcomes between those with high-TMB versus low-TMB. The second cohort included patients with tumor harboring high-TMB to compare outcomes between those who received an ICI monotherapy versus those who received other therapies. Figure 1 shows the cohort selection of the study. Institutional review board approval of the study protocol was obtained before study conduct and included a waiver of informed consent on the basis of the observational, noninterventional nature of the study (WCG IRB, Protocol No. 420180044).

CGP

Hybrid capture–based NGS assays (FoundationOne or FoundationOne CDx) were performed on patient tumor specimens in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited laboratory (Foundation Medicine, Inc). Samples were evaluated for alterations as previously described.[12] TMB was determined on up to 1.1 Mb of sequenced DNA and the minimum tumor purity required to call TMB was 20%, with the clinical and analytic validation previously described.[13,14] To determine MSI status via NGS, we used a fraction-based MSI algorithm to categorize a tumor as MSI-H, MSI-low (MSI-L), or MSS. This algorithm calculates the fraction of unstable microsatellite loci on the

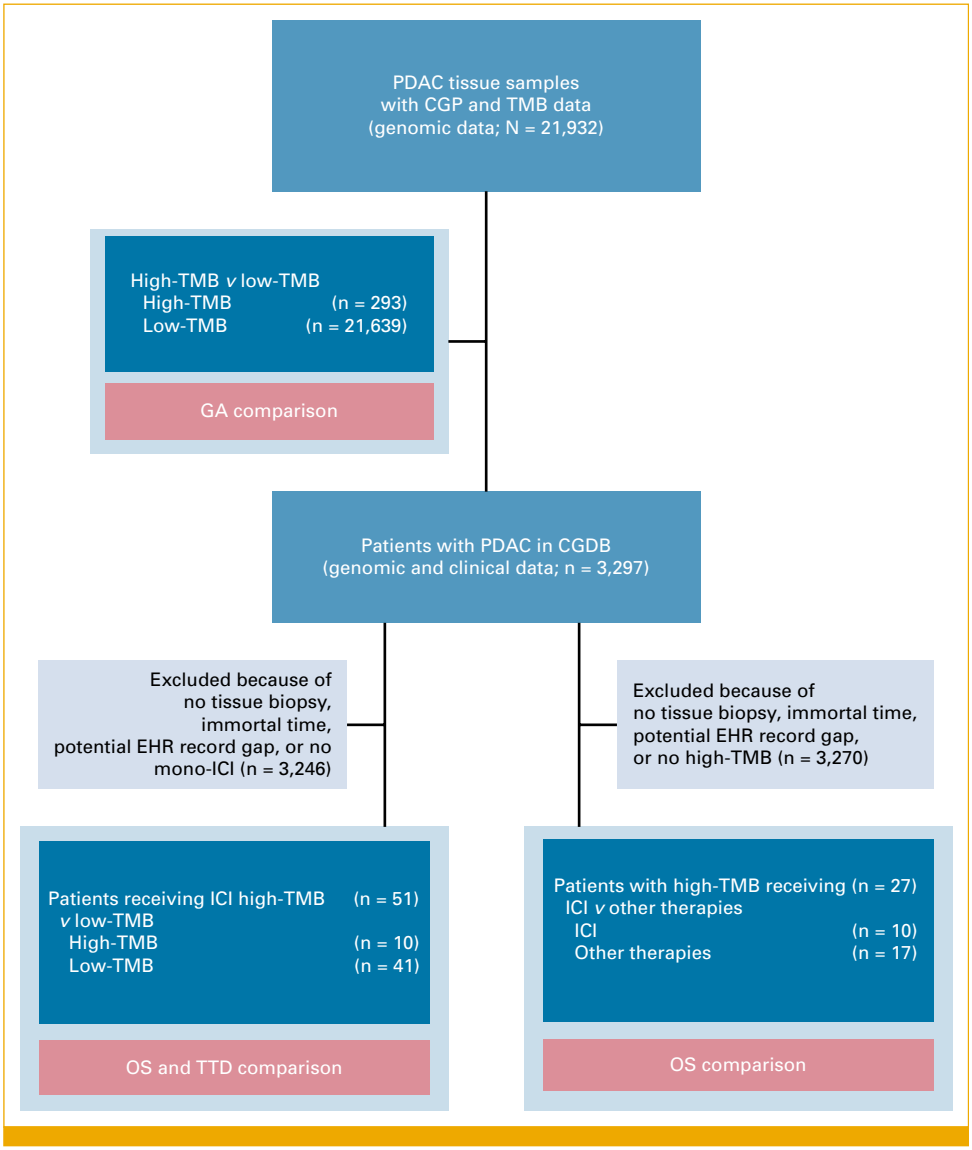


FIG 1. Cohort selection and main analyses overview. CGDB, Flatiron Health and FoundationMedicine clinicogenomic database; CGP, comprehensive genomic profiling; EHR, electronic health records; GA, genomic alterations; ICI, immune checkpoint inhibitor; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; TMB, tumor mutational burden; TTD, time to treatment discontinuation.

basis of an analysis across >2,000 loci. Known and likely pathogenic MMR alterations were determined by FoundationOne or FoundationOne CDx. Variants of unknown significance were excluded. Zygosity was determined as previously described.[15]

Outcomes

Overall survival (OS) was used as the primary end point, and time to treatment discontinuation (TTD) as a secondary end point for this study. OS was calculated from the start of treatment to death from any cause, and patients with no record of mortality were right-censored at the date of the last clinic visit or structured activity. OS risk intervals were left truncated to the date of the CGP report to account for immortal time, as patients cannot enter the database until a CGP report is provided.[16,17] TTD was calculated from the treatment start date until the treatment discontinuation for any reason or death, and patients not yet reaching treatment discontinuation or death were right-censored at the date of last clinical visit, laboratory result, or medication use.

The mortality information in the Flatiron Health database is a composite derived from deidentified patient-level data within the EHR, the public Social Security Death Index, and a commercial death data set mining data from obituaries and funeral homes. This mortality information has been externally validated in comparison with the National Death Index with >85% sensitivity and >95% agreement within 15 days.[18] The CGDB has previously replicated associations with survival observed in biomarker subgroup analyses of randomized controlled trials.[19-21]

Statistical Analysis

Pathogenic genomic alterations were compared between tissue specimens with high-TMB and low-TMB by chi-square, adjusted for multiple comparisons. The outcome analyses performed in this study were prespecified in a prospectively declared statistical analysis plan (SAP). The SAP also prespecified inclusion and exclusion criteria, potential biases, primary and secondary outcome measures, and handling of missing data. All methods herein described are consistent with ISPOR guidelines.[22] The SAP included the comparison of OS and TTD of patients with high-TMB receiving an ICI versus patients with low-TMB receiving an ICI and the comparison of OS of patients with high-TMB receiving an ICI versus other therapy regimens not containing an ICI. We also compared OS and TTD of patients with high-TMB receiving chemotherapy versus patients with low-TMB receiving chemotherapy.

Chi-square tests and Wilcoxon rank-sum tests were used to assess differences in baseline characteristics between groups of categorical and continuous variables, respectively. Baseline characteristics assessed included age at treatment start, sex, race, ECOG status, line of therapy, year of treatment start, CA19-9, practice type (academic or community), albumin, alkaline phosphatase, serum creatinine, hemoglobin, lactate dehydrogenase, neutrophil-to-lymphocyte ratio, opioid pretherapy, and steroid pretherapy. Differences in OS and TTD were evaluated with the log-rank test and Cox proportional hazard models. Multiple comparison adjustments for the outcome analyses were not performed, and the *P* values are reported to quantify the strength of association for the treatment group and outcome not for null hypothesis significance testing. R version 4.1.3 software program (R Core Team, Vienna, Austria) was used for all statistical analyses.

RESULTS

Genomic Comparison of High-TMB Versus Low-TMB

We identified 21,932 patients with PDAC who had tissue CGP data available: 21,639 (98.7%) with low-TMB and 293 (1.3%) with high-TMB (Fig 1). Table 1 shows the age, sex, and MSI status of these patients. In addition, we report pancreatic cancer subtypes other than PDAC with CGP data and their

TABLE 1. Characteristics of Patients With PDAC With Tissue CGP Data

Characteristic	High-TMB (n = 293)	Low-TMB (n = 21,639)	All (N = 21,932)
Age, years			
Median (Q1-Q3)	66 (59-73)	66 (59-72)	66 (59-72)
Sex, No. (%)			
Female	133 (45.4)	10,145 (46.9)	10,278 (46.8)
Male	160 (54.6)	11,494 (53.1)	11,654 (53.1)
MSI status,[a] No. (%)			
MSI-H	105 (36.8)	8 (0.04)	113 (0.5)
MSS	145 (50.9)	20,638 (97.5)	20,783 (96.9)
MSI-L	35 (12.3)	527 (2.5)	562 (2.6)

Abbreviations: CGDB, Flatiron Health and Foundation Medicine clinicogenomic database; CGP, comprehensive genomic profiling; MSI-H, microsatellite instability-high; MSI-L, MSI-low; MSS, microsatellite stable; PDAC, pancreatic ductal adenocarcinoma; Q1, first quartile; Q3, third quartile; TMB, tumor mutational burden.

[a]Percentage reported on the basis of samples with MSI assessment available, (n = 285 high-TMB/n = 21,173 low-TMB/n = 21,458 total).

respective prevalence of high-TMB and MSI-H in Appendix Table A1.

Overall, patients with high-TMB had higher prevalence of genomic alterations (Figs 2A and 2B). Among actionable alterations, patients with high-TMB had higher prevalence of *BRCA2* mutations ($P < 1 \times 10^{-10}$), *BRAF* mutations ($P = 6.90 \times 10^{-10}$), *PALB2* mutations ($P = 1.49 \times 10^{-9}$), and alterations in genes of the mismatch repair pathway (*MSH2*, *MSH6*, *MLH1*, and *PMS2*; $P < 1 \times 10^{-10}$), but lower prevalence of *KRAS* mutations ($P < 1 \times 10^{-10}$; Figs 2C and 2D). The most common *KRAS* mutation in both groups of patients was G12D (41.9% and 42.8% of *KRAS* mutations in high-TMB and low-TMB groups, respectively), while *KRAS* G12C was prevalent in 3.0% and 1.7% of patients with *KRAS* mutations in high-TMB and low-TMB groups, respectively (Appendix Table A2). The most common *BRAF* mutation in patients with high-TMB was *BRAF* V600E (class I), while the most common *BRAF* mutation in patients with low-TMB was *BRAF* N486_P490del (class II; Appendix Table A3). Targetable fusions (*ALK*, *NTRK1/2/3*, *ROS1*, *FGFR2*, *RET*, and *NRG1*) were overall rare (0.7%) and were not associated with high-TMB (1.4% *v* 0.7%; $P = .163$) but were enriched in *KRAS* wild-type (6.5% *v* 0.2%; $P < .0001$).

Of the 293 patients with high-TMB, 105 (36.8%) were MSI-H, 35 (12.3%) MSI-L, and 145 (50.9%) MSS (Table 1). Among patients with MSS tumors, patients with high-TMB also had higher prevalence of *BRCA2* mutations ($P < 1 \times 10^{-10}$), *PALB2* mutations ($P = .05$), and alterations in genes of the mismatch repair pathway, but there was no difference between the prevalence of *BRAF* mutations (Appendix Fig A1). Among patients with MSS tumors, patients with high-TMB still also had lower prevalence of *KRAS* mutations ($P < 1 \times 10^{-10}$; Appendix Fig A1). Appendix Figure A2 shows a contrast between genomic alterations across patients with PDAC: MSI-H and high-TMB, MSS and high-TMB, and MSS and low-TMB.

Comparative Effectiveness of ICI Therapy Among Patients With High-TMB Versus Low-TMB

In the CGDB, data on 3,297 patients with PDAC was available. After excluding patients for lack of tissue biopsy and/or unavailable follow-up data, we identified 51 patients with PDAC who received an ICI, 41 with TMB-L and 10 with TMB-H (Fig 1). No differences with $P < .05$ were observed for any baseline feature evaluated (Table 2; Appendix Table A4).

Among patients receiving an ICI, those with high-TMB had more favorable OS (median, 25.7 *v* 5.2 months; hazard ratio [HR], 0.32; 95% CI, 0.11 to 0.91; $P = .034$; Fig 3A) and TTD (median, 12.4 *v* 2.3 months; HR, 0.41; 95% CI, 0.16 to 1.0; $P = .05$; Fig 3B). To further evaluate the association of TMB and patient outcomes, we compared OS and TTD of 1,782 patients with PDAC who received chemotherapy by high-TMB versus low-TMB, and did not observe differences in outcomes between the two groups (Figs 3C and 3D). Chemotherapy regimens for this analysis included gemcitabine with or without paclitaxel protein-bound, fluorouracil, leucovorin, and irinotecan (FOLFIRI), FOLFIRINOX, infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX), olaparib, and paclitaxel protein-bound monotherapy.

Six of the 10 patients (60%) with high-TMB had longer mOS when receiving an ICI compared with the mOS for patients receiving first-line line preferred chemotherapy (gemcitabine + paclitaxel or FOLFIRINOX) or ≥2 line chemotherapy (Fig 4). In comparison, only 29.3% of patients with low-TMB had higher mOS when receiving ICI compared with the mOS for patients receiving first-line line preferred chemotherapy or ≥2 line chemotherapy (Fig 4).

Comparative Effectiveness of ICI Versus Other Therapies in Patients With High-TMB

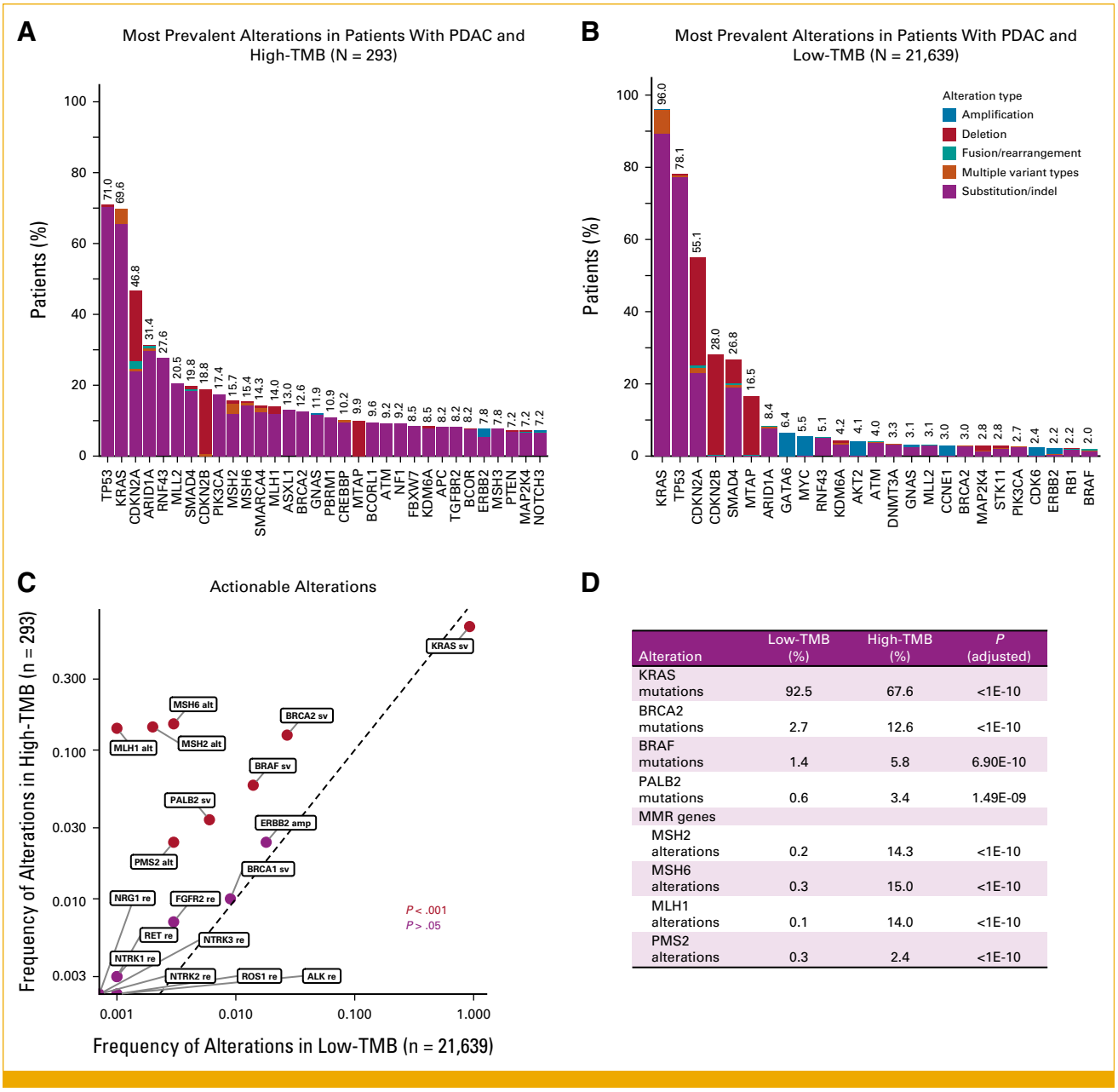
In our study population, 10 patients with high-TMB received an ICI and 17 patients received other non-ICI therapies (Fig 1). Other therapies included gemcitabine ± paclitaxel protein bound, FOLFIRI, FOLFIRINOX, FOLFOX, and olaparib. More patients received other therapies in the first line and more patients received ICI in later lines of therapy ($P < .038$). No differences with $P < .05$ were observed for other baseline features (Table 2; Appendix Table A5). Among patients with high-TMB, those receiving ICI had more favorable OS (25.7 months) compared with those receiving other therapies (6.6 months) with an HR of 0.31, 95% CI, 0.10 to 0.96, nominally favoring ICI monotherapy in this small cohort (n = 10 *v* 17; $P = .043$; Appendix Fig A3).

Genomic Alterations in Real-World Patients With PDAC and Favorable Outcomes on ICI

To explore the genomic alterations present in patients with favorable outcomes on ICI, we assessed the alterations present in tumors from 11 patients who received an ICI for at least 6 months (TTD >6 months). The most prevalent alterations (in at least two of the 11 patients) include *KRAS* and *TP53* mutations (short variants), and *CDKN2A* mutations and amplifications (Appendix Fig A4A). We observed that some gene mutations were present only in patients with high-TMB (n = 4), such as *BCOR*, *MSH6*, and *SMAD4* (Appendix Fig A4B), and that *MYC* amplification was present in two of seven patients with low-TMB (Appendix Fig A4C). With small numbers, it is difficult to make any firm conclusions regarding alterations that could predict prolonged treatment response to ICIs.

DISCUSSION

This is one of the first studies to evaluate the role of ICIs in patients with PDAC with respect to TMB. We demonstrate that patients with high-TMB who received an ICI had significantly favorable outcomes compared with those with low-TMB who received an ICI and those with high-TMB who received other therapies. In addition, we reported pathogenic



Alteration	Low-TMB (%)	High-TMB (%)	P (adjusted)
KRAS mutations	92.5	67.6	<1E-10
BRCA2 mutations	2.7	12.6	<1E-10
BRAF mutations	1.4	5.8	6.90E-10
PALB2 mutations	0.6	3.4	1.49E-09
MMR genes			
MSH2 alterations	0.2	14.3	<1E-10
MSH6 alterations	0.3	15.0	<1E-10
MLH1 alterations	0.1	14.0	<1E-10
PMS2 alterations	0.3	2.4	<1E-10

FIG 2. Genomic alterations in PDAC patients with high-TMB and low-TMB. (A) Bar plots showing the most prevalent alterations (>7%) for high-TMB 10 and (B) the most prevalent alterations (>2%) for low-TMB. (C) Scatter plot showing the difference in prevalence of actionable alterations in patients with TMB ≥10 versus TMB <10, and (D) the respective prevalence for those actionable alterations that are statistically significant different between the two groups. alt, alterations; amp, amplification; del, deletion; MMR, mismatch repair; PDAC, pancreatic ductal adenocarcinoma; re, rearrangements; sv, short variants; TMB, tumor mutational burden.

genomic alterations seen in patients with high-TMB and compared with those observed in patients with low-TMB. We found lower rates of *KRAS* mutation among patients with high-TMB, which to our knowledge, has not been previously reported in PDAC.

Survival among patients with high-TMB receiving an ICI (mOS of 25.7 months) was longer compared with that observed among noncolorectal solid tumors with high-TMB in the KEYNOTE-158 trial, which demonstrated a mOS of 11.7 months.[10] and compared with the subcohort of patients with MSI-H/dMMR in KEYNOTE-158, which demonstrated a mOS of only 4.0 months. These differences may be in part due to line of therapy in which an ICI was received, as in our study, 60% with high-TMB received an ICI in the first or second lines, whereas in KEYNOTE-158, nearly 60% of all patients had received two or more previous lines of therapy.

TABLE 2. Baseline Characteristics of CGDB PDAC Patients

Characteristic	ICI Low-TMB (N = 41)	ICI High-TMB (N = 10)	Chemo High-TMB (N = 17)
Age at Tx start, years			
Median (Q1-Q3)	71.0 (67.0-77.0)	68.0 (66.0-73.0)	69.0 (64.0-73.0)
Sex, No. (%)			
Female	12 (29.3)	2 (20.0)	4 (23.5)
Male	29 (70.7)	8 (80.0)	13 (76.5)
Practice type, No. (%)			
Academic	2 (4.9)	1 (10.0)	2 (11.8)
Academic/community	6 (14.6)	1 (10.0)	0 (0.0)
Community	33 (80.5)	8 (80.0)	15 (88.2)
ECOG, No. (%)			
0	6 (16.2)	1 (10.0)	6 (20.7)
1	22 (59.5)	8 (80.0)	15 (51.7)
2	8 (21.6)	1 (10.0)	7 (24.1)
≥3	1 (2.7)	0 (0.0)	1 (3.4)
N-Miss	4	0	4
Line of Tx, No. (%)			
1	6 (14.6)	3 (30.0)	14 (82.4)
2	14 (34.1)	3 (30.0)	2 (11.8)
3	9 (22.0)	2 (20.0)	0 (0.0)
≥4	12 (29.3)	2 (20.0)	1 (5.9)
CA19-9, No. (%)			
Median (Q1, Q3)	461.0 (55.1, 3,062.2)	40.2 (22.0, 182.0)	53.0 (12.5, 668.6)
N-Miss	8	5	6
Albumin, No. (%)			
<LLN	7 (17.5)	4 (40.0)	4 (25.0)
≥LLN	33 (82.5)	6 (60.0)	12 (75.0)
N-Miss	1	0	1
ALK, No. (%)			
≤ULN	21 (52.5)	3 (33.3)	8 (50.0)
>ULN	19 (47.5)	6 (66.7)	8 (50.0)
N-Miss	1	1	1
Serum creatinine, No. (%)			
≤ULN	36 (92.3)	10 (100.0)	15 (93.8)
>ULN	3 (7.7)	0 (0.0)	1 (6.2)
N-Miss	2	0	1
Hemoglobin, No. (%)			
<LLN	31 (75.6)	8 (80.0)	13 (81.2)
≥LLN	10 (24.4)	2 (20.0)	3 (18.8)
N-Miss	0	0	1
LDH, No. (%)			
≤ULN	6 (60.0)	1 (100.0)	3 (60.0)
>ULN	4 (40.0)	0 (0.0)	2 (40.0)
N-Miss	31	9	12
NLR, No. (%)			
≤2.5	12 (34.3)	4 (57.1)	3 (21.4)
>2.5	23 (65.7)	3 (42.9)	11 (78.6)
N-Miss	6	3	3

Abbreviations: ALK, alkaline phosphatase; CA19-9, cancer antigen 19-9; Chemo, chemotherapy; CGDB, Flatiron Health and Foundation Medicine clinicogenomic database; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitors; LDH, lactate dehydrogenase; LLN, lower limit of normal; NLR, neutrophil-to-lymphocyte ratio; PDAC, pancreatic ductal adenocarcinoma; Q1, first quartile; Q3, third quartile; TMB, tumor mutational burden; Tx, therapy; ULN, upper limit of normal.

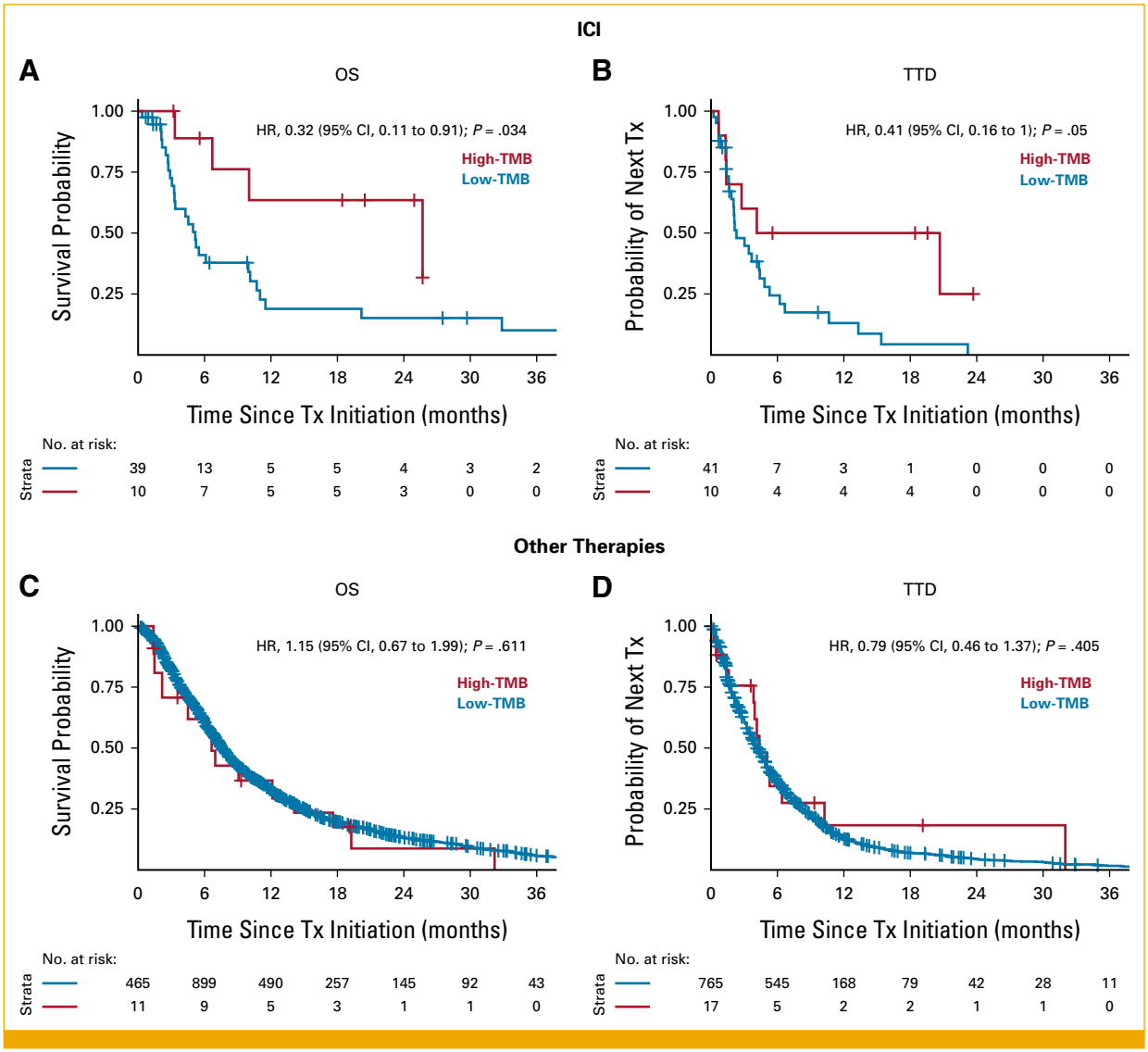


FIG 3. Real-world patients with PDAC and high-TMB have better outcomes receiving ICI compared with patients with low-TMB, while no statistical difference in outcomes is observed for patients receiving other therapies. Kaplan-Meier plots by TMB status for patients receiving ICI for (A) OS and (B) TTD, and for patients receiving other therapies (n = 1,765 for low-TMB and n = 17 for high-TMB) for (C) OS and (D) PFS. ICI, immune checkpoint inhibitor; HR, hazard ratio; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; TMB, tumor mutational burden; TTD, time to treatment discontinuation; Tx, therapy.

The mOS of 5.2 months manifested by low-TMB patients receiving an ICI in this study is comparable with survival reported in a phase II study evaluating tremelimumab plus durvalumab and durvalumab monotherapy in patients with PDAC not selected on the basis of MMR/MSI/TMB status, which showed mOS of 3.1 and 3.6 months, respectively.[6] However, we demonstrate that 29.3% of patients with low-TMB receiving an ICI demonstrated higher OS than mOS observed in patients receiving first-line preferred chemotherapy or two or more lines of chemotherapy. This suggests that TMB, MSI, and MMR may not be the only biomarkers of response to immunotherapy in patients with PDAC. For example, in the case of *MYC*, which plays a role in the regulation of immune cells in the tumor microenvironment and expression of immune checkpoint gene products, amplification has been associated with positive response to immune checkpoint inhibition.[23,24] In this study, among patients with low-TMB who were on an ICI for at least 6 months, two of six patients had *MYC* amplification, which suggests that this may be an independent biomarker predictive of response to ICIs in PDAC.

Multiple theories have been proposed regarding the lack of efficacy of ICIs in PDAC. Within the PDAC tumor microenvironment, tumor stroma may account for 50% of the tumor mass, and consists of various immunosuppressive cellular components, which function through inhibition of T-cell response to the tumor.[25-28] In addition to cellular

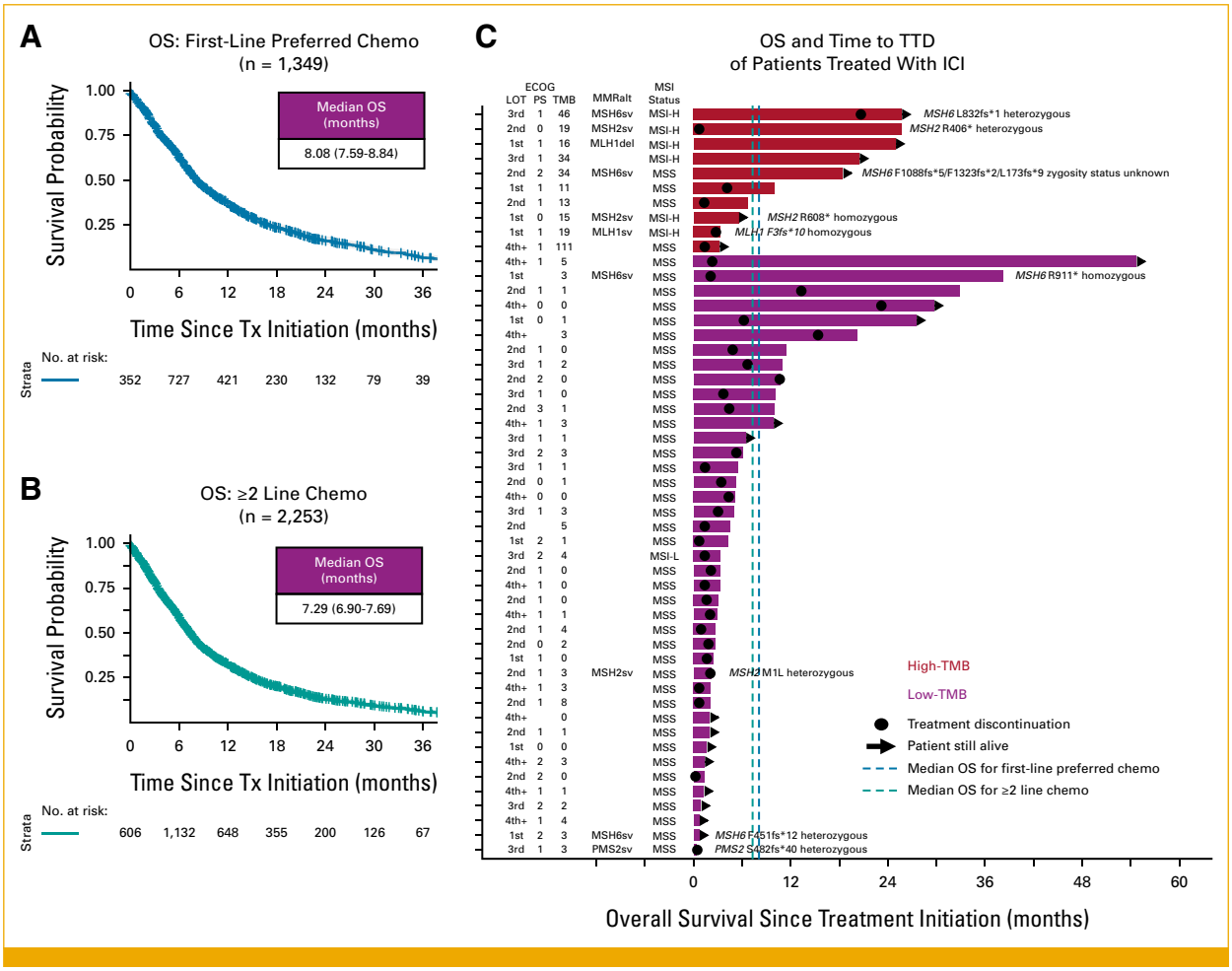


FIG 4. Patients with high-TMB have better outcomes receiving ICI compared with patients with TMB low-TMB and compared with the median OS of patients receiving other therapy regimens. Kaplan-Meier plots showing OS for patients (A) receiving first-line preferred chemotherapy according to NCCN (gemcitabine + paclitaxel protein bound or FOLFIRINOX) and (B) receiving ≥2 therapies without ICI. (C) Swimmer plot showing OS and TTD for patients with TMB ≥10 receiving ICI and other therapies. The swimmer plot does not account for OS left-truncation. chemo, chemotherapy; ECOG, Eastern Cooperative Oncology Group; FOLFIRINOX, oxaliplatin, irinotecan, fluorouracil, and leucovorin; ICI, immune checkpoint inhibitor; LOT, line of therapy; MMR alt, alteration in the mismatch repair genes (MSH2, MSH6, MLH1, and PMS2); MSI, microsatellite instability; NCCN, National Comprehensive Cancer Network; NR, not reached; OS, overall survival; TMB, tumor mutational burden; TTD, time to treatment discontinuation; Tx, therapy.

components, molecular components contribute to immune evasion, such as expression of focal adhesion kinase (Fak), which is associated with increased fibrosis and reduced CD8+ T-cell infiltration.[29] PDAC cells are generally noted to have low TMB, correlated with fewer neoepitopes, which may limit efficacy of ICIs.[30] This mechanism can explain the efficacy of ICIs in the subset of patients with PDAC with high-TMB and highlights the importance of patient selection when deciding whether to use an ICI in a patient with PDAC. Our study suggests that TMB status might be an independent factor for clinical benefit with ICI irrespective of MSI status.

In the comparison of pathogenic genomic alterations seen in patients with high-TMB versus those with low-TMB, higher rates of mutations within MMR genes, including *MSH2*, *MSH6*, *MLH1*, and *PMS2*, were observed as anticipated, considering previously reported abundance of high TMB in dMMR/MSI-H tumors.[31] We also report increased rates of *BRCA2* and *BRAF* mutations in patients with high-TMB, which has previously been demonstrated in PDAC, as well as other malignancies.[8,32,33] In addition to these previously reported correlations, we also found lower rates of *KRAS* mutation among patients with high-TMB. A previous analysis of *KRAS* mutation in lung adenocarcinoma found a correlation between *KRAS* mutation and increased TMB; however, subgroup analysis found lower TMB among patients with G12D mutations.[34] Interestingly, the most commonly observed *KRAS* mutation in the reported population was G12D, seen in approximately 42% of patients. *KRAS* has been found to play a role in resistance to ICIs, and inhibition of *KRAS* G12C in mouse models for non–small-cell

lung cancer has led to decreased myeloid-derived suppressor cells and increased inflammatory infilitrate.[35] In our retrospective analysis, we report lower survival among patients with low-TMB receiving an ICI, but we also see higher rates of *KRAS* mutation in this population, which may further impede the efficacy of ICIs. Future investigation may assess concomitant *KRAS* inhibition with ICI therapy in this population.

Limitations of this study include its retrospective nature, lack of clinical data for all patients, and small sample size with respect to some of the cohorts. However, as PDAC with high-TMB is quite rare, undertaking comparative effectiveness studies in this population is challenging. To our knowledge, this is one of the largest studies evaluating efficacy of ICI therapy in patients with PDAC and high-TMB. In addition, our database has previously replicated associations with survival observed in biomarker subgroup analyses of randomized controlled trials in other disease areas.[19,21] An additional limitation of this study is lack of adjusted analysis, again because of small size; however, the cohorts within the study are relatively balanced.

In conclusion, high-TMB is rare in PDAC, occurring in approximately 1% of patients. PDAC has largely been considered a nonimmunogenic tumor, with poor response to ICI therapy. In the present retrospective study, we show improved OS among patients with PDAC and high-TMB receiving ICI compared with those with low-TMB, as well as compared with those with high-TMB receiving other therapies. This study supports the FDA-approved use of ICIs in patients with PDAC and high-TMB and demonstrates the importance of TMB assessment for all patients with PDAC. In addition, we report higher rates of *KRAS* mutation among PDAC patients with low-TMB, which to our knowledge is a novel finding and may further impede the efficacy of ICI in the low-TMB cohort.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

TABLE A1. Pancreatic Cancer Subtypes and Prevalence of High-TMB and MSI-H

Pancreatic Cancer Subtype	No.	High-TMB, No. (%)	MSI-H, No. (%)
Islet cell tumor	1,034	66 (6.4)	4 (0.4)
Acinar cell carcinoma	236	16 (6.8)	3 (1.3)
Adenosquamous carcinoma	160	7 (4.4)	4 (2.5)
Mucinous cystadenocarcinoma	40	0	0
Solid and papillary tumor	10	0	0

Abbreviations: MSI-H, high microsatellite instability; TMB, tumor mutational burden.

TABLE A2. Types of *KRAS* Mutation Present in Patients With PDAC With TMB ≥10 and TMB <10

KRAS Mutation	High-TMB (n = 198), No. (%)	Low-TMB (n = 20,021), No. (%)
G12D	83 (41.9)	8,578 (42.8)
G12V	51 (28.8)	6,193 (30.9)
G12R	25 (12.6)	3,140 (15.7)
Q61H	7 (3.5)	876 (4.4)
G12C	6 (3.0)	339 (1.7)
Q61R	4 (2.0)	285 (1.4)
G13D	5 (2.5)	114 (0.6)
G12A	5 (2.5)	90 (0.4)
Q61L	1 (0.5)	83 (0.4)
Q61K	1 (0.5)	66 (0.3)
G12L	0	65 (0.3)
G12S	0	27 (0.1)
G12I	1 (0.5)	22 (0.1)
G13P	0	13 (0.1)
A146V	0	12 (0.1)
G13E	0	10 (0.1)
Others	9 (4.5)	108 (0.5)

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; TMB, tumor mutational burden.

TABLE A3. Types of *BRAF* Mutation Present in Patients With PDAC With TMB ≥10 and TMB <10

BRAF Mutation	High-TMB (n = 17), No. (%)	Low-TMB (n = 297), No. (%)
Class I		
V600E	7 (41.2)	57 (19.2)
V600_K601 > E	0	22 (7.4)
Class II		
N486_P490del	2 (11.8)	77 (25.9)
V487_P492 > A	0	7 (2.4)
K601E	0	4 (1.3)
Class III		
G466E	2 (11.8)	2 (0.7)
G469V	0	5 (1.7)
D594G	3 (17.6)	10 (3.4)
D594N	0	10 (3.4)
N581S	0	4 (1.3)
T599_V600insT	0	7 (2.4)
Others	3 (17.6)	92 (31.0)

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; TMB, tumor mutational burden.

TABLE A4. Baseline Characteristics of Patients With PDAC Receiving ICI (low-TMB *v* high-TMB)

Characteristic	Low-TMB (n = 41)	High-TMB (n = 10)	Total (n = 51)	*P*
Age at Tx start, years				.23
Median (Q1-Q3)	71.0 (67.0-77.0)	68.0 (66.0-73.0)	70.0 (66.0-76.5)	
Sex, No. (%)				.556
Female	12 (29.3)	2 (20.0)	14 (27.5)	
Male	29 (70.7)	8 (80.0)	37 (72.5)	
Race, No. (%)				.926
Asian	1 (2.4)	0 (0.0)	1 (2.0)	
Black or African American	3 (7.3)	1 (10.0)	4 (7.8)	
Other race	5 (12.2)	1 (10.0)	6 (11.8)	
Unknown/not documented	2 (4.9)	0 (0.0)	2 (3.9)	
White	30 (73.2)	8 (80.0)	38 (74.5)	
Practice type, No. (%)				.785
Academic	2 (4.9)	1 (10.0)	3 (5.9)	
Academic/community	6 (14.6)	9 (90.0)	7 (13.7)	
Community	33 (80.5)		41 (80.4)	
ECOG, No. (%)				.793
0	6 (16.2)	1 (10.0)	8 (17.0)	
1	22 (59.5)	8 (80.0)	29 (61.7)	
2	8 (21.6)	1 (10.0)	9 (19.1)	
≥3	1 (2.7)	0 (0.0)	1 (2.1)	
N-Miss	4	0	4	
Year of Tx start, No. (%)				.089
2014-2017	14 (34.1)	1 (10.0)	15 (29.4)	
2018	9 (22.0)	0 (0.0)	9 (17.6)	
2019	6 (14.6)	4 (40.0)	9 (17.6)	
2020	7 (17.1)	3 (30.0)	10 (19.6)	
2021	3 (7.3)	2 (20.0)	6 (11.8)	
CA19-9, No. (%)	2 (4.9)		2 (3.9)	
Median (Q1-Q3)	461.0 (55.1-3,062.2)	40.2 (22.0-182.0)	308.6 (38.8-3,009.4)	.16
N-Miss	8	5	13	
Albumin, No. (%)				
<LLN	7 (17.5)	4 (40.0)	11 (22.0)	.124
≥LLN	33 (82.5)	6 (60.0)	39 (78.0)	
N-Miss	1	0	1	
ALK, No. (%)				
≤ULN	21 (52.5)	3 (33.3)	24 (49.0)	
>ULN	19 (47.5)	6 (66.7)	25 (51.0)	.299
N-Miss	1	1	2	
Serum creatinine, No. (%)				
≤ULN	10 (100.0)	10 (100.0)	46 (93.9)	
>ULN	0 (0.0)	0 (0.0)	3 (6.1)	.365
N-Miss	0	0	2	
Hemoglobin, No. (%)				
<LLN	8 (80.0)	8 (80.0)	39 (76.5)	
≥LLN	2 (20.0)	2 (20.0)	12 (23.5)	.769
LDH, No. (%)				
≤ULN	1 (100.0)	1 (100.0)	7 (63.6)	
>ULN	0 (0.0)	0 (0.0)	4 (36.4)	.428
N-Miss	9	9	40	

(continued on following page)

TABLE A4. Baseline Characteristics of Patients With PDAC Receiving ICI (low-TMB *v* high-TMB) (continued)

Characteristic	Low-TMB (n = 41)	High-TMB (n = 10)	Total (n = 51)	*P*
NLR, No. (%)				
≤2.5	3 (42.9)	4 (57.1)	15 (35.7)	
>2.5	4 (57.1)	3 (42.9)	27 (64.3)	.666
N-Miss	3	3	9	
Opioid pretherapy, No. (%)				
No	6 (60.0)	6 (60.0)	34 (66.7)	
Yes	4 (40.0)	4 (40.0)	17 (33.3)	.618
Steroid pretherapy, No. (%)				
No	3 (30.0)	4 (40.0)	16 (31.4)	
Yes	7 (70.0)	6 (60.0)	35 (68.6)	.917
Line of therapy, No. (%)				
1	4 (40.0)	3 (30.0)	10 (19.6)	
2	3 (30.0)	3 (30.0)	17 (33.3)	.276
3	2 (20.0)	2 (20.0)	10 (19.6)	
≥4	1 (10.0)	2 (20.0)	17 (33.3)	

Abbreviations: ALK, alkaline phosphatase; CA19-9, cancer antigen 19-9; Chemo, chemotherapy; CGDB, Flatiron Health and Foundation Medicine clinicogenomic database; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitors; LDH, lactate dehydrogenase; LLN, lower limit of normal; NLR, neutrophil-to-lymphocyte ratio; PDAC, pancreatic ductal adenocarcinoma; Q1, first quartile; Q3, third quartile; TMB, tumor mutational burden; Tx, therapy; ULN, upper limit of normal.

TABLE A5. Baseline Characteristics of Patients With PDAC and High-TMB (chemotherapy *v* ICI)

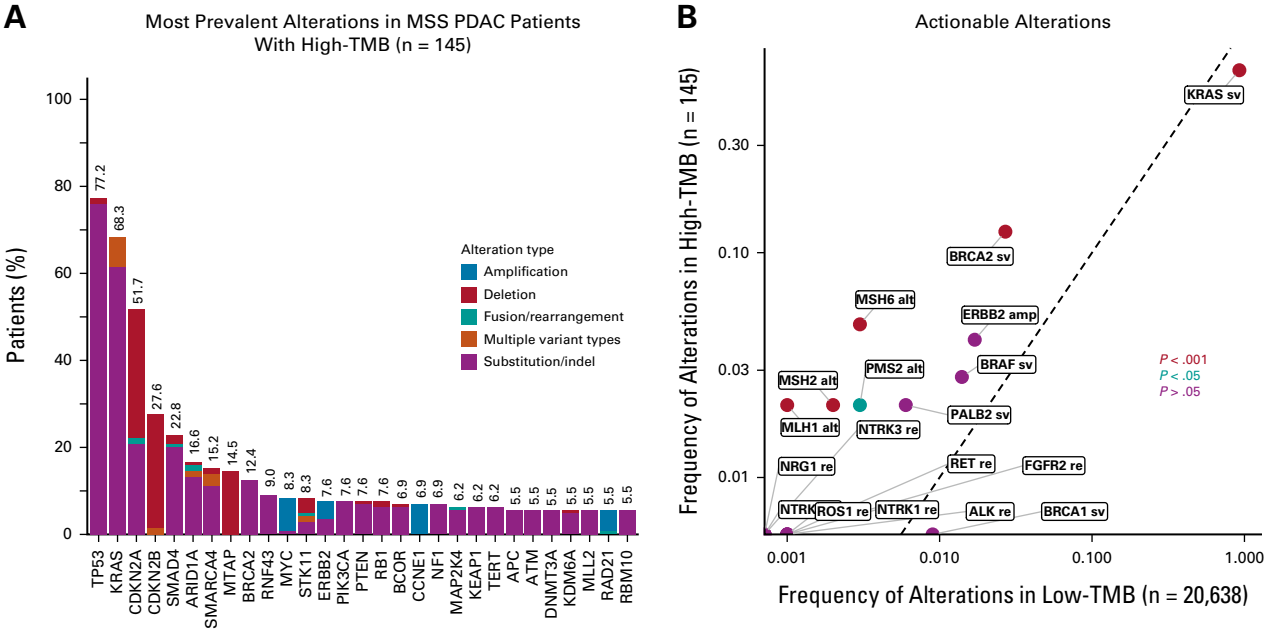
Characteristic	Chemo (n = 17)	ICI (n = 10)	Total (n = 27)	*P*
Age at Tx start, years				.98
Median (Q1-Q3)	69.0 (64.0-73.0)	68.0 (66.0-73.0)	68.0 (64.0-73.0)	
Sex, No. (%)				.831
Female	4 (23.5)	2 (20.0)	6 (22.2)	
Male	13 (76.5)	8 (80.0)	21 (77.8)	
Race, No. (%)				.818
Black or African American	1 (5.9)	1 (10.0)	2 (7.4)	
Other race	3 (17.6)	1 (10.0)	4 (14.8)	
White	13 (76.5)	8 (80.0)	21 (77.8)	
Practice type, No. (%)				.888
Academic	2 (11.8)	1 (10.0)	3 (11.1)	
Community	15 (88.2)	9 (90.0)	24 (88.9)	
ECOG, No. (%)				.04
0	9 (56.2)	1 (10.0)	10 (38.5)	
1	7 (43.8)	8 (80.0)	15 (57.7)	
2	0 (0.0)	1 (10.0)	1 (3.8)	
N-Miss	1	0	1	
Year of Tx start, No. (%)				.208
2014-2017	8 (47.1)	1 (10.0)	9 (33.3)	
2018	1 (5.9)	0 (0.0)	1 (3.7)	
2019	2 (11.8)	4 (40.0)	6 (22.2)	
2020	2 (11.8)	3 (30.0)	5 (18.5)	
2021	3 (17.6)	2 (20.0)	5 (18.5)	
2022	1 (5.9)	0 (0.0)	1 (3.7)	
CA19-9				.692
Median (Q1-Q3)	53.0 (12.5-668.6)	40.2 (22.0-182.0)	46.6 (15.2-437.5)	
N-Miss	6	5	11	
Albumin, No. (%)				.42
<LLN	4 (25.0)	4 (40.0)	8 (30.8)	
≥LLN	12 (75.0)	6 (60.0)	18 (69.2)	
N-Miss	1	0	1	
ALK, No. (%)				.42
≤ULN	8 (50.0)	3 (33.3)	11 (44.0)	
>ULN	8 (50.0)	6 (66.7)	14 (56.0)	
N-Miss	1	1	2	
Serum creatinine, No. (%)				.42
≤ULN	15 (93.8)	10 (100.0)	25 (96.2)	
>ULN	1 (6.2)	0 (0.0)	1 (3.8)	
N-Miss	1	0	1	
Hemoglobin, No. (%)				.937
<LLN	13 (81.2)	8 (80.0)	21 (80.8)	
≥LLN	3 (18.8)	2 (20.0)	5 (19.2)	
N-Miss	1	0	1	
LDH, No. (%)				.439
≤ULN	3 (60.0)	1 (100.0)	4 (66.7)	
>ULN	2 (40.0)	0 (0.0)	2 (33.3)	
N-Miss	12	9	21	
NLR, No. (%)				.102
≤2.5	3 (21.4)	4 (57.1)	7 (33.3)	

(continued on following page)

TABLE A5. Baseline Characteristics of Patients With PDAC and High-TMB (chemotherapy *v* ICI) (continued)

Characteristic	Chemo (n = 17)	ICI (n = 10)	Total (n = 27)	*P*
>2.5	11 (78.6)	3 (42.9)	14 (66.7)	
N-Miss	3	3	6	
Opioid pretherapy, No. (%)				.952
No	10 (58.8)	6 (60.0)	16 (59.3)	
Yes	7 (41.2)	4 (40.0)	11 (40.7)	
Steroid pretherapy, No. (%)				.088
No	2 (11.8)	4 (40.0)	6 (22.2)	
Yes	15 (88.2)	6 (60.0)	21 (77.8)	
Line of therapy, No. (%)				.038
1	14 (82.4)	3 (30.0)	17 (63.0)	
2	2 (11.8)	3 (30.0)	5 (18.5)	
3	0 (0.0)	2 (20.0)	2 (7.4)	
≥4	1 (5.9)	2 (20.0)	3 (11.1)	

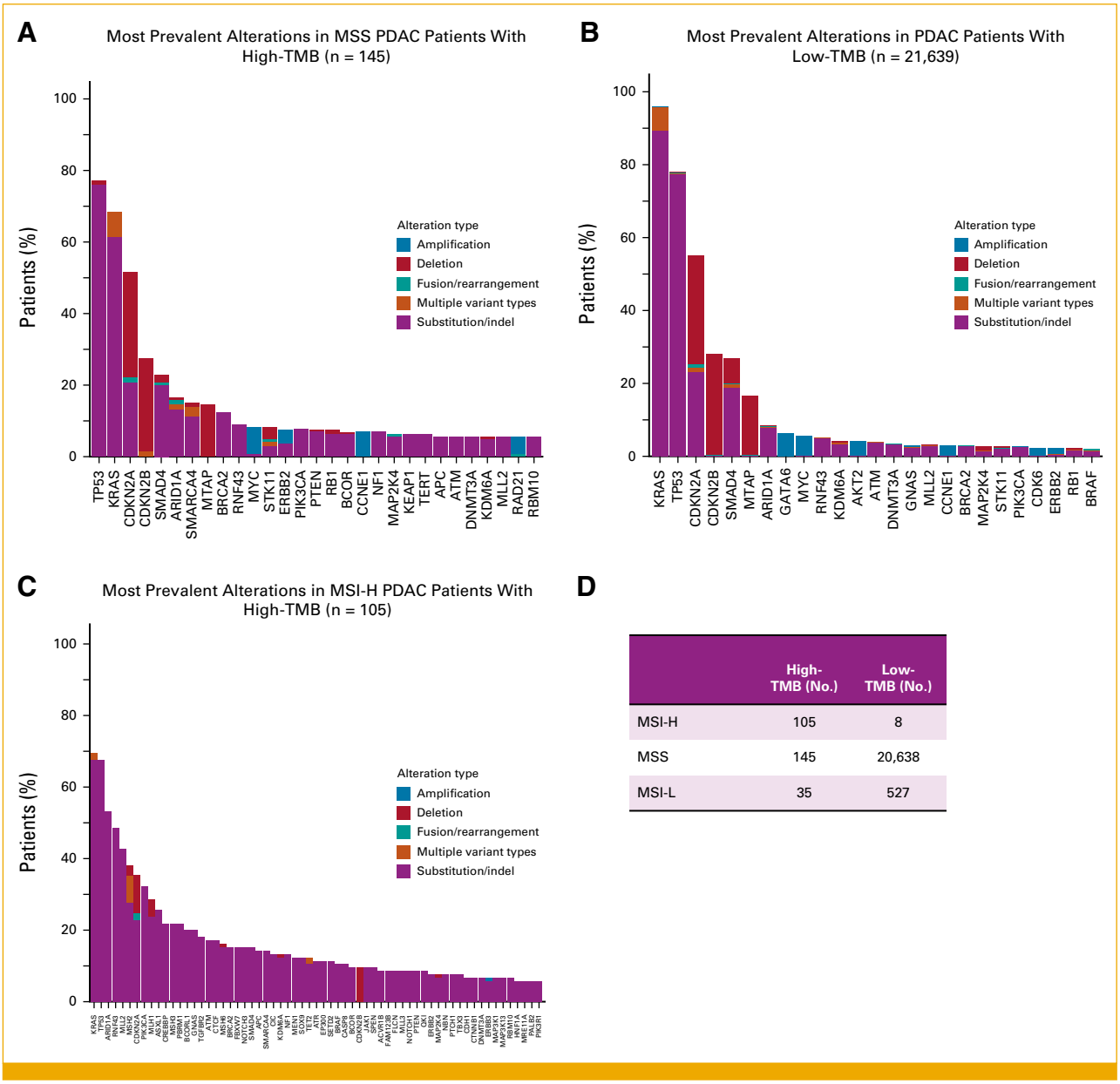
Abbreviations: ALK, alkaline phosphatase; CA19-9, cancer antigen 19-9; Chemo, chemotherapy; CGDB, Flatiron Health and Foundation Medicine clinicogenomic database; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitors; LDH, lactate dehydrogenase; LLN, lower limit of normal; NLR, neutrophil-to-lymphocyte ratio; PDAC, pancreatic ductal adenocarcinoma; Q1, first quartile; Q3, third quartile; TMB, tumor mutational burden; Tx, therapy; ULN, upper limit of normal.



C

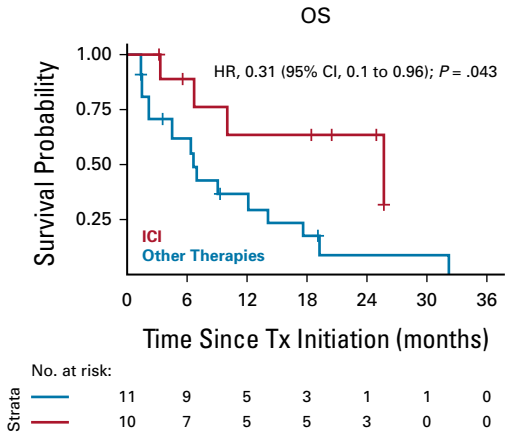
Alteration	Low-TMB (%)	High-TMB (%)	*P* (adjusted)
KRAS mutations	92.6	64.8	<1E-10
BRCA2 mutations	2.7	12.4	<1E-10
PALB2 mutations	0.6	2.1	.05
MMR genes			
MSH2 alterations	0.2	2.1	5.4E-7
MSH6 alterations	0.3	4.8	<1E-10
MLH1 alterations	0.1	2.1	<1E-10
PMS2 alterations	0.3	2.1	3.1E-4

FIG A1. Genomic alterations in MSS PDAC patients with high-TMB. (A) Bar plot showing the most prevalent alterations (>5%) for MSS patients with high-TMB. Scatter plot showing (B) the difference in prevalence of actionable alterations in MSS patients with high-TMB versus low-TMB, and (C) the respective prevalence for those actionable alterations that are statistically significant different between the two groups. alt, alterations; amp, amplification; del, deletion; MMR, mismatch repair; MSS, microsatellite stable; PDAC, pancreatic ductal adenocarcinoma; re, rearrangements; sv, short variants; TMB, tumor mutational burden.



	High-TMB (No.)	Low-TMB (No.)
MSI-H	105	8
MSS	145	20,638
MSI-L	35	527

FIG A2. Contrast between genomic alterations across patients with PDAC: MSI-H and high-TMB, MSS and high-TMB, and MSS and low-TMB. Bar plots showing (A) the most prevalent alterations (>5%) for MSS patients with high-TMB, (B) the most prevalent alterations (>2%) for MSS patients with low-TMB, and (C) the most prevalent alterations (>5%) for MSI-H patients with high-TMB. (D) Number of patients with each MSI and TMB status. MSI-H, microsatellite instability-high; MSI-L, MSI-low; MSS, microsatellite stable; PDAC, pancreatic ductal adenocarcinoma; TMB, tumor mutational burden.



	No.	Median OS (months)
ICI	10	25.7 (10.0-NR)
Other therapies	17	6.6 (2.17-NR)

FIG A3. Patients with high-TMB have better outcomes receiving ICI compared with other therapies. HR, hazard ratio; ICI, immune checkpoint inhibitor; NR, not reached; OS, overall survival; TMB, tumor mutational burden; Tx, therapy.

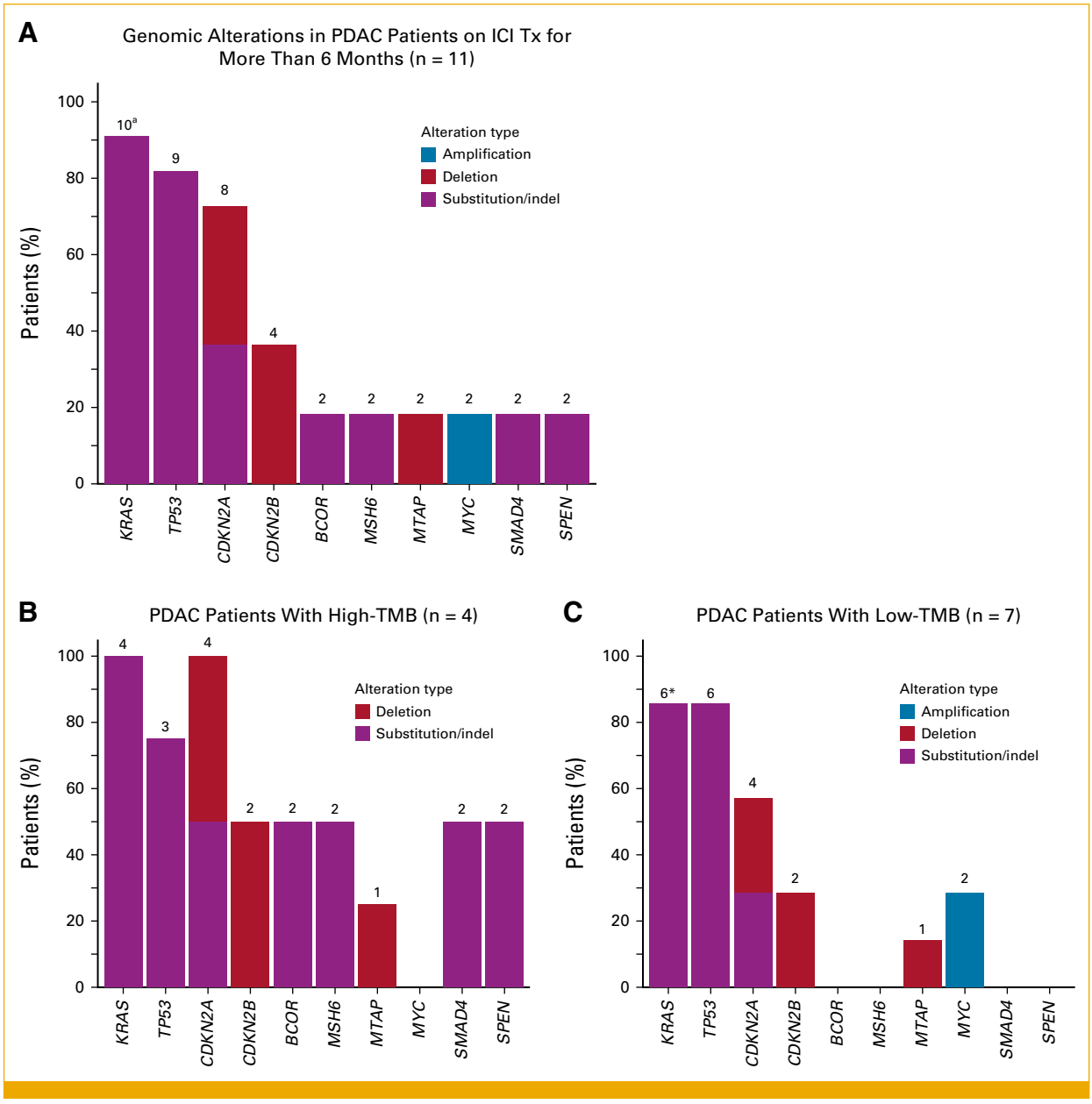


FIG A4. Most common genomic alterations prevalent in patients treated with ICI for at least 6 months: (A) all patients, (B) only patients with high-TMB, and (C) only patients with low-TMB. amp, amplification; ICI, immune checkpoint inhibitor; PDAC, pancreatic ductal adenocarcinoma; TMB, tumor mutational burden; Tx, therapy. [a]Two patients have also KRAS amp in addition to mutations.