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Alterations in Driver Genes are Predictive of Survival in Patients with Resected Pancreatic Ductal Adenocarcinoma

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Abstract

Background: *KRAS, TP53, CDKN2A* and *SMAD4* are established driver genes in pancreatic ductal adenocarcinoma (PDAC). We aimed to determine if the mutational status of driver genes, and those involved in DNA repair pathways, are associated with clinical outcomes in individuals who undergo resection.

Methods: Eligible individuals were those who underwent resection of PDAC and consented to targeted sequencing of their primary tumor using MSK-IMPACT. Genomic alterations were determined based on MSK-IMPACT results from formalin-fixed, paraffin-embedded samples. Associations between genomic alterations and clinical outcomes were assessed.

Results: Targeted sequencing was performed on *N*=283 primary tumors resected between 2004–2017. Median follow up was 23 months among survivors. Alterations in *KRAS* and *TP53* were associated with worse overall survival (OS) as compared to wildtype (median[95%CI], 38.8[33.0–45.5] vs 91.0[34.8-NA] months, p=0.043; 37.4[32.1–42.8] vs 65.0[33.0-NA] months, p=0.035). *KRAS* G12D mutations were associated with worse OS (31.6[25.3–45.5] vs 39.2[37.4–75.2] months, p=0.012). *TP53* truncating mutations (39.6[32.4–75.2] vs 33.9[24.0–39.0] months, p=0.020) and those associated with loss of heterozygosity (LOH) (26.6[21.6–44.2] vs 39.2[34.5–49.1] months, p=0.048) had decreased OS. *TP53* alterations were independently associated with OS on multivariate analysis (HR[95% CI], 1.54[1.01–2.33], p=0.042). Individuals with germline alterations in homologous repair deficiency (HRD) genes had improved OS as compared to those without (median OS not reached vs 37.0[33.0–49.8] months, p=0.035).

Conclusion: In patients with resected PDAC, genomic alterations in *KRAS* and *TP53* are associated with worse outcomes, whereas alterations in HRD genes are associated with favorable prognosis. Further studies are needed to better define these alterations as biomarkers in resected PDAC.

Precis:

Results from a routinely used, clinically-actionable targeted sequencing panel demonstrate that alterations in KRAS and TP53 are associated with worse outcomes in patients undergoing resection for PDAC. Specifically, KRAS G12D mutations, and TP53 alterations resulting in truncations and those in areas with loss of heterozygosity (LOH), were associated with poorer prognosis; additionally, germline HRD mutations are associated with improved overall survival.

Keywords

Pancreatic Ductal Adenocarcinoma; Driver Gene Alterations; Resection; Homologous Recombination; Survival Outcomes

Introduction

Pancreatic adenocarcinoma (PDAC) is predicted to be the second leading cause of cancer deaths by 2030.[1] Over the past decade, the genomic landscape of pancreatic cancer has been well characterized.[2–5] *KRAS, TP53, CDKN2A* and *SMAD4* are the main genes that drive

pancreatic tumorigenesis. Alterations in *KRAS* and *CDKN2A* are early events in tumorigenesis, whereas mutations in *TP53* and *SMAD4* occur at a later stage.[6,7] Additionally, a subset of genes involved in multiple oncogenic pathways mutated at a lesser frequency have been identified.[2–5]

Previous data has demonstrated an association between alterations in driver genes and overall survival (OS) and recurrence-free survival (RFS) following resection for PDAC.[8] Alterations in *KRAS, TP53* and *CDKN2A* were associated with worse RFS, and *CDKN2A* alterations with worse OS. Other studies have demonstrated that loss of *SMAD4* is associated with worse disease-free survival,[9] and wildtype *KRAS* and *CDKN2A* are associated with improved OS.[10,11] Additionally, studies have demonstrated that increasing numbers of driver gene mutations are associated with worse outcomes.[8,11,12]

While there has been an increased understanding of the genomics of PDAC, relatively few clinically actionable mutations have been identified. Previous data from our institution using targeted genomic profiling identified potentially targetable alterations in a minority of individuals with PDAC and other groups have suggested a greater frequency of actionability.[13,14] Many targeted therapies are under investigation, but few have been validated in the treatment of PDAC.[14–18] Several studies suggest that patients with germline *BRCA1* and *BRCA2* mutations have improved outcomes following treatment with platinum-based chemotherapy and poly-ADP ribose polymerase (PARP) inhibitors.[5,19–22] No targeted agents against any of the four driver genes have been validated and there are no FDA approved drugs for use in this setting.

In this study, we evaluated the use of a targeted sequencing panel, MSK-IMPACT, in individuals with PDAC, and investigated the associations of the four driver genes, and other commonly altered genes, including those involved in DNA repair pathways, with clinical and pathologic outcomes in those who underwent surgical resection for PDAC at Memorial Sloan Kettering (MSK).

Methods

Patient cohort

A prospectively maintained database was queried for consecutive individuals who underwent resection for PDAC at MSK and had targeted next generation sequencing (NGS) performed on their primary tumor. This study was reviewed by the MSK Institutional Review Board (IRB). Individuals were consented under an institutional protocol to have their tumors sequenced with Memorial Sloan Kettering-Integrated Mutational Profiling of Actionable Cancer Targets (MSK-IMPACT, NCT01775072) as part of clinical practice.[23] Demographic, clinical, pathologic, and outcome data were abstracted from the database and electronic medical record.

Genetic analysis

Samples were obtained from the resection specimen or pre-treatment biopsy of the primary pancreatic tumor. All tumor samples were reviewed by a gastrointestinal pathologist and macrodissected from formalin-fixed paraffin-embedded blocks to ensure adequate

cellularity. DNA was extracted from blood for somatic mutation calling and for germline testing when consent was obtained for the latter. Tumors were sequenced using MSK-IMPACT, which consists of deep, targeted sequencing of all exons and selected introns of 351 (n=4), 410 (n=176) or 468 (n=103) cancer-related genes. Tumor purity was estimated based on pathologic review of samples. Single nucleotide variants (SNVs) and insertions and deletions (Indels) were called using MuTect, Pindel, and Somatic Indel Detector, as previously described.[24] MSISensor was used to determine microsatellite instability.[25,26] FACETS was used for evaluation of copy number alterations (CNA), including loss of heterozygosity (LOH),[27] and results were manually reviewed to identify the best fit. To characterize LOH, mutant allele frequency and overall coverage at that locus was assessed. A region of LOH was considered present if the lower copy number was 0 or the mutant allele frequency was consistent with the variant allele frequency (VAF) that would be expected from LOH favoring the mutant allele.[28] Three individuals without clear CNA profiles were removed from *TP53* LOH analysis, and one patient with two *TP53* alterations was removed from LOH and truncation analyses. Mutations with OncoKB annotation of "Predicted Oncogenic," "Likely Oncogenic," or "Oncogenic" are considered putative drivers, while other mutations are labeled as unknown significance.[29]

Homologous recombination (HRD) genes were analyzed, and included *ARID1A*, *ATM, BAP1, BARD1, BLM, BRCA1, BRCA2, BRIP1, CHEK2, FAM175A, FANCA, FANCC, NBN, PALB2, RAD50, RAD51, RAD51C*, and *RTEL1*. Mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6*, and *PMS2*, were also evaluated. Mutational frequencies seen in this cohort were compared to those of a larger population of both primary and metastatic PDAC tumors (n=1,702) sequenced with MSK-IMPACT.

Germline Sequencing

A subset of individuals in the cohort consented to germline testing with MSK-IMPACT (n=154; NCT01775072), and serves as the denometer for germline analyses. This platform consists of up to 88 genes associated with cancer predisposition. Blood was used to call all germline mutations.

Statistical analysis

Continuous data are expressed as median and range, and categorical variables are expressed as frequency and percentage. Wilcoxon rank-sum and Fisher's exact tests were used to compare pathological variables and binary gene mutation status. Overall survival (OS) was defined as the time from surgery to the date of death or date of last follow up. Postoperative imaging was reviewed for site of first recurrence. Recurrence-free survival (RFS) was defined as the time between surgery and time of first recurrence or death, whichever occurred first; if the endpoint was not met, then patients were censored at the date of last imaging. Individuals were excluded from RFS analysis if they had metastatic disease at the time of operation (n=4) or incomplete follow up imaging (n=1). OS and RFS were estimated with Kaplan-Meier methods and compared using the log-rank test. Univariate and multivariate survival analyses were completed using a Cox proportional hazard model to evaluate the impact of genomic alterations on OS in the setting of potential cofounding variables (stage (AJCC 8th edition), LN status (positive or negative), lymphovascular

invasion, perineural invasion, tumor size and neoadjuvant therapy. The multivariable model was constructed by including factors significantly associated with OS ($p < 0.05$) on univariate analysis.

Cumulative incidences of patterns of first recurrence (CIR; distant only, local only, local and distant) and death without evidence of recurrence, as well as specific site of distant recurrence, were estimated using competing risks methods from the date of operation. Associations between site of recurrence and gene mutation status were evaluated using Gray's test and the Fine and Gray competing risks method. All tests were two-sided and $p<0.05$ was considered significant. The nominal p-value is shown unless otherwise noted. SAS (version 9.4, SAS institute Inc., Cary, NC) or R (Version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria) using 'cmprsk' were used for all analyses.

Results

Patient cohort

There were 283 individuals who underwent resection for PDAC at MSK between January 2004 and August 2017, and had targeted sequencing performed on the primary tumor (Table S1). All data was finalized in August 2018. Demographic and clinical characteristics are illustrated in Table 1. There were 58 patients (20%) who received neoadjuvant therapy, 72% (n=205) of the cohort underwent pancreaticoduodenectomy, and 77% (n=218) received adjuvant therapy. In total, 81% (n=229) had genomic sequencing of their tumors performed prior to receipt of systemic treatment.

The median OS of the entire cohort was 39.0 [95%CI: 33.3–46.3] months and median RFS was 12.8 [95%CI: 11.7–15.5] months, with a median follow up of 23 months among survivors. Sequencing with MSK-IMPACT was performed routinely beginning in 2014, and therefore a subset of individuals alive when MSK-IMPACT testing was initiated consented to have their primary tumor sequenced retrospectively (n=43), resulting in a survivor bias between those sequenced before and after 2014. The median OS for individuals resected after 2014 was 32.0 [95%CI: 27.2–37.6] months and RFS was 12.0 [95%CI: 10.1–13.9] months, as compared to 82.0 [95%CI: 51.6-NA] months and 28.0 [95%CI: 22.5–52.6] months for those resected before 2014. At the time of analysis, cumulative incidences of distant, isolated local, and simultaneous local and distant recurrences at 3-years postoperatively were 40.7% [95%CI: 34.3%−46.9%], 21.5% [16.3%−27.3%], and 15.0% [10.9%−19.8%], respectively (Figure S1a). The most common site of distant recurrence was the liver (n=75; 3-year CIR [95% CI], 27.9% [22.5–33.5%]; Figure S1b).

Genomic alterations in driver and HRD genes

The average sequencing depth of all variants was 722X and the median cellularity of all samples was 23%. An oncoprint demonstrating genetic alterations in the cohort is shown in Figure 1. *KRAS* was altered in 93% (n=262) of the cohort, of which, 42% (n=109) had G12D mutations. Alterations in *TP53* occurred in 72% (n=203), and specifically, 27% (n=54) had truncating mutations and 29% (n=80) had alterations in the presence of LOH.

Truncating mutations and LOH co-occurred in 10% (n=29) of patients. Mutations in *CDKN2A* were noted in 28% (n=79), and *SMAD4* in 20% (n=57) of the cohort.

ARID1A, RNF43, and *PTPRT* were altered in ≥5% of the cohort. There were 47 patients with somatic HRD mutations, and 4 patients had somatic alterations in MMR genes. There were 154 (54%) individuals who underwent germline testing with MSK-IMPACT, and of these, 29 (19%) had at least one germline mutation identified with 21 (14%) having a mutation predicted to be oncogenic. There were 18 patients with germline HRD (gHRD) mutations, of which 7 had germline *BRCA1* or *BRCA2* mutations.

Following the implementation of routine genomic profiling with MSK-IMPACT, there have been a total of 1,702 PDAC tumors sequenced using this platform. We compared the alterations in our cohort of 283 resected tumors to all other primary and metastatic PDAC tumors sequenced at MSK. Mutational frequencies of the four driver genes, genes altered at a frequency of ≥5%, and both HRD and MMR genes were similar between the two cohorts (p>0.05).

Driver gene alterations and association with clinical and pathologic variables

Alterations in *KRAS* and *TP53* were compared to pathologic variables associated with poor prognosis, including tumor diameter, lymphovascular invasion, perineural invasion and the presence of positive lymph nodes. The only positive association observed was between *KRAS* alterations and larger tumor size (median, 3.0 vs 2.2cm, p=0.009; Table S2). There were no significant associations between *CDKN2A* status, *SMAD4* status, number of genes altered, and pathologic variables.

Driver gene alterations and association with survival

Gene-level alterations in both *KRAS* and *TP53* demonstrated an association with OS on univariate analysis, but not RFS (Table 2). There was no association between alterations in *CDKN2A, SMAD4,* or the number of driver genes (0–4) and OS or RFS. Individuals with mutations in *PTRPT* had worse RFS than those with wildtype *PTRPT* (median [95%CI], 5.7 [3.2-NA] vs 14.0 [12.0–17.9] months, p=0.019).

Median survival in individuals with *KRAS* alterations was 38.8 [95%CI: 33.0–45.5] months versus 91.0 [95%CI: 34.8-NA] months in those with wildtype *KRAS* (p=0.043, Figure 2a). Moreover, G12D mutations were associated with worse OS as compared to all other KRAS mutations (median [95% CI], 31.6 [25.3–45.5] vs 39.2 [37.4–75.2] months, p=0.012; Figure 2b), and specifically other G12 mutations (median [95%CI], 31.6 [25.3–45.5] vs 39.3 [37.2–51.6] months, p=0.027).

There were 21 individuals (7.5%) with *KRAS* wildtype tumors (Figure S2). Of these, 5 patients had no other SNV or CNA identified while 16 individuals had other somatic alterations, 12 of which were exonic. The mean cellularity of all *KRAS* wildtype patients was 23.6%; it was 30.4% in patients with exonic somatic alterations, but only 16% in those without any identified alterations. One patient had a BRAF-KDM7A fusion, however, the breakpoint in KDM7A occurred 2Kb after the stop codon making the significance of this alteration unknown.

The median OS in patients with *TP53* alterations was 37.4 [95%CI: 32.1–42.8] months and 65.0 [95%CI: 33.0-NA] months in those with wildtype *TP53* (p=0.035, Figure 3a). *TP53* mutations that resulted in truncations were associated with worse OS (median [95%CI], 33.9 [24.0–39.0] vs 39.6 [32.4–75.2] months, p=0.020, Figure 3b) and RFS (median [95%CI], 10.9 [8.5–13.7] vs 15.5 [11.4–21.6] months, p=0.014). *TP53* mutations in regions with LOH were associated with worse OS compared to no LOH (median [95%CI], 26.6 [21.6–44.2] vs 39.2 [34.5–49.1] months, p=0.048, Figure 3c), although there was no significant difference in RFS (9.8 [6.8–15.5] vs 15.9 [12.3–21.8] months, p=0.079). Individuals who had truncating mutations in a region with LOH (n=29) had worse RFS than either truncating or LOH alone (median [95%CI], 8.7 [5.0–12.0] vs 12.7 [10.9–28.5] months, p=0.026 and 11.4 [6.8–35.9] months, p=0.019), however there was no significant difference in OS (Figure S3). Alterations in *KRAS* and *TP53* co-occurred in 70% (n=197) of patients, and this was associated with worse OS than a single mutation alone (median [95% CI], 37.4 [32.0–42.8] vs 65.0 [33.0-NA] months, p=0.027).

We then conducted univariate and multivariate analyses looking at the association of *KRAS* and *TP53* status with OS in the setting of other potential confounding variables. Results of univariate analysis demonstrated that *KRAS* alterations, *TP53* alterations, Stage III/IV tumors, lymphovascular invasion and neoadjuvant therapy were associated with OS (Table 3). When adjusting for other variables, *KRAS* was no longer associated with OS (HR [95%CI], 1.74 [0.75–4.01], yet TP53 alterations remained significantly associated with a worse prognosis (HR [95% CI], 1.54 [1.01–2.33], p=0.042).

Time to site of first recurrence and association with genomic alterations

Associations between genotype and cumulative incidence of site of first recurrence was evaluated for all patients. *TP53* truncations and mutations associated with LOH were associated with simultaneous local and distant recurrences (3-year CIR [95%CI], 25.6% [14.3–38.5%] vs 10.4% [5.9–16.5%], p=0.009; 22.2% [13.2–32.7%] vs 9.1% [4.6–15.6%], p=0.030; Table 4). *KRAS* alterations were associated with an increased incidence of distant recurrences although not statistically significant (3-year CIR [95%CI], 14.2% [10.1–19.0%] vs 23.1% [6.4–45.9%], p=0.056).

KRAS alterations were associated with liver recurrences (Table 4, 3-year CIR [95%CI], 29.7% [23.9–35.6%] vs 5.8% [0.3–24.5%], p=0.013), as was *TP53* LOH (40.5% [29.0–51.7%] vs 24.5% [16.6–33.1%], p=0.017). Additionally, co-occurrence *KRAS*/*TP53* alterations trended towards significance in patients with liver metastases (3-year CIR [95%CI], 31.6 [24.8–38.6%] vs 8.0% [0.4–32.2%], p=0.054). No genomic alterations were associated with lung metastases as the first site of recurrence.

Alterations in HRD and association with clinical outcomes

Associations between somatic and germline mutations in genes involved in DNA repair pathways and survival outcomes were examined. There were 47 individuals with somatic HRD alterations, and there was no difference in OS or RFS in patients with somatic HRD mutations and those without (median [95%CI], 39.3 [24.3-NA] vs 39.0 [33.2–47.2] months, p=0.705; 11.3 [8.2–21.2] vs 14.2 [12.4–18.6] months, p=0.120). In this group, there were 11

patients who received platinum-based therapy perioperatively and 31 who received chemotherapy without platinum. There was no difference in survival outcomes in individuals with somatic HRD gene alterations who received or did not receive platinum-based chemotherapy (OS: 18.0 [14.0-NA] vs 51.6 [33.3-NA] months, p=0.114; RFS: 10.9 [6.7-NA] vs 11.9 [9.7–24.5] months, p=0.420). There were 18 individuals with mutations in gHRD genes, and these patients had significantly improved OS as compared to wildtype (median OS not reached vs 37.4 [33.0–49.8] months, p=0.035), but there was no difference in RFS (median [95%CI], 14.5 [9.4-NA] vs 13.9 [12–18.6] months, p=0.259). There were 7 individuals (2.5%) with a germline *BRCA1* or *BRCA2* mutations, and 3 patients demonstrated evidence of biallelic loss through LOH. Of these, one received neoadjuvant FOLFIRINOX and two received adjuvant gemcitabine and cisplatin. Two of these patients subsequently developed metastatic disease and are maintained on olaparib with ongoing disease control at the time of data analysis.

There were two individuals in this cohort with MSI high tumors, and both patients had MMR germline alterations. One individual had PDAC arising within an IPMN and developed metastatic disease to retroperitoneal lymph nodes following resection. This patient was subsequently diagnosed with Lynch Syndrome based on outside genetic testing, and later developed urothelial, gastric, and prostate cancers. This patient was treated with pembrolizumab, to which his prostate and PDAC had a favorable response during two years of treatment, however ultimately died of progressive urothelial cancer (with controlled small volume metastatic PDAC). The other individual was diagnosed with PDAC arising in association with an IPMN, subsequently metastasized to retroperitoneal lymph nodes. Upon testing with MSK-IMPACT, they were noted to have a germline *PMS2* heterozygous loss of exons 11–14. To date, this individual has not developed any other malignancy.

Discussion

Pancreatic ductal adenocarcinoma (PDAC) is associated with a poor prognosis, with an overall five-year survival rate of less than 10%.[30] Only 15–20% of patients have resectable disease at diagnosis while most present with locally advanced or metastatic disease.[31] Biomarkers are expanding, and recently several actionable targets have been identified in PDAC. The most promising of these have been the use of platinum agents and PARP-inhibitors in patients with germline BRCA mutations.[19–22] Prior studies have evaluated targeting driver genes, yet results have thus far not demonstrated any clinical benefit.[16] Therefore, there exists a need for novel predictive and prognostic biomarkers to improve outcome and refine therapeutic options.

Herein, we demonstrate that alterations in both *KRAS* and *TP53* are associated with worse outcomes in patients who undergo resection for PDAC. Patients with *KRAS* alterations have decreased OS, and specifically, *KRAS* G12D mutations confer a worse prognosis as compared to other *KRAS* alterations. Furthermore, *KRAS* alterations are also associated with larger tumor size, and distant recurrences following resection, specifically liver metastases. *TP53* alterations are associated with worse survival and time to concurrent local and distant metastases as first site of recurrence. In particular, truncating *TP53* mutations and those associated with LOH were indicative of a poor prognosis. On multivariate OS

analysis, alterations in *TP53* were independently associated with OS after controlling for disease stage, vascular invasion, and exposure to neoadjuvant therapy.

KRAS and *TP53* have been shown to be mutated in approximately 90% and 75% of PDAC, respectively.[32–34] The results of the current study demonstrate that these two frequently altered genes are associated with poorer survival as well as recurrence patterns with higher rates of systemic failure. Previous data have demonstrated that alterations in driver genes are associated with worse outcomes in patients who underwent resection for PDAC.[8–10] Using NGS and immunohistochemistry (IHC), Qian et al. demonstrated that *KRAS*, *TP53* and *CDKN2A* alterations are associated with decreased RFS, and *CDKN2A* with worse OS.[8] Specifically, patients with *KRAS* G12D mutations had worse RFS and OS.[8] Additionally, NGS analysis of the CONKO-001 trial showed that p53 protein overexpression was associated with worse OS and RFS in the setting of adjuvant gemcitabine.[35] Given the poor outcomes associated with *KRAS* and *TP53* variants, these should be further evaluated for use as biomarkers to provide prognostic information and help guide treatment decisions. For example, adjuvant therapies could be intensified for patients with an unfavorable genomic profile.

Furthermore, such alterations could serve as therapeutic targets in the future, and several studies have demonstrated potential in targeting *KRAS*. *KRAS* is commonly altered in other solid tumors, and results of a recent phase I trial using AMG 510, a small molecule inhibitor of *KRAS* G12C, demonstrated a partial response or stable disease in *KRAS* G12C mutated colorectal and non-small cell lung cancers.[36] In locally advanced pancreatic cancer, an initial phase 1/2a trial evaluating siG12D-LODER, a particle which releases small amounts of RNAi against *KRAS* G12D and to a lesser extent in *KRAS* G12V, in combination with gemcitabine, demonstrated stable disease or a partial response in all patients.[37] Currently, there is an ongoing randomized phase 2 trial evaluating gemcitabine and nab-paclitaxel with or without siG12D-LODER in locally advanced pancreatic cancer patients (NCT01676259).

Patients with wildtype *KRAS* have been shown to have improved OS as compared to *KRAS*-mutated individuals following resection for PDAC.[11] Our results are consistent with these data. True wildtype *KRAS* should be confirmed by evaluation of CNA or the presence/absence of other mutations given the low cellularity of PDAC tumors. *KRAS* wildtype individuals have been shown to have other genomic alterations driving pancreatic tumorgenesis. In our cohort, two individuals had oncogenic *BRAF* mutations (as predicted by OncoKB[24]) and one was noted to have a BRAF-KDM7A of uncertain significance. Pishvaian et al. identified that 14 of 81 *KRAS* wildtype patients had alterations in BRAF.[14] Additionally, ALK fusions have been shown to drive tumorgenesis in *KRAS* wildtype PDAC, and can be targeted with tyrosine kinase inhibitors.[38,39] Although these alterations are noted in patients with metastatic disease, this demonstrates the importance of accurate identification of *KRAS* wildtype patients, as a subset of these patients may have other actionable targets.

Given recent data that has emerged with the use of platinum agents and PARP-inhibitors in PDAC, we evaluated the outcomes of individuals with both germline and somatic alterations in HRD genes. Preliminary data implies that some *ARID1A* alterations result in an HRD-

like phenotype (unpublished data), and therefore we have included *ARID1A* mutations in this group, although we recognize this is not a point of consensus. Our results suggest that individuals with gHRD alterations have improved survival. Patients with gHRD mutations were diagnosed at a younger age (median, 59 vs 68 years), however given the heterogeneity of treatments received and small sample size, we are unable to accurately assess for confounding factors to account for this difference. No survival differences were observed in our cohort for individuals with and without somatic HRD alterations or with the use of platinum agents. However, the number of individuals with somatic HRD alterations in our cohort was small (17%), and likely underpowered to detect differences between groups. We had limited power to consider the effect of biallelic loss of HRD genes, given there were only 8 patients that had VAFs consistent with biallelic loss. This is important, as one wild-type copy of a gene is often sufficient for normal function. Additionally, only a small subset of these patients received platinum-based chemotherapy, as many patients underwent resection before this was routinely adopted in clinical practice.

In this study, we evaluated genomic alterations obtained from a routinely used, clinically-actionable targeted sequencing panel. This can be done using samples obtained from cytology or core needle biopsies of a tumor, whereas IHC often requires additional tissue. However, a limitation of this study is that we only evaluated genomic alterations, and did not account for downstream effects of gene products using IHC. Prior data has shown that CDKN2A is altered in up to 90% of tumors, either through genetic or epigenetic modifications;[40,41] therefore, this could account for differences of association CDKN2A and SMAD4 status and outcome in this study as compared to prior studies.[8–10] Additionally, the large stromal component in many PDAC tumors and use of bulk sequencing can lead to decreased mutation detection in some patients. There is an inherent selection bias in the subset of patients who underwent resection prior to 2014 for increased OS, as these patients were alive at the time that MSK-IMPACT was implemented. We included this subgroup of patients in our analysis as we evaluated all patients who had their primary tumor sequenced with MSK-IMPACT. Lastly, several of the subgroups analyzed are small in size and therefore limited in power.

In conclusion, our results demonstrate that alterations in *KRAS* and *TP53,* specifically *KRAS* G12D and *TP53* truncations and LOH, are associated with a worse prognosis in patients who undergo surgical resection for PDAC. Patients with KRAS wildtype tumors and gHRD alterations have improved OS. Given the potential of *KRAS*, *TP53,* and HRD status as biomarkers for stratification of outcome or refinement of therapeutic choices, further studies are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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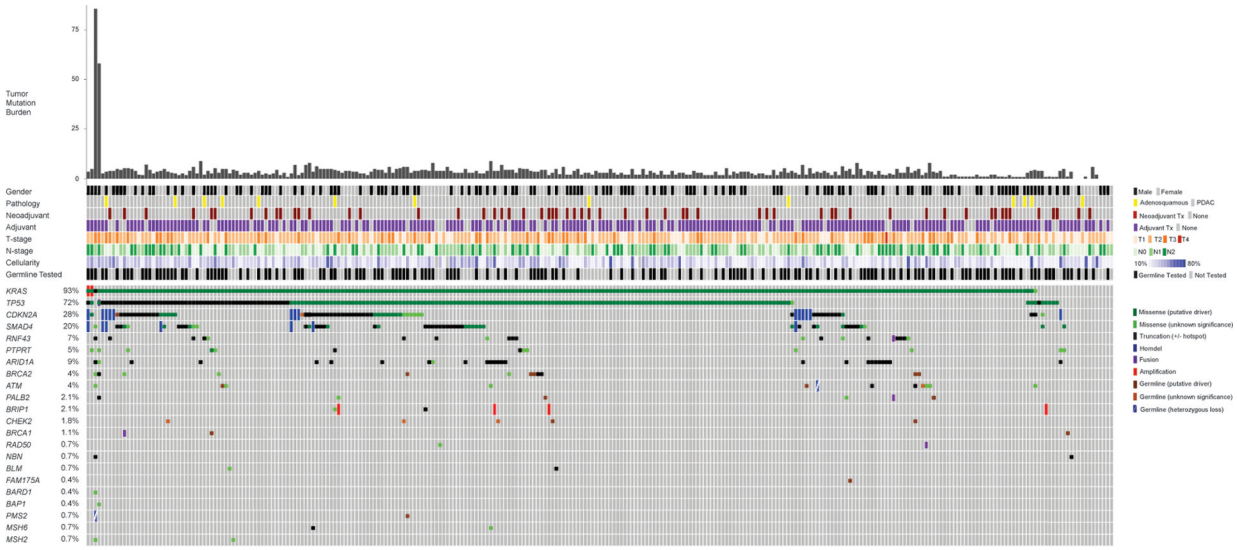
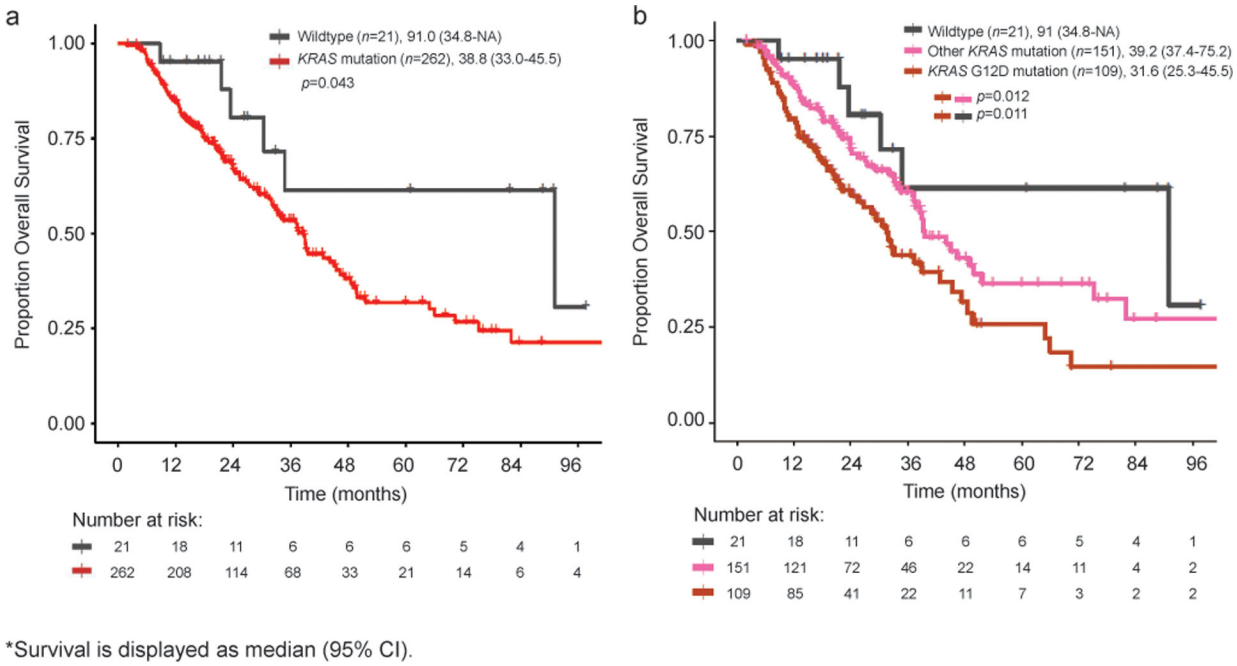


Figure 1.
Oncoprint of Frequently Altered Genes and those Involved in DNA Repair Pathways.



*Survival is displayed as median (95% CI).

Figure 2.

Kaplan-Meier Curves for Individuals with Gene-Level Alterations in *KRAS* (a) and *KRAS* G12D mutations (b).

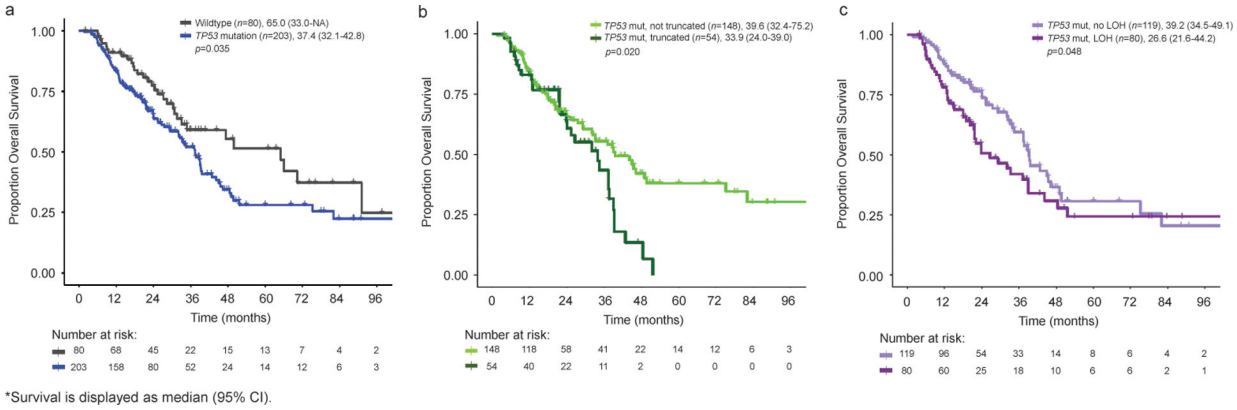


Figure 3.

Kaplan-Meier Curves for OS Individuals with Gene-Level Alterations in *TP53* (a), Truncating *TP53* Mutations (b), and *TP53* Mutations Associated with LOH (c).

Table 1.

Demographic, Clinical and Pathologic Data for Entire Cohort of Resected PDAC.

	***n* = 283**
Epidemiology	
Age, years	67 (59–73)
Gender	
Male	137 (48)
Female	146 (52)
Race/Ethnicity[≠]	
White/Caucasian	250 (88)
African American	7 (2)
Asian	10 (4)
Hispanic/Latino	13 (5)
Ashkenazi Jewish Descent[*]	
Yes	41 (15)
No	224 (79)
Smoking History	
Yes	158 (56)
No	125 (44)
Diabetes	
Yes	82 (29)
No	201 (71)
Personal history of cancer	
Yes	80 (28)
No	203 (72)
Family history of pancreas cancer[**]	
Yes	50 (18)
No	226 (80)
Germline BRCA1/2 mutation	7 (2)
Treatment	
Neoadjuvant Therapy	58 (20)
Chemotherapy alone	42 (72)
Chemotherapy + Radiation	16 (28)
Platinum-based chemotherapy	47 (81)
Procedure	
Pancreaticoduodenectomy	205 (72)
Distal Pancreatectomy	76 (27)
Central Pancreatectomy	1 (0.5)
Total Pancreatectomy	1 (0.5)
Adjuvant Therapy[#]	220 (78)
Chemotherapy alone	179 (81)

	***n* = 283**
Chemotherapy + Radiation	40 (18)
Platinum-based chemotherapy	24 (11)
Pathology	
Tumor Diameter, cm	2.8 (2.2–3.8)
Pathology	
Ductal adenocarcinoma	265 (93)
IPMN/MCN-associated	5 (2)
Adenosquamous	13 (5)
Lymphovascular Invasion	
Yes	194 (69)
No	89 (31)
Perineural Invasion	
Yes	253 (89)
No	30 (11)
Tumor Stage (AJCC 8th Edition)	
T1	57 (20)
T2	171 (60)
T3	54 (19)
T4	1 (0.5)
Lymph Node Stage (AJCC 8th Edition)	
N0	90 (32)
N1	113 (40)
N2	20 (28)
Metastasis Stage	
M0	4 (1)
M1	279 (99)

Variables expressed as n (%) or median (IQR).

$^{\neq}$unknown in 3 patients;

*unknown in 18 patients;

**unknown in 7 patients;

$^{\#}$unknown in 20

Table 2.

Associations of Driver Genes, and Other Frequently Mutated Genes (> 5%), with Overall and Recurrence-Free Survival.

	Alteration *n* (%)	Overall Survival					Recurrence Free Survival				
		Median (95% CI), months		3-year (95% CI), %			Median (95% CI), months		3-year (95% CI), %		
		Wildtype	Altered	Wildtype	Altered	*p*-value	Wildtype	Altered	Wildtype	Altered	*p*-value
KRAS	262 (93)	91.0 (34.8–NA)	38.8 (33.0–45.5)	61.4 (38.9–96.8)	53.5 (46.7–61.2)	***0.043***	21.7 (14.2–76.5)	12.9 (11.7–15.9)	32.9 (16.5–65.5)	19.8 (14.6–26.9)	0.117
TP53	203 (72)	65.0 (33.0–NA)	37.4 (32.1–42.8)	59.0 (47.4–73.5)	52.0 (44.3–61.0)	***0.035***	15.1 (12.8–21.7)	12.5 (10.9–18.1)	21.2 (12.8–35.3)	20.9 (15.0–29.1)	0.352
CDKN2A	77 (27)	39.0 (33.0–47.2)	38.8 (29.1–NA)	54.5 (47.2–63.0)	51.7 (38.4–69.6)	0.846	14.2 (12.3–18.6)	11.7 (8.9–19.2)	22.1 (16.3–30.1)	17.8 (9.5–33.3)	0.244
SMAD4	57 (20)	38.8 (32.4–47.2)	39.2 (33.3–NA)	53.8 (46.5–62.1)	55.0 (41.1–73.6)	0.375	13.2 (11.9–17.3)	14.6 (10.3–23.6)	19.9 (14.4–27.6)	24.5 (14.3–42.1)	0.594
ARID1A	26 (9)	39.0 (33.9–46.3)	33.3 (14.3–NA)	54.6 (47.7–62.5)	48.4 (30.9–75.8)	0.722	14.0 (12.3–18.5)	10.5 (7.2–24.5)	22.1 (16.7–29.3)	9.3 (1.8–48.7)	0.119
RNF43	20 (7)	37.6 (33.2–45.5)	NR	53.1 (46.2–61.0)	61.7 (42.8–88.9)	0.178	13.7 (12.0–17.6)	15.5 (9.8–NA)	21.5 (16.2–28.6)	12.0 (2.4–60.5)	0.708
PTPRT	13 (5)	39.0 (33.3–47.2)	34.8 (21.3–NA)	54.9 (48.2–62.5)	40.0 (17.8–89.6)	0.428	14.0 (12.0–17.9)	5.7 (3.2–NA)	22.1 (16.8–29.1)	NA	***0.019***

*NR: median survival not reached

Table 3.

Univariate and Multivariate Analyses of Association between Genomic Alterations and Pathologic Variables with OS.

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
KRAS Alteration	2.29 (1.00–5.22)	0.049	1.74 (0.75–4.01)	0.2
TP53 Alteration	1.54 (1.03–2.31)	0.037	1.54 (1.02–2.33)	0.042
AJCC Stage (8th Edition)		<0.001		0.002
I–II	Ref		Ref	
III–IV	1.89 (1.33–2.69)		1.78 (1.23–2.57)	
Lymphovascular Invasion	1.73 (1.16–2.59)	0.007	1.96 (1.26–3.04)	0.003
Perineural Invasion	1.33 (0.68–2.63)	0.41		
Tumor Size	1.06 (0.94–1.20)	0.36		
Neoadjuvant therapy	1.96 (1.33–2.87)	<0.001	2.66 (1.77–4.01)	<0.001
Lymph Nodes	1.20 (0.82–1.75)	0.34		

Table 4.

Cumulative Incidence of Site of First Recurrences for *KRAS* and *TP53* Alterations.

	***KRAS* Alteration**		***KRAS* Wildtype**		**p-value**
	1-year	**3-year**	**1-year**	**3-year**	
Distant only	28.8 (23.4–34.5)	42.4 (35.7–48.9)	10.0 (1.6–27.8)	22.0 (6.3–43.0)	0.056
Liver only	23.3 (18.3–28.7)	29.7 (23.9–35.6)	0 (0–0)	5.8 (0.3–24.5)	**0*.013***
Local only	7.2 (4.4–10.8)	21.6 (16.2–27.7)	5.0 (0.3–21.2)	22.3 (6.3–44.3)	0.356
Local + Distant	9.9 (6.6–14.0)	14.2 (10.1–19.0)	10.0 (1.6–27.8)	23.1 (6.4–45.9)	0.669
	***KRAS* G12D**		**Other *KRAS* mutation**		**p-value**
	1-year	**3-year**	**1-year**	**3-year**	
Distant only	37.1 (27.9–46.3)	49.9 (38.7–60.1)	23.2 (16.7–30.4)	37.0 (28.5–45.6)	***0.039***
Liver only	26.7 (18.6–35.4)	32.8 (23.9–42.0)	21.2 (14.9–28.1)	27.7 (20.2–35.6)	0.268
Local only	5.7 (2.3–11.3)	16.0 (8.8–25.0)	8.3 (4.5–13.5)	25.3 (17.6–33.7)	0.089
Local + Distant	8.6 (4.2–14.9)	13.7 (7.8–21.2)	11.0 (6.5–16.7)	14.6 (9.2–21.3)	0.906
	***TP53* Alteration**		***TP53* Wildtype**		***p*-value**
	1-year	**3-year**	**1-year**	**3-year**	
Distant only	28.9 (22.7–35.3)	39.7 (32.3–47.0)	23.8 (14.8–33.9)	43.0 (30.6–54.8)	0.751
Liver only	25.8 (19.9–32.1)	30.7 (24.1–37.5)	10.5 (4.9–18.7)	20.6 (12.1–30.7)	0.084
Local only	8.7 (5.2–13.1)	22.4 (16.1–29.3)	2.7 (0.5–8.4)	19.7 (10.9–30.4)	0.875
Local + Distant	10.7 (6.8–15.5)	14.6 (9.9–20.2)	7.9 (3.2–15.4)	16.1 (8.3–26.2)	0.862
	***TP53* Truncation**		***TP53* Mut, No Truncation**		***p*-value**
	1-year	**3-year**	**1-year**	**3-year**	
Distant only	26.6 (15.4–39.1)	37.5 (22.7–52.2)	29.2 (22.0–36.8)	40.4 (31.6–48.9)	0.505
Liver only	25.8 (18.9–33.1)	31.2 (23.5–39.3)	26.6 (15.4–39.1)	30.5 (17.2–44.9)	0.831
Local only	13.3 (5.8–24.1)	27.3 (15.1–41.0)	7.0 (3.6–12.0)	20.5 (13.4–28.7)	0.173
Local + Distant	19.1 (9.7–30.8)	25.6 (14.3–38.5)	7.7 (4.1–12.8)	10.4 (5.9–16.5)	***0.009***
	***TP53* LOH**		***TP53* Mutation, No LOH**		***p*-value**
	1-year	**3-year**	**1-year**	**3-year**	
Distant only	34.2 (23.7–44.9)	41.8 (29.6–53.5)	24.8 (17.3–32.9)	38.4 (28.8–48.0)	0.257
Liver only	35.5 (24.9–46.3)	40.5 (29.0–51.7)	19.6 (13.0–27.3)	24.5 (16.6–33.1)	0***.017***
Local only	7.9 (3.2–15.4)	15.1 (7.5–25.2)	9.4 (5.0–15.6)	28.1 (19.0–38.0)	0.079
Local + Distant	17.1 (9.6–26.4)	22.2 (13.2–32.7)	6.0 (2.6–11.4)	9.1 (4.6–15.6)	***0.030***
	***KRAS/TP53* Co-mutation**		***KRAS/TP53* Wildtype**		***p*-value**
	1-year	**3-year**	**1-year**	**3-year**	
Distant only	29.8 (23.4–36.3)	41.0 (33.4–48.5)	14.3 (2.1–37.5)	30.4 (8.4–56.3)	0.255
Liver only	26.6 (20.6–33.0)	31.6 (24.8–38.6)	0 (0–0)	8.0 (0.4–32.3)	0.054
Local only	8.4 (5.0–12.9)	21.9 (15.5–28.9)	0 (0–0)	14.3 (2.0–37.8)	0.742
Local + Distant	10.5 (6.6–15.3)	14.5 (9.7–20.2)	7.1 (0.4–28.7)	26.4 (5.1–55.2)	0.976

*Displayed as % CIR (95% CI)