Published in final edited form as:

Clin Cancer Res. 2022 June 13; 28(12): 2704–2714. doi:10.1158/1078-0432.CCR-21-3581.

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All authors: Final approvals

Conflict of Interests:

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Honoraria - Array BioPharma; AstraZeneca; Bayer; Blueprint Medicines; Celgene; Ipsen; Merck; syncore; TriSalus Life Sciences

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Speakers' Bureau - Bayer; Celgene; Incyte; Ipsen; Novartis

Research Funding - Advanced Accelerator Applications (Inst); ASLAN Pharmaceuticals (Inst); Bayer (Inst); boston biomedical (Inst); Caris Life Sciences (Inst); Caris Life Sciences (Inst); Genentech (Inst); halozyme (Inst); Immunomedics (Inst); incyte (Inst); incyte (Inst); Karyopharm Therapeutics (Inst); Lilly (Inst); Merck (Inst); merus (Inst); Momenta Pharmaceuticals (Inst); novartis (Inst); Plexxikon (Inst); QED Therapeutics (Inst); QED Therapeutics (Inst); Regeneron (Inst); Taiho Pharmaceutical (Inst); Taiho Pharmaceutical (Inst); TYME (Inst); tyme (Inst)

Travel, Accommodations, Expenses - Abbvie; celgene; Rafael Pharmaceuticals (OPTIONAL) Uncompensated Relationships - Caris MPI; Rafael Pharmaceuticals

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Patents, Royalties, Other Intellectual Property - I share a patent with several Fox Chase investigators for a novel method to investigate hereditary CRC genes (Inst)

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Research Funding - Novocure

Travel, Accommodations, Expenses - GlaxoSmithKline

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Research Funding - Boehringer Ingelheim (Inst); Boehringer Ingelheim (Inst); Boehringer Ingelheim (Inst); Boehringer Ingelheim (Inst); Caris Centers of Excellence (Inst); Caris Centers of Excellence (Inst); Caris Centers of Excellence (Inst); Caris Centers of Excellence (Inst)

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Molecular characterization of *KRAS* wild type tumors in patients with pancreatic adenocarcinoma

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Research Funding - Alkermes; Astellas Pharma; AstraZeneca; Bayer; Boehringer Ingelheim; Boston Biomedical; Caris Life Sciences; Daiichi Sankyo; Eisai; Esperas Pharma; Esperas Pharma; Exelixis; Five Prime Therapeutics; H3 Biomedicine; Halozyme; Hutchison China Meditech; ImaginAb; Incyte; Inovio Pharmaceuticals; Jiangsu Alphamab Biopharmaceuticals; Lexicon; LSK BioPharma; MSK Pharma; Nouscom; Plexxikon; Repertoire Immune Medicines; Seattle Genetics; Shanghai HaiHe Pharmaceutical; Taiho Pharmaceutical; Telix Pharmaceuticals; Xencor
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Abstract

Purpose: KRAS mutation (MT) is a major oncogenic driver in PDAC. A small subset of PDACs harbor KRAS-wild-type (WT). We aim to characterize the molecular profiles of KRAS-WT PDAC to uncover new pathogenic drivers and offer targeted treatments.

Experimental Design: Tumor tissue obtained from surgical or biopsy material was subjected to next-generation DNA/RNA sequencing, microsatellite-instability (MSI) and mismatch-repair (MMR) status determination.

Results: Of the 2,483 patients (male 53.7%, median-age 66 years) studied, 266 tumors (10.7%) were KRAS-WT. The most frequently mutated gene in KRAS-WT-PDAC was TP53 (44.5%), followed by BRAF (13.0%) . Multiple mutations within the DNA-damage-repair (BRCA2, ATM, BAP1, RAD50, FANCE, PALB2), chromatin-remodeling (ARID1A, PBRM1, ARID2, KMT2D, KMT2C, SMARCA4, SETD2), and cell-cycle-control pathways (CDKN2A, CCND1, CCNE1) were detected frequently. There was no statistically-significant difference in PDL1-expression between KRAS-WT (15.8%) and MT (17%) tumors. However, KRAS-WT-PDAC were more likely to be MSI-high (4.7% vs 0.7%; $p<0.05$), TMB-high (4.5% vs 1%; $p<0.05$), and exhibit increased infiltration of CD8+ T-cells, NK-cells and myeloid dendritic cells. KRAS-WT-PDACs exhibited gene fusions of BRAF (6.6%), FGFR2 (5.2%), ALK (2.6%), RET (1.3%) and NRG1 (1.3%), as well as amplification of FGF3 (3%), ERBB2 (2.2%), FGFR3 (1.8%), NTRK (1.8%) and MET (1.3%). Real-world evidence reveals a survival advantage of KRAS-WT patients in overall cohorts as well as in patients treated with gemcitabine/nab-paclitaxel or 5FU/oxaliplatin.

Conclusions: KRAS-WT PDAC represents 10.7% of PDAC and is enriched with targetable alterations, including immuno-oncologic markers. Identification of KRAS-WT patients in clinical practice may expand therapeutic options in a clinically meaningful manner.

Keywords

Pancreatic adenocarcinoma; *KRAS*; molecular profiling; next generation sequencing

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a challenging disease with overall 5-year survival rate of only 10% (1,2). There is increasing global burden of this disease.

The majority of patients with PDAC harbor metastatic disease, present either on initial presentation or subsequently, emphasizing the need for more effective systemic treatments. Progress in drug therapy of PDAC overall has been very limited especially with the use of targeted agents and immunotherapy (3). Outside of germline BRCA mutations (4), there have been very few actionable alterations in PDAC identified thus far. As a result, the adoption of molecular profiling as part of routine clinical practice in this cancer type has been inconsistent.

KRAS (Kirsten rat sarcoma) mutation, a hallmark of PDAC, is an early event in its natural history and is linked to critical aspects of its biology such as inflammation, immune evasion, and altered metabolism (3–6). Mutated *KRAS* is the major oncogenic driver for PDAC and an attractive treatment target (7,8). To date, no effective treatments have been identified for patients with *KRAS* mutated PDAC except for the very rare *KRAS* G12C mutations (9). However, prior studies in patients with PDAC have identified a subset of patients (10–20%) whose tumors harbor no known *KRAS* mutations at all. These *KRAS* wild type tumors may have biological differences from *KRAS* mutated PDACs and may offer treatment opportunities that are unique to this subset. Advances in molecular techniques including whole genome sequencing provide the opportunity to improve our understanding of the molecular biology of PDAC and particularly in tumors that have no *KRAS* mutations. This may offer a personalized approach in treating patients with PDAC and rationalize the wider use of molecular diagnostics to improve patient outcome. A recent study by Pishvaian (10) et al. reported actionable mutations in 26% of a cohort of 677 patients with PDAC that impacted patient outcome. Their findings demonstrated the potential value matching targeted therapies with molecular profiling in PDAC.

Our hypothesis was that in the absence of *KRAS* activating mutations, other genomic abnormalities that drive carcinogenesis and are medically targetable will be present in *KRAS* wild type PDACs. We report on the molecular profiling of PDAC in 2483 tumor samples. The objective of the study was to perform a detailed molecular characterization of *KRAS* wild type PDAC to better understand the biology of PDAC and to explore therapeutic targets beyond current standard of care that use cytotoxic therapy alone.

Methods

Subjects:

Tumor tissue from patients diagnosed with PDAC was obtained through surgical specimens or tissue obtained by image guided biopsy. Tumors that went through comprehensive molecular profiling testing between 2016 and 2020 at Caris Life Sciences (Phoenix, AZ) were included in the comparative biomarker analyses. In addition, treatment and survival information from insurance data from a large cohort of real-world evidence (RWE) database updated in 2021 were included for survival analysis. Tumors in the biomarker analyses were part of the RWE database.

Next generation sequencing (NGS) of DNA:

NGS was performed on genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples using the NextSeq or NovaSeq platform (Illumina, Inc., San Diego, CA). For NextSeq, a custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA). All variants were detected with > 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of > 500x and an analytic sensitivity of 5%. For NovaSeq, a hybrid pull-down panel of baits designed to enrich for more than 700 clinically relevant genes at high coverage (>500x) and high read-depth was used, along with another panel designed to enrich for an additional >20,000 genes at lower depth (>250x). Prior to molecular testing, tumor enrichment was achieved by harvesting targeted tissue using manual microdissection techniques. Genetic variants identified were interpreted by board-certified molecular geneticists and categorized as ‘pathogenic,’ ‘likely pathogenic,’ ‘variant of unknown significance,’ ‘likely benign,’ or ‘benign,’ according to the American College of Medical Genetics and Genomics (ACMG) standards. When assessing mutation frequencies of individual genes, ‘pathogenic,’ and ‘likely pathogenic’ were counted as mutations while ‘benign’, ‘likely benign’ variants and ‘variants of unknown significance’ were excluded. All variants were detected with greater than 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of greater than 500 and an analytic sensitivity of 5%.

Tumor mutational burden (TMB):

TMB was measured by counting all non-synonymous missense, nonsense, in-frame insertion/deletion and frameshift mutations found per tumor that had not been previously described as germline alterations in dbSNP151, Genome Aggregation Database (gnomAD) databases or benign variants identified by Caris geneticists. A cutoff point of ≥10 mutations per MB was used (11,12).

MSI/MMR status:

A combination of multiple test platforms was used to determine the MSI, or MMR proficiency status of the tumors, including fragment analysis (FA, Promega, Madison, WI), immunohistochemistry (IHC) (MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 anti-body; and PMS2, EPR3947 antibody [Ventana Medical Systems, Inc., Tucson, AZ, USA]) and NGS. The three platforms generated highly concordant results as previously reported (13) and in the rare cases of discordant results, the MSI or MMR status of the tumor was determined in the order of IHC, FA and NGS.

RNA sequencing:

All tumors included in the molecular study were also tested using next-generation RNA sequencing (RNA-Seq). FFPE specimens underwent pathology review to diagnose percent tumor content and tumor size; a minimum of 10% of tumor content in the area for microdissection was required to enable enrichment and extraction of tumor-specific RNA. Gene fusion detection was performed on mRNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina NovaSeq platform (Illumina, Inc., San Diego,

CA) and Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies, Santa Clara, CA). Qiagen RNA FFPE tissue extraction kit was used for extraction to detect fusions and the RNA quality and quantity was determined using the Agilent TapeStation. Biotinylated RNA baits were hybridized to the synthesized and purified cDNA targets and the bait-target complexes were amplified in a post capture PCR reaction. The resultant libraries were quantified, normalized and the pooled libraries were denatured, diluted, and sequenced; the reference genome used was GRCh37/hg19 and analytical validation of this test demonstrated ≥97% Positive Percent Agreement (PPA), ≥99% Negative Percent Agreement (NPA) and ≥99% Overall Percent Agreement (OPA) with a validated comparator method. Transcripts per million molecules were generated using the Salmon expression pipeline for transcription counting.

Tumor microenvironment characterization and MAPK activation evaluation:

MCP-counter was used for quantification of the abundance of stromal cell populations using transcriptomic data (14), while QuantiSeq (15) was used to quantify the immune cell infiltration. MAPK activation was evaluated by MPAS (MAPK Pathway Activity Score) calculation (16).

PD-L1 expression:

IHC was performed on FFPE sections of glass slides. PD-L1 testing was performed using the SP142 anti-PD-L1 antibody (Spring Biosciences). The staining was regarded as positive if its intensity on the tumor cells was ≥2+ and the percentage of positively stained cells was > 5%. On a semiquantitative scale of 0–3: 0 represented no staining, 1+ weak staining, 2+ moderate staining, and 3+ strong staining.

Statistical methods:

The comparison of molecular alterations between *KRAS* wild type and mutant tumors was performed using Chi-square, Fisher's exact or Mann-Whitney test when appropriate. Benjamini-Hochberg method was used to calculate adjusted p values (i.e., q values) and a $q<0.05$ was regarded as statistically significant to reduce false discovery rate in multiple testing; $p<0.05$ but $q>0.05$ was regarded as trending differences. Real-world overall survival (rwOS) information was obtained from insurance claims data and calculated from either tissue collection or first of treatment time to last of contact. Kaplan-Meier estimates were calculated for molecularly defined patient cohorts. In order to explore biomarker differences in cohorts with survivals longer or shorter than the median overall survival, a volcano plot was drawn to display the significance versus fold changes of biomarker alterations and the most significantly different biomarkers were investigated further for their prognostic effects. Significance was determined as p values <0.05.

Data availability statement:

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. The NGS raw data are owned by Caris Life Sciences and cannot be publicly shared due to the data usage agreement signed by Dr. Philip

Philip. Qualified researchers can apply for access to these data by contacting Joanne Xiu (jxiu@carisls.com) and signing a data usage agreement.

Compliance statement:

This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont report, and U.S. Common rule. In keeping with 45 CFR 46.101(b)(4), this study was performed utilizing retrospective, deidentified clinical data. Therefore, this study is considered IRB exempt and no patient consent was necessary from the subjects.

Results

Study Population and *KRAS* status

The study population comprised 2,483 patients, with 53.7% males, and a median age of 66 years (range 22–95 years) (Table 1). Of the total, 266 (10.7%) were *KRAS* WT. PDAC constituted 92.5% of the analyzed samples with a similar *KRAS* WT percentage 10.1%. The study also included the additional histological subtypes including mucinous, adenosquamous carcinomas, and sarcomatoid tumors, etc comprising the other 7.5%. Pancreatic acinar cell carcinomas were particularly enriched in *KRAS* WT (82%). Two of 2 pseudopapillary tumors were *KRAS* WT. Sex or age were not associated with *KRAS* wild type status (11% in men and 10% in women). The median age of *KRAS* WT and MT was 66 and 67 years, respectively. The spectrum of *KRAS* mutations is listed in Supplemental Table 1. The majority of mutations were G12D (43%), G12V (31%) and G12R (14%). The most common *KRAS* alteration was the substitution of Glycine at position 12 by Aspartic Acid (G12D) (962/2234 = 43.1%). Replacement of Glycine at position 12 by Valine (G12V; 30.8 %), Arginine (G12R; 14.2%) complete the top three. The potentially targetable G12C mutation was seen in only 1.9% of patients. Seventeen tumors expressed more than one *KRAS* mutation. Overall, recurring *KRAS* mutations involved exclusively Glycine 12, glutamine 61 and glycine 13. The quasi-totality of mutations (>99%) were single point mutations with negligible numbers of insertions and deletions.

Alterations in *RAS* WT subgroup

Alterations in the 233 *KRAS* WT PDAC were analyzed by whole-exome sequencing, whole-transcriptome sequencing and IHC (Figure 1). The most frequently mutated gene was *TP53* (44.5%). *BRAF*, a downstream effector of *KRAS*, was mutated the second most frequently (13.0%). Unlike melanoma and CRC where V600E mutation constitute the vast majority of *BRAF* mutations, in the *KRAS* WT PDAC group approximately a third (37%) of *BRAF* mutations involved V600 (Class 1), and the *RAS*-independent mutations (Class 2) were most common (43%); RAS-dependent mutations (Class 3) were the least common (17%) (Figure 1C, Supplemental Table 2). Deletion of a 5 AA stretch (N486_P490del) was the most common *RAS*-independent BRAF mutation, while D594N was the most common *RAS*-dependent mutation. Multiple mutations were seen in DNA-damage repair pathway genes (BRCA2: 5.2%, ATM: 4.7%, BAP1: 2.9%, RAD50: 2.3%, FANCE: 2.1%, PALB2: 2.1%), genes involved in chromatin remodeling (ARID1A: 11.6%, PBRM1: 5.6%, ARID2: 3.9%, KMT2D: 3.4%, KMT2C: 3.8%, SMARCA4: 2.2%, SETD2: 1.8%), and cell cycle regulation (CDKN2A: 10.3%, CCND1 amp: 2.6%, CCNE1 amp: 2.3%). *KRAS* WT PDACs

also exhibited copy number amplification of *FGF3* (3%), *ERBB2* (2.2%), *FGFR3* (1.8%), *NTRK* (1.8%), *MET* (1.3%). Taken together, over 10% of *KRAS* WT PDACs exhibited amplifications that may be amenable to known targeted therapies. Consistent with previous reports, we observed targetable fusion events in *KRAS* WT cohorts, the most prevalent included BRAF, FGFR2, ALK and RET (Supplemental Table 3).

Analysis of significant differences in genomic alterations between *KRAS* WT and MT PDAC revealed associations with alterations in key cancer-related genes. When alterations relevant to immune checkpoint inhibitor therapies were analyzed, *KRAS* WT PDAC were likelier to be MSI-high/MMR-deficient (4.7% vs 0.7%; $p<0.05$) and TMB-high (4.5% vs 1%; $p<0.05$) when compared to *KRAS* MT tumors (Figure 2). However, there was no statistically significant difference in PDL1 expression between *KRAS* WT (15.8%) and *KRAS* MT (17%). Canonical tumor suppressor genes well-known to be involved in tumorigenesis and metastasis including *TP53, CDKN2A* and *SMAD4* mutations were more prevalent in *KRAS* MT PDAC, however, the vast majority of alterations that were significantly different between WT and MT were seen to be enriched in the WT tumors, including targetable alterations (e.g., *BRAF, ALK, ROS1, FGFR2, NRG1, MSI-H, IDH1, RET*) (Figure 3). A comprehensive listing of significantly different alterations in *KRAS* MT and WT PDAC tumors is listed in Supplemental Table 4. An oncoprint of the 233 *KRAS* WT PDAC tumors detailing immunotherapy-associated markers, gene fusions, mutations and amplifications is shown in Figure 4. Notably, while genomic fusion events are largely absent in *KRAS* MT tumors, the only exception being a *MET* fusion (seen in 1 case in *KRAS* MT cohort), 21% of WT tumors (50 out of 233) were identified to harbor a gene fusion event, involving BRAF (6.6%), FGFR2 (5.2%), ALK (2.6%), RET (1.3%), NRG1 (1.3%), and RAF1 (1.3%) etc. These gene fusion events and other oncogenic alterations demonstrated mutual exclusivity in the majority of cases. As many of genomic alterations identified in *KRAS* WT PDAC have been associated with activation of MAPK pathway, potentially serving as an alternative oncogenic driver in PDAC in the absence of *KRAS* mutation, we evaluated the MAPK pathway activity using a previously published 10-gene signature, MAPK Pathway Activity Score (MPAS) (16). *KRAS* MT PDAC demonstrated a significant elevation of MAPK activity when compared to the KRAS WT cohort ($p<0.0001$); NF1 mutation, RAF1 fusion, BRAF fusion and mutation all showed higher MPAS when compared to a group of *KRAS* WT tumors without MAPK pathway mutations (Supplemental Figure 1).

RNA deconvolution analysis was utilized to characterize the immune-microenvironment (Quantiseq, (15)). A statistically significant over-representation of myeloid dendritic cells, NK cells and CD8+ T-cells was observed (Figure 5A) in *KRAS* WT PDAs. Conversely, *KRAS* WT PDACs harbored significantly less neutrophils. B-cells were numerically increased in *KRAS* WT PDAC but this difference did not achieve statistical significance when adjusted for multiple comparison. Estimation of the stromal cell population showed a trend for decreased fibroblasts in the WT tumors and increased endothelial cells in *KRAS* WT tumors (14) (Figure 5B). As MSI-H/dMMR causes genomic mutations and production of the neoantigens on the cell surface which may induce infiltrations of different cell lymphocyte populations, we further analyzed the immune cell populations in tumors with confirmed MSS/pMMR status and compared tumors with or without KRAS mutation. All

significant results as shown in Figure 5 hold true in the MSS cohort, suggesting that the observed immune-microenvironment differences are specific to KRAS mutations and not secondary effect to MSI-H/dMMR.

Treatment and outcome analysis

Treatment and survival information were available from a total of 5324 pancreatic cancer patients tested as either *KRAS* WT (N=705) or *KRAS* MT (N=4619) using NextGen sequencing platforms (NextSeq or Novaseq). *KRAS* WT patients had a small but statistically significant prolongation of overall survival (calculated from tissue collection to last day of contact) compared to *KRAS* MT patients (Fig 6A. HR=1.152, p=0.002); the improvement of survival was more prominent when data on only patients with distant metastases profiled were analyzed (Fig 6B. HR=1.259, p<0.0001). In patients treated with 5FU and oxaliplatin (Fig 6C, HR=1.432, p<0.0001) and those treated with gemcitabine and nab-paclitaxel (Fig 6D, HR=1.362, p=0.0003), *KRAS* WT patients had increased overall survival compared with their *KRAS* MT counterpart. Within *KRAS* WT cohort, we explored molecular alterations that are more enriched in patients with longer overall survival by using a volcano plot analysis and observed that *TP53* wild type status is associated with longer survival: patients with *TP53* mutations had significantly decreased overall survival compared to the wild type (Fig 6E HR=1.456, p<0.0001).

Discussion:

Routine molecular profiling of PDAC is currently established to identify germline *BRCA* mutations, and DNA mismatch repair deficiency (10,17). Additionally, certain gene fusions have been described as effective therapeutic targets in PDAC (18–20). However, the utility of comprehensive multigene tumor profiling in patients with pancreatic cancer remains to be established (21). This is largely a result of the low prevalence of actionable mutations in unselected cases as well as the difficulty to obtain adequate tumor tissue, especially in the locally advanced, unresectable setting. Here, we demonstrate enrichment of *KRAS* WT PDAC for therapeutically targetable molecular alterations as suggested by previous reports (22). To our knowledge, this is the largest study investigating the molecular profiles of PDAC with a focus on molecular characteristics of 233 *KRAS* WT PDAC. The entire cohort of tumors were interrogated for a broad spectrum of molecular alterations that include mutations, gene amplifications, fusions, changes in gene expression levels as well as protein expression of selected markers such as PD-L1 and MMR proteins. DNA sequencing data of 592 cancer-related genes (NextSeq) and whole exome sequencing were analyzed for mutations while whole transcriptome sequencing was used for gene expression analysis and sensitive fusion detection (23). We are also the first to report on tumor immune infiltration in KRAS WT vs. KRAS MT PDAC. Additionally, our work represents the largest the largest outcome data for PDAC by KRAS status. *KRAS* WT comprised 10.7% of the study population, and no differences in median age or gender when compared to *KRAS* MT patients was seen. The frequency of the *KRAS* WT was within the previously reported range from smaller cohorts of PDAC tumors (24). Fusion events reported here include therapeutically targetable ALK, ROS1, NRG1, BRAF, FGFR2, RET, RAF1, MET (25).

Our data highlight the distinctly different molecular composition of *KRAS* WT tumors compared with *KRAS* MT PDAC, suggesting potentially different molecular pathogenesis mechanisms and adding to our understanding of inter-individual tumor heterogeneity in this disease. Despite being the most altered gene in *KRAS* WT PDAC, *TP53* is even more frequently mutated in *KRAS* MT tumors. Activating mutations of *BRAF,* a downstream effector of *KRAS* signaling, were found in 13% of the KRAS WT tumors and were mutually exclusive with *KRAS* mutations, a difference that was highly statistically significant (p-value 8.19E-27) when compared with *KRAS* MT tumors. While class 1 *BRAF* mutations, including the frequent V600E mutation can be effectively targeted using *BRAF* inhibitors with or without MEK inhibitors for numerous cancer types, class 2 and class 3 *BRAF* mutants have also become targetable. As reported in the PancSeq study (26), the in-frame BRAF deletions, considered as a class 2 mutant, confer MAPK sensitivity in preclinical models and were treated with MEK inhibitors accordingly with promising clinical activity. Class 3 mutants have been reported to rely on additional MAPK activation signals and would be more effectively targeted in combination with inhibitors of receptor tyrosine kinases. (27–29). Similarly, gene fusions involving *BRAF* were the most prevalent fusions in *KRAS* WT compared to *KRAS* MT (6.6% vs. 0%) with statistical significance ($p<0.001$); these fusions are known to dimerize and present elevated kinase activity, highlighting the importance of the MAPK kinases pathway in PDAC biology. Targeting BRAF fusions clinically with MEK inhibitor or BRAF inhibitors has been reported: A case series of 2 melanoma tumor harboring BRAF fusions treated with trametinib showed a 90% reduction in extracranial metastases in one patient with PPFIBP2-BRAF fusion, and improvement in symptoms with slight disease progression in imaging in another patient with a KIAA1549-BRAF fusion (30). Another study examining BRAF fusions across solid tumors reported a Spitzoid melanoma harboring a ZKSCAN1-BRAF fusion responded to trametinib treatment and the lung metastasis was rendered resectable after the treatment; as well as response of a malignant spindle cell tumor of the chest wall harboring a KIAA1549-BRAF fusion to combination therapy with sorafenib, bevacizumab and temsirolimus (31, 32). In our study, the fraction of *KRAS* WT tumors harboring BRAF alterations was substantially higher than reported in previous studies, possibly reflecting differences in analytical methods and highlight the advantage of fusion detection using RNA-based assays (22). Reliable detection of these alterations is critical from a clinical perspective since they represent meaningful therapeutic target (23).

In agreement with previous reports from our group and others, NRG1 fusion events were seen exclusively in *KRAS* WT PDAC cohort (N=3). Targeting the protein product of these gene fusions using monoclonal antibodies including seribantumab has recently been shown to result in clinical responses in a subset of patients (33). Recently, zenocutuzumab (MCLA-128), a bispecific humanized IgG1 monoclonal antibody, showed an ORR of 40% and DCR of 90% in a cohort of 10 pancreatic cancer patients. All 9 patients who had disease control had a >50% decline in their CA19–9. Duration of response is pending (34). In addition, *FGFR* and *IDH* alterations were exclusively seen in *KRAS* WT disease, potentially representing meaningful therapeutic targets as seen in biliary tract cancers (35,36). So far, FGFR inhibitors have been tested in combination with chemotherapy in

unselected metastatic PDAC populations as with the addition of dovutinib to gemcitabine and capecitabine and BGJ398 to mFOLFIRINOX (37).

Additional targetable alterations (*ALK, RET* and *MET*) were also more prevalent in our *KRAS* WT PDAC cohort. A previously reported case series (18) of 4 patients with PDAC exhibiting ALK-fusions treated with ALK-inhibitors with promising results. In particular, one patient with metastatic PDAC exhibiting and exon 13 EML4–exon 20 ALK translocation treated sequentially with crizotinib, ceritinib and alectinib remained alive for 52 months after diagnosis. Another case report (38) described a patient with locally advanced PDAC with STRN-ALK rearrangement initially resistant mFOLFIRINOX with sustained response to a combination of IMRT and crizotinib that led to an avoidance of surgery. Interestingly, six out of 7 patients with ALK translocations were reported in patients under the age of 50 (39). Preliminary data from the basket phase I/II ARROW trial showed partial responses with 2 patients with pancreatic cancer with RET-fusions treated with pralsetinib with a duration of response of 5.5 months and 7.4 months in each (40). Pralsetinib has been recently approved for thyroid and NSCLC with RET fusions. Of note, RET fusions have been reported in a subset of pancreatic acinar cell carcinomas (41). Additionally, one patient with a novel MET fusion was reported to have a complete response that lasted over 12 months.

Using MPAS, a RNA signature previously reported to evaluate activation of MAPK pathway, we show that numerous alterations seen in the *KRAS* WT PDAC are associated with significant MAPK activation with some reaching MPAS scores comparable to the *KRAS* mutant PDAC cohort (16). While targeting this pathway with an anti-EGFR or anti-IGFR strategy failed in the past when treatment was tested without appropriate selection strategies (42, 43), such strategies should be reconsidered in *KRAS* WT PDAC especially with MAPK activation profile in PDAC cells lacking the *KRAS* function (44).

Findings of this study also suggest that immunotherapy with checkpoint inhibitors that has shown no benefit in unselected PDAC patients thus far (45) may have potential activity in the *KRAS* WT population based on the finding of a higher frequency of MSI-high/MMR-deficient and TMB-high tumors. Treatment of such tumors with pembrolizumab can result in objective responses as demonstrated by a subset analysis of the KEYNOTE-158 study which showed an ORR of 18% in pancreatic tumors, lagging most other MSI-High tumors (46). These findings agree with prior observations of an association between *KRAS* WT and immune response biomarkers (22,47) and recent data on the role of *KRAS* MT on immune evasion in pre-clinical models (48).

Increasing evidence from our data as well as data from others suggest that there are systematic differences between *KRAS* MT and WT tumors at the genomic level that result in altered transcriptomic profiles and are expected to drive differences in clinical behavior of the two disease subtypes. In agreement with this, our real-world evidence confirms prolonged survival of patients with *KRAS* WT tumors which is consistent with previously reported smaller series (49). Molecular features that potentially lead to improved survival include higher frequency of MSI-high/MMR-deficient and associated increased lymphocyte infiltration. In addition, it is conceivable that the slightly prolonged overall survival observed

in the platinum-treated patients compared to gemcitabine-nab-pacltiaxel treated patients may be a result of higher rate of DDR genes mutations, including *RAD50* and *PALB2* in the *KRAS* WT cohort. Our results adds to the current literature which provides inconsistent information on the prognostic or predictive impact of *KRAS* in PDAC (49,50). One weakness of our study is that there was no breakdown of the study population based on the stage of the disease at presentation. However, with the typically advanced nature of PDAC at diagnosis it is unlikely that disease stage will have a significant impact on its molecular profile. This study included tumor tissue from primary or metastatic sites. Given that *KRAS* mutations are very early events in PDAC oncogenesis, it is highly unlikely a difference would be seen if studied separately as was supported recent reports (51,52), despite the recent study potentially challenging the notion (53). To further study activation of oncogenic pathways in *KRAS* WT tumors is an important consideration because genetic models of PDAC are mostly focused on the *KRAS* MT genotype despite a growing evidence of a complex pattern of PDAC evolution based on single-cell sequencing of precursor lesions (54). Another challenge of tissue-based study in pancreatic cancer is the paucity of material obtained through standard diagnostic procedures such as using endoscopic ultrasound. The adoption of liquid biopsies will help address this issue in the future.

In conclusion

In conclusion, patients with PDAC with *KRAS* WT status represent a distinct subgroup who may benefit from comprehensive molecular profiling to improve their treatment outcomes. In this subgroup of PDACs with an estimated incidence of over 5,000 patients per year in the USA, early identification of targetable mutations will optimize treatment planning at a personalized level. Collectively, multigene profiling, including determination of the *KRAS* status as part of initial diagnostic workup, should be considered in the routine management of PDAC (Figure 7). Future studies will focus on identification of predictive transcriptomic signatures in the *KRAS* WT population. This is in particular relevant for a large range of drugs targeting non-oncogene dependencies compared to mutated genes (55).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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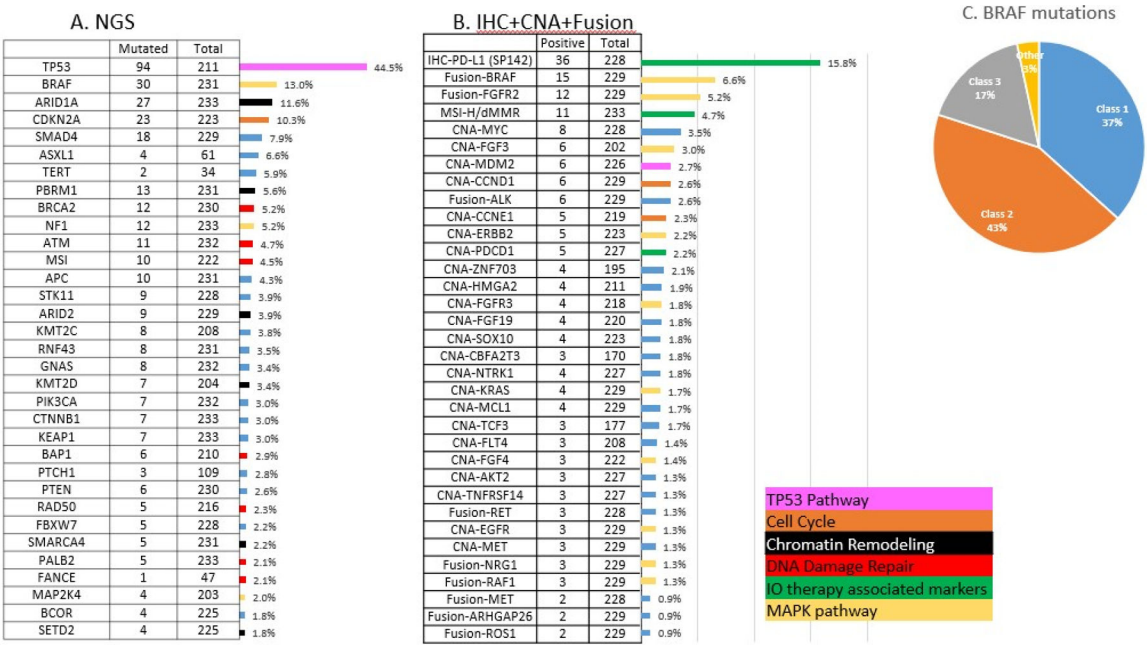
Statement of translational relevance:

Pancreatic Ductal adenocarcinoma (PDAC) carries a dismal prognosis. KRAS mutation is considered a main oncogenic driver in the vast majority of PDACs. Current treatment of metastatic PDAC consists of combination cytotoxic agents. So far, targeted and immunotherapy have failed in PDAC all-comers. In this manuscript, we characterize the molecular profiles of KRAS-wild type (WT) PDACs to uncover molecular drivers that offer targeted treatment opportunities. We show that KRAS WT pancreatic adenocarcinomas represent about 10% of all PDACs and are enriched in alterations that can be targeted with targeted therapies that have been FDA-approved in other organ systems. BRAF mutations and mutations in DNA-damage repair pathway were present. KRAS-WT tumors were also more likely to MSI-high opening up the doors for checkpoint inhibitors. Real-world evidence data showed that KRAS-WT tumors exhibited an overall survival advantage. Our work establishes the importance of incorporating molecular characterization of KRAS-WT tumors into routine clinical practice

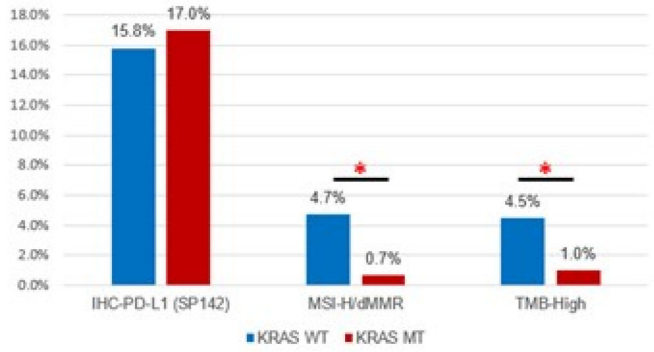


	Mutated	Total	
TP53	94	211	44.5%
BRAF	30	231	13.0%
ARID1A	27	233	11.6%
CDKN2A	23	223	10.3%
SMAD4	18	229	7.9%
ASXL1	4	61	6.6%
TERT	2	34	5.9%
PBRM1	13	231	5.6%
BRCA2	12	230	5.2%
NF1	12	233	5.2%
ATM	11	232	4.7%
MSI	10	222	4.5%
APC	10	231	4.3%
STK11	9	228	3.9%
ARID2	9	229	3.9%
KMT2C	8	208	3.8%
RNF43	8	231	3.5%
GNAS	8	232	3.4%
KMT2D	7	204	3.4%
PIK3CA	7	232	3.0%
CTNNB1	7	233	3.0%
KEAP1	7	233	3.0%
BAP1	6	210	2.9%
PTCH1	3	109	2.8%
PTEN	6	230	2.6%
RAD50	5	216	2.3%
FBXW7	5	228	2.2%
SMARCA4	5	231	2.2%
PALB2	5	233	2.1%
FANCE	1	47	2.1%
MAP2K4	4	203	2.0%
BCOR	4	225	1.8%
SETD2	4	225	1.8%

	Positive	Total	
IHC-PD-L1 (SP142)	36	228	15.8%
Fusion-BRAF	15	229	6.6%
Fusion-FGFR2	12	229	5.2%
MSI-H/dMMR	11	233	4.7%
CNA-MYC	8	228	3.5%
CNA-FGF3	6	202	3.0%
CNA-MDM2	6	226	2.7%
CNA-CCND1	6	229	2.6%
Fusion-ALK	6	229	2.6%
CNA-CCNE1	5	219	2.3%
CNA-ERBB2	5	223	2.2%
CNA-PDCD1	5	227	2.2%
CNA-ZNF703	4	195	2.1%
CNA-HMGA2	4	211	1.9%
CNA-FGFR3	4	218	1.8%
CNA-FGF19	4	220	1.8%
CNA-SOX10	4	223	1.8%
CNA-CBFA2T3	3	170	1.8%
CNA-NTRK1	4	227	1.8%
CNA-KRAS	4	229	1.7%
CNA-MCL1	4	229	1.7%
CNA-TCF3	3	177	1.7%
CNA-FLT4	3	208	1.4%
CNA-FGF4	3	222	1.4%
CNA-AKT2	3	227	1.3%
CNA-TNFRSF14	3	227	1.3%
Fusion-RET	3	228	1.3%
CNA-EGFR	3	229	1.3%
CNA-MET	3	229	1.3%
Fusion-NRG1	3	229	1.3%
Fusion-RAF1	3	229	1.3%
Fusion-MET	2	228	0.9%
Fusion-ARHGAP26	2	229	0.9%
Fusion-ROS1	2	229	0.9%

Figure 1:

Molecular alterations seen in KRAS-WT tumors. 1A: Mutation rates detected by NGS. 1B: Alteration rates detected by immunohistochemistry, copy number amplification rates detected by NGS and fusion rates detected by RNA sequencing. Bars are color coded according to the oncogenic pathways of each biomarker. 1C: BRAF mutations seen in the cohort categorized into class 1, 2 and 3 based on mechanism of action.



	KRAS WT Tumors				KRAS MT Tumors					
	Positive N	Negative N	Total	Percent	Positive N	Negative N	Total	Percent	p values	q values
IHC-PD-L1 (SP142)	36	192	228	15.8%	338	1654	1992	17.0%	0.652474	1
MSI-H/dMMR	11	222	233	4.7%	14	2035	2049	0.7%	1.22E-05	0.001814
TMB-High	10	213	223	4.5%	20	1946	1966	1.0%	0.000472	0.0424

Figure 2:

Comparison of immune checkpoint inhibitor-associated biomarkers in *KRAS* WT and MT tumors. An asterisk indicates a significant difference.

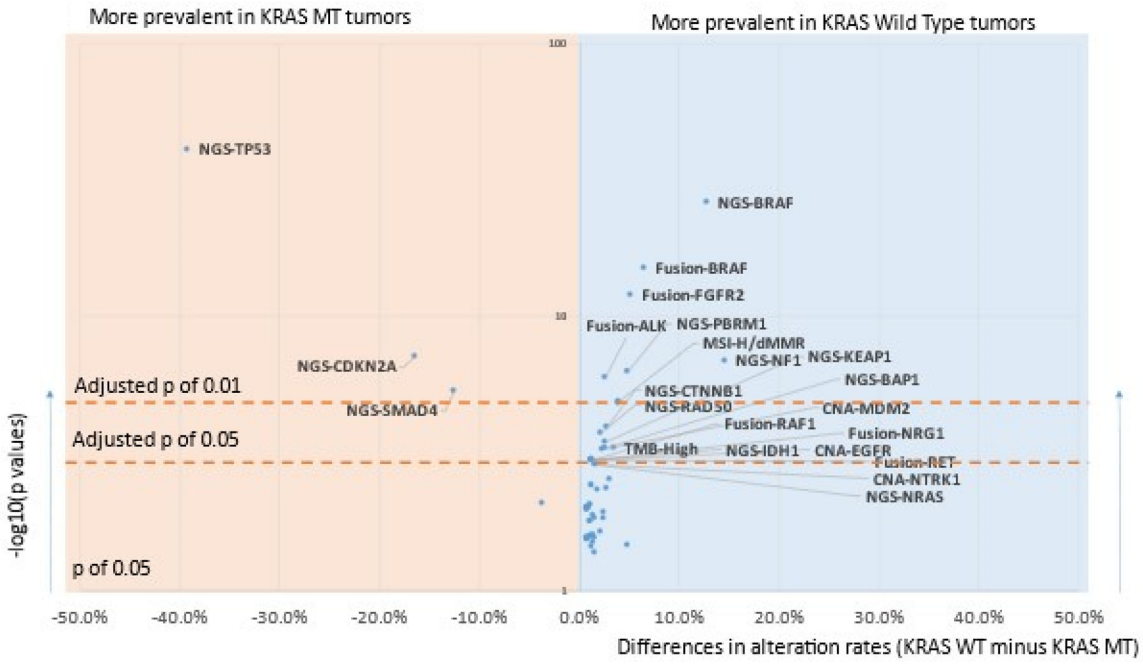


Figure 3:

Volcano plot comparing molecular alterations of *KRAS* MT vs. WT tumors. NGS: Next-Gen Sequencing detected mutations. Only molecular alterations significantly different (adjusted p<0.05) are labeled. Full results can be found in Supplemental table 4.

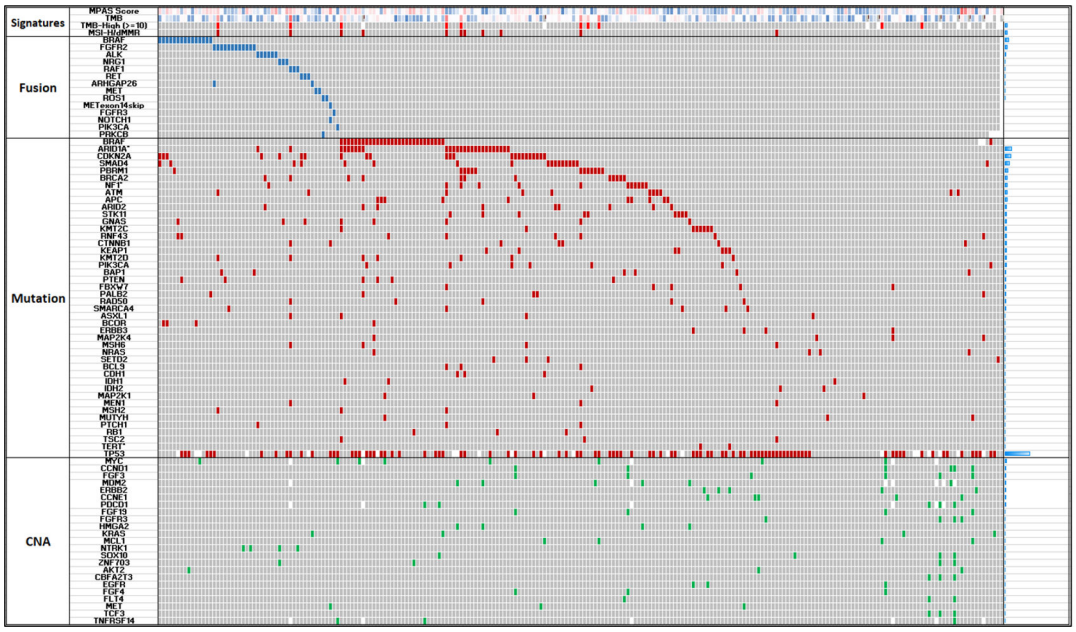


Figure 4:

An oncoprint displaying the molecular alteration patten of the 233 PDAC tumors. Each row represents a biomarker of either fusion, mutation or copy number amplification, as well as genomic signatures such as TMB or MSI/MMR. Red, blue and green represents TMB-H, MSI-high/MMR-deficient or mutations detected using DNA-sequencing; green represents copy number amplification detected by DNA sequencing, while navy blue represents fusions detected by RNA Sequencing. Grey represents no alteration detected while blanks represent unavailable data (indeterminate results due to low coverage or noisy signals). Bars on the right represents the prevalence of molecular alterations of each row.

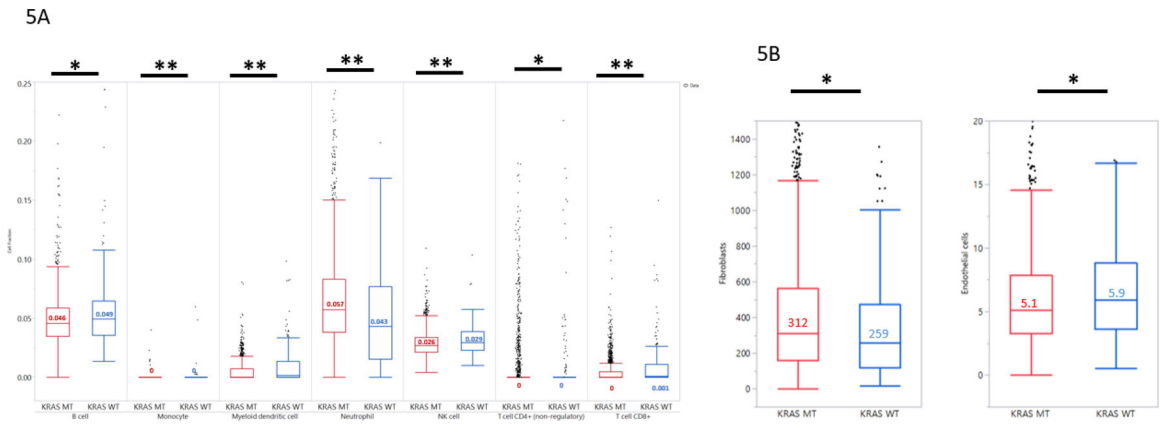


Figure 5:

Comparison of Tumor Microenvironment (TME) characteristics in KRAS MT vs. WT tumors. 5A: Lymphocyte cell fractions estimated by RNA sequencing using Quantiseq.5B: Stromal cell populations estimated by RNA sequencing using MCP counter.**: significantly different after correcting for multiple comparison; * trending differences.

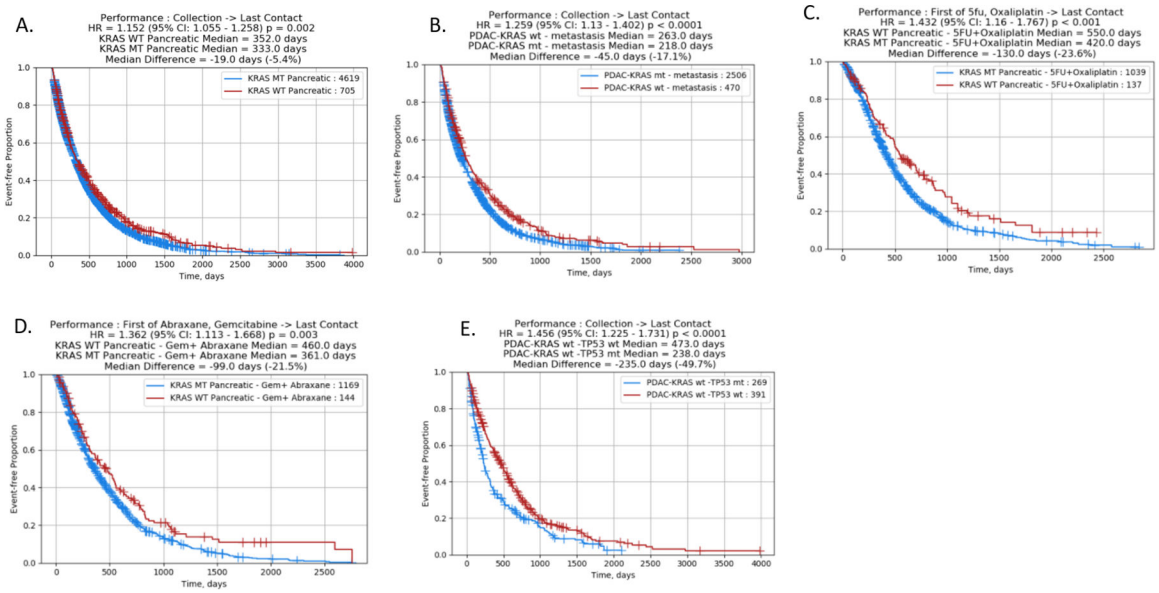


Figure 6:

rwOS in KRAS MT and KRAS WT tumors. 6A: overall survival (calculated from tissue collection to last day of contact) of KRAS WT compared to KRAS MT patients; 6B: overall survival of KRAS MT compared to KRAS MT patients with metastatic tumors; 6C: comparison of survival of KRAS WT patients treated with 5FU and oxaliplatin (calculated from start of treatment to last day of contact) with KRAS MT; 6D: comparison of survival of KRAS WT patients treated with gemcitabine and abraxane with KRAS MT; 6E: overall survival (from tissue collection to last day of contact) of KRAS WT patients with or without TP53 mutation.

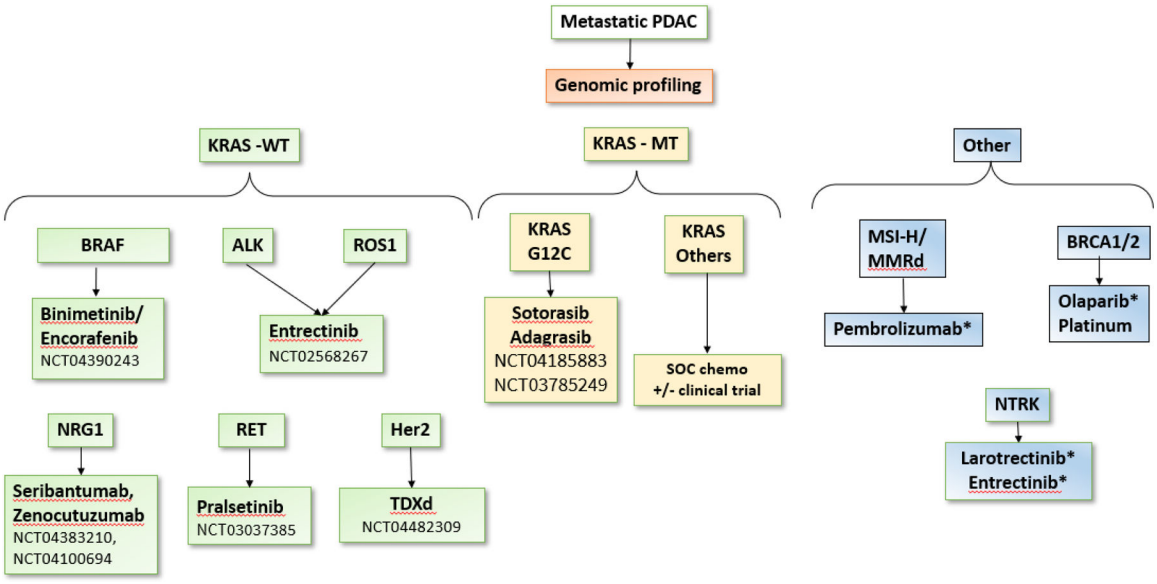


Figure 7:

Genomic profiling of advanced pancreatic adenocarcinoma to determine targetable molecular abnormalities. Drugs with an * indicate FDA-approved agents for treatment in pancreatic adenocarcinoma. KRAS WT tumors are enriched with several targetable mutations when compared to KRAS MT tumors. Currently, olaparib approval by the FDA is limited to treating patients with germline BRCA1/2 mutations only. WT: wild type. MT: mutant. PDAC: pancreatic ductal adenocarcinoma. SOC: standard of care.

Table 1:

Tumor characteristics. 1a: tumor histology of analyzed cohort

Groups	KRAS WT (N)	KRAS WT (%)	KRAS MT (N)	KRAS MT (%)	Total
Adenocarcinoma, NOS	233	10.1%	2064	89.9%	**2297**
Mucinous	16	13.3%	104	86.7%	**120**
Squamous/Adenosquamous	5	11.1%	40	88.9%	**45**
Acinar	9	81.8%	2	18.2%	**11**
Sarcomatoid	1	14.3%	6	85.7%	**7**
Pseudopapillary	2	100.0%		0.0%	**2**
Pleomorphic			1	100.0%	**1**
Total	**266**	**10.7%**	**2217**	**89.3%**	**2483**

Table 1b:

patient gender and age

Gender	KRAS WT (N)	KRAS WT (%)	KRAS MT (N)	KMRAS MT (%)	Total
Female	115	10.0%	1034	90.0%	1149
Male	151	11.3%	1183	88.7%	1334
	KRAS WT		**KRAS MT**		
Age-Median	66		67		
Age Range	33–92		26–95		