Personalized Medicine and Imaging

Clinical Cancer Research

Genomics-Driven Precision Medicine for Advanced Pancreatic Cancer: Early Results from the COMPASS Trial

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Abstract

Purpose: To perform real-time whole genome sequencing (WGS) and RNA sequencing (RNASeq) of advanced pancreatic ductal adenocarcinoma (PDAC) to identify predictive mutational and transcriptional features for better treatment selection.

Experimental Design: Patients with advanced PDAC were prospectively recruited prior to first-line combination chemotherapy. Fresh tumor tissue was acquired by image-guided percutaneous core biopsy for WGS and RNASeq. Laser capture microdissection was performed for all cases. Primary endpoint was feasibility to report WGS results prior to first disease assessment CT scan at 8 weeks. The main secondary endpoint was discovery of patient subsets with predictive mutational and transcriptional signatures.

Results: Sixty-three patients underwent a tumor biopsy between December 2015 and June 2017. WGS and RNASeq were successful in 62 (98%) and 60 (95%), respectively. Genomic results were reported at a median of 35 days (range, 19–52 days) from biopsy, meeting the primary feasibility endpoint. Objective responses to first-line chemotherapy were significantly better in patients with the classical PDAC RNA subtype compared with those with the basal-like subtype ($P = 0.004$). The best progression-free survival was observed in those with classical subtype treated with m-FOLFIRINOX. *GATA6* expression in tumor measured by RNA *in situ* hybridization was found to be a robust surrogate biomarker for differentiating classical and basal-like PDAC subtypes. Potentially actionable genetic alterations were found in 30% of patients.

Conclusions: Prospective genomic profiling of advanced PDAC is feasible, and our early data indicate that chemotherapy response differs among patients with different genomic/transcriptomic subtypes. *Clin Cancer Res; 24(6); 1344–54. ©2017 AACR.*

Introduction

Despite decades of research and exhaustive phase III trials, median survival for patients with advanced pancreatic ductal carcinoma (PDAC) remains less than 12 months (1, 2). Based on Level I evidence (1–3), chemotherapy is the mainstay of treatment and biologic agents, either alone or combined with chemotherapy, currently have no significant impact on survival (3, 4). Without biomarkers for treatment selection, patients with

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Trial Registration: This study is registered at www.clinicaltrials.gov with registration number NCT02750657.

Data Sharing: Genomic data generated within the COMPASS study are being submitted to the European Genome-phenome Archive (EGA) under the accession number EGAS00001002543.

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Translational Relevance

COMPASS is the first prospective translational study that establishes the feasibility of comprehensive real-time genomic analysis of advanced PDAC (metastatic and locally advanced) using whole genome and RNA sequencing with a clinically meaningful turnaround time, and with rigorous clinical annotation. Early results from COMPASS demonstrate that there are unique advanced PDAC genomic and transcriptomic subtypes with molecular heterogeneity between individual cases, and with differing responses to chemotherapy. This is the first prospective evidence that molecular profiling may predict differential response to chemotherapy among advanced PDAC patients with different RNA subtypes. Approximately 30% of patients harbor important germline and somatic genetic aberrations, thus confirming that a significant subset of patients with advanced PDAC will benefit from tailored treatment approaches. Our early results also have implications in designing neoadjuvant, adjuvant, and palliative chemotherapy approaches, considering objective responses to chemotherapy measured by RECIST were mainly observed in patients with "classical" RNA subtype.

advanced PDAC undergo toxic chemotherapy, often with futile results. Thus, better patient stratification is needed to prevent harmful chemotherapy and to develop personalized treatment strategies to improve outcomes. Genomics-driven precision medicine may fulfill this urgent unmet need (5, 6).

Data from resected PDAC genome sequencing studies indicate that PDAC lacks highly actionable simple somatic mutations (7–10). Almost all PDAC harbor a key driver mutation in *KRAS*, and over half have mutations and/or copy number losses of *TP53*, *SMAD4*, and *CDKN2A* (7, 8, 11). Yet none of these changes are directly druggable. However, recent studies have also revealed unique and complex subtypes of early-stage PDAC with potential therapeutic implications (8, 9, 11–14) based on structural genomic aberrations, mutational signatures, and RNA expression profiles. Most of these features appear to be retained in metastases (15, 16), suggesting they are relevant for biomarker development in advanced PDAC.

A major impediment in implementing precision medicine strategies in advanced PDAC is the technical inaccessibility of high cellularity biospecimens in most patients (17). To date, clinically meaningful real-time whole genome sequencing (WGS) and RNA sequencing (RNASeq) to identify predictive mutational signatures and RNA profiles has not been established (17). To overcome this challenge, Comprehensive Molecular Characterization of Advanced Pancreatic Ductal Adenocarcinoma for Better Treatment Selection (COMPASS; a prospective study: NCT02750657) was launched at the Princess Margaret Cancer Centre in December 2015. Here, we report the feasibility and novel early results from this ongoing study.

Materials and Methods

Study population

Eligible patients required a radiologic or histologic diagnosis of locally advanced or metastatic PDAC, an ECOG performance status of 0 or 1, a tumor amenable to percutaneous core needle biopsy, adequate organ function, and expected life expectancy >90 days. Patients were to receive modified FOLFIRINOX (m-FOLFIRINOX) or gemcitabine/nab-paclitaxel (GP) as standard first-line therapy, or investigational agent(s) combined with m-FOLFIRINOX or GP in trial settings. Modified FOLFIRINOX includes 2 weekly intravenous leucovorin 400 mg/m^2, 5-fluorouracil 2,400 mg/m^2 given over 46 hours, irinotecan 150 mg/m^2, and oxaliplatin 85 mg/m^2. GP includes a 4 weekly regime of gemcitabine 1,000 mg/m^2 and nab-paclitaxel 125 mg/m^2 given intravenously at days 1, 8, and 15 of each cycle. Dose modifications were made with the discretion of the treating physician. Patients with a performance status $\geq$2 were excluded, as the likelihood of receiving second line treatment was low. Those with metastatic disease required a tumor lesion measurable by RECIST 1.1 in addition to the lesion being biopsied. Patients were followed by their treating physician as per standard of care. Response to therapy was assessed every 8 weeks using CT or MRI and RECIST 1.1. Patients were also consented for a repeat biopsy at the time of progression if they were well enough and the biopsy was deemed safe. The target lesions for RECIST 1.1 measurement were selected by independent radiologists blinded to molecular profiling data. For patients with metastatic disease, any lesion that meets the criteria for a target lesion as defined by RECIST can be used for assessment, except the one which was biopsied. For patients with locally advanced disease, the primary tumor is used as the target lesion. Patient demographics, treatment details and grade $\geq$3 nonhematologic adverse events (AE) and $\geq$grade 2 peripheral neuropathy related to chemotherapy and all grade AE related to study procedures were prospectively collected using an electronic MEDIDATA database.

Collection of fresh tumor and whole blood samples and genomic analysis by WGS and RNASeq

A minimum of 3 $\times$ 18G good quality cores from primary or metastatic PDAC tumors were obtained by image-guided percutaneous core needle biopsy and an EDTA whole blood sample by venipuncture. Tumor biospecimens were immediately embedded in optimal cutting temperature compound and snap-frozen in liquid nitrogen prior to sectioning. Hematoxylin–eosin stained frozen sections were reviewed by a specialist pathologist and prioritized based on tumor cellularity for (i) WGS, (ii) RNASeq, and (iii) future use (stored frozen). Biospecimens for WGS and RNASeq underwent laser capture microdissection (LCM) for tumor enrichment before nucleic acid extraction, as previously described (15). A separate formalin fixed-paraffin embedded (FFPE) tumor core was used to confirm PDAC in patients with no prior histologic diagnosis. WGS of tumor and germline DNA was performed at the Ontario Institute for Cancer Research (OICR) using established protocols (15, 16). Germline and somatic variant calling, ploidy status determination, neoantigen calling, and classification of PDAC genomic subtypes are described in Online Methods. RNASeq and RNA immune signature analysis were performed at OICR as described elsewhere (15) with additional transcriptomic subtyping analysis at the University of North Carolina, Chapel Hill, North Carolina (detailed in Online Methods). Differentially activated pathways between Moffitt's tumor RNA subtypes, classical and basal-like, were identified by gene set enrichment analysis (GSEA). Genes expressed in more than 90% of samples were selected for GSEA and to make genes comparable, gene expressions were median normalized for each gene. Moffitt tumor RNA subtype was treated

as the phenotype. The analysis was conducted using GSEA-P v3.0 (18, 19) with C2 gene sets in MSigDB v6.1 (18, 20, 21) that includes the Kyoto encyclopedia of genes and genomes (KEGG) pathways (22) and Reactome pathways (23, 24). Normalized enrichment score (NES) were used to identify positively and negatively related pathways.

RNA *in situ* hybridization for *GATA6* detection

Thick (4–5 μm) FFPE tumor tissue sections were deparaffinized in xylene, followed by dehydration in an ethanol series. Tissue sections were then incubated in citrate buffer (10 nmol/L, pH 6) maintained at boiling temperature (100 to 103°C) using a hot plate for 15 minutes, rinsed in deionized water, and treated with 10 μg/mL protease (Sigma-Aldrich) at 40°C for 30 minutes in a HybEZ hybridization oven (Advanced Cell Diagnostics). The probe targeting *GATA6* was designed and synthesized by Advanced Cell Diagnostics. Further steps were performed using the RNAscope® 2.5 High Definition (HD)-RED Assay according to the manufacturer's instructions (https://acdbio.com/technical-support/user-manuals). *GATA6* RNA signal in the tumor cells was scored using a semiquantitative method based on the following criteria: score 0, absent to rare discernable dots under 40× objective lens; score 1, few discernable dots at 20×; score 2, dots (4–9/cell) resolved at 10×; score 3, individual dots (more than 10 dots/cell) or clusters resolved at 5×. Samples containing less than a hundred tumor cells were excluded from analysis.

Molecular tumor board

COMPASS results were reviewed regularly at a monthly multidisciplinary molecular tumor board comprising oncologists, pathologists, genome scientists, a genetic counselor, and core COMPASS research personnel. All putative deleterious germline mutations were verified in a CLIA [College of American Pathologists (CAP)-Certified Laboratory Improvements Amendment] certified laboratory and actionable somatic aberrations validated by orthogonal methodology as indicated.

Endpoints, sample size, and statistical considerations

The study meets its primary endpoint if 80% of the first 50 patients biopsied have robust WGS results (tumor cellularity >20%, tumor sequencing depth >45×, and normal DNA sequencing depth >30×) reported within 8 weeks of baseline tumor biopsy. If the primary endpoint is met, at least 200 patients will be recruited over 3 to 4 years. The secondary endpoints are: percentage of patients with distinct genomic features that inform tailored therapy choices; percentage of patients who receive matched targeted second line therapy; progression-free survival (PFS) and overall survival (OS). PFS is defined as the time from the first dose of chemotherapy to the first date where disease progression or death occurs. OS is defined as the time from the date of tumor biopsy to death for any reason. Data are presented using descriptive statistics and statistical tests used as appropriate. Survival curves were estimated using the Kaplan–Meier method

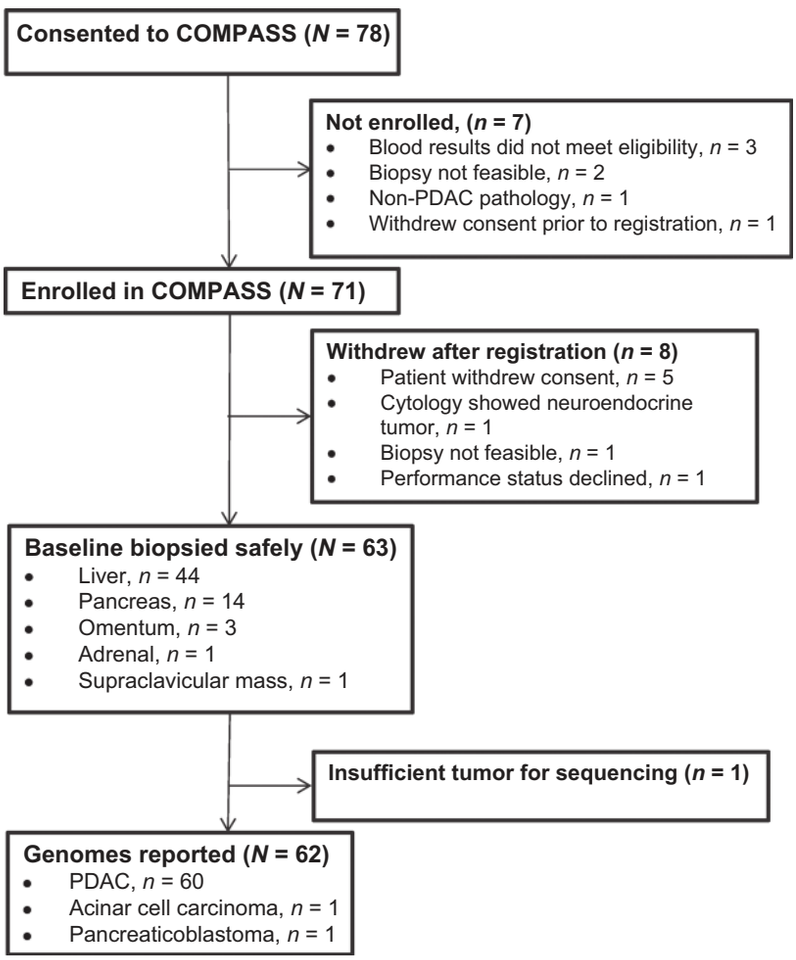

Figure 1.
COMPASS Consort Diagram.

and hazard ratios were calculated using Cox proportional hazard regressions with *P*-values calculated using the Wald statistic. All tests were two-sided. Statistical significance was set at $P = 0.05$. All the analyses were conducted in R (Vienna, Austria).

Results

Patients

Between December 17, 2015, and June 15, 2017, 71 patients were enrolled and 63 underwent a baseline biopsy (44 liver, 14 pancreas, 3 omentum, 1 adrenal, and 1 supraclavicular mass; Fig. 1) at a median of 5 days (range 0–13 days) from consent, without delay in planned treatment. Sixty-one patients had PDAC, one acinar cell carcinoma, and one pancreaticoblastoma (Table 1). Median age was 62 years (range 27–74 years) and the majority (87%) had metastatic disease (Table 1). One PDAC patient did not have sufficient tumor material for genome sequencing and another two had insufficient material for RNASeq. Thus, WGS and RNASeq results were available for 62 and 60 patients, respectively.

Treatments

Of 61 PDAC patients (60 with sequencing data and one without), four did not start planned therapy due to rapid deterioration from disease progression ($N = 3$) and death due to a stroke ($N = 1$). Fifty-seven had at least one cycle of m-FOLFIRINOX ($N = 41$) or GP ($N = 16$). Five patients came off treatment before radiologic assessment at 8 weeks with clinical progression. Two were not radiologically evaluable. Therefore, 50 PDAC patients (37 m-FOLFIRINOX and 13 GP) were chemotherapy response evaluable. The patient with acinar cell carcinoma was treated with m-FOLFIRINOX and considered response evaluable. The pancreaticoblastoma case was treated with cisplatin/doxorubicin and excluded from response analysis. For patients who had at least one cycle of m-FOLFIRINOX ($N = 41$), ≥grade 3 nonhematologic toxicities were reported in three patients (one grade 3 diarrhea; one grade 4 neutropenic sepsis; one grade 3 pain at chemotherapy administration site) and one grade 2 peripheral sensory neuropathy related to treatment was also reported. In patients who had at least one cycle of GP, ≥grade 3 nonhematologic toxicities related to treatment were reported in two patients (one grade 3 diarrhea; one grade 3 fatigue).

Table 1. Patient characteristics

Characteristics	Patients, *n* (%)
Number of patients biopsied	63
Age (years)	
Median	62
Range	26–74
Gender	
Male	33 (52)
Female	30 (48)
Race	
White	44 (70)
Asian	18 (29)
Black or African American	1 (1)
Stage of the disease	
III (locally advanced)	8 (13)
IV	55 (87)
Histology	
Adenocarcinoma	57 (90)
Adenosquamous	3 (5)
Acinar cell carcinoma	1 (2)
Colloid carcinoma	1 (2)
Pancreaticoblastoma	1 (2)

Feasibility of fresh tumor acquisition and WGS

Adequate tumor material for WGS was obtained from biopsies of 62 of 63 patients (98%); in one case, the liver biopsy was devoid of tumor cells. One patient (<2%) experienced a biopsy-related serious AE; pancreatitis requiring hospital admission for supportive management that resolved without sequelae. On average, we obtained five tumor cores per patient (range 3–11). Only 20 (32%) samples demonstrated high (>70%) tumor cellularity while 19 (30%) had low (≤35%) tumor cellularity. LCM dramatically increased tumor cellularity (median: 79%; range 37–93%), and enabled high-resolution genomic analysis in all cases. Performance targets for tumor and normal DNA WGS were achieved in all 62 cases analyzed and WGS results reported at a median of 35 days (range 19–52 days) from biopsy (Supplementary Fig. S1), meeting the primary feasibility endpoint.

Genomic subtypes and treatment response

Unstable subtype. We identified three tumors harboring >200 structural variants, classified as "unstable" according to Waddell and colleagues (8). Two of those showed a novel "duplicator" mutational phenotype characterized by high numbers of structural duplication variants of sizes ranging between 10k-1mbp. One "duplicator" case (COMP-0019) achieved partial response (PR) to m-FOLFIRINOX sustained for 15 months at the time of this report. The second "duplicator" case (COMP-0043) achieved stable disease for 6 months after m-FOLFIRINOX with tumor shrinkage of 20% as the best tumor response. The third unstable tumor (COMP-0047) harbored an activating *BRAF* mutation with numerous chromosomal translocations and had a deep PR to m-FOLFIRINOX at 16 weeks and is currently on a treatment break.

Tumors with BRCA mutations and double-strand break repair deficiency. Two patients carried the same pathogenic *BRCA2* p.1982fs germline mutation, one (COMP-0037) with somatic loss of heterozygosity (LOH) of the wild-type allele and one (COMP-0057) without a "second-hit" (no LOH or somatic mutation) (Supplementary Fig. S2). Hallmarks of DSBR deficiency were observed in COMP-0037 but not in COMP-0057. COMP-0057 had two previous diagnoses of breast cancer: one triple negative invasive ductal carcinoma and one hormone receptor positive lobular carcinoma in the contralateral breast, 13 and 8 years prior to the diagnosis of PDAC, respectively. We performed targeted sequencing of archival material from the breast tumors and found that there was *BRCA2* LOH in both tumors. In contrast, the PDAC had no *BRCA2* LOH, it was *KRAS* wild type, and harbored an activating *PIK3CA* mutation (Supplementary Fig. S3 and Table S1). COMP-0037 had a PR to m-FOLFIRINOX that is now sustained for 10 months and ongoing, whereas COMP-0057 achieved stable disease (SD) as best tumor response (5.6% shrinkage) after 4 months of therapy. Interestingly, another patient (COMP-0068), who was treated with a GP based regimen and achieved SD with minor shrinkage, had a *BRCA* wild-type tumor that displayed hallmarks of DSBR deficiency. No germline or somatic mutations in genes encoding the homologous recombination (HR) pathway were found in this latter patient.

Transcriptomic subtypes and chemotherapy response

Similar to other recent studies including that of the Cancer Genome Atlas Research Network,[11] we reproduced the most robust PDAC subtypes, the basal-like and classical RNA subtypes

by Moffitt and colleagues (refs. 11, 13; Fig. 2; Supplementary Fig. S4). Of 50 chemotherapy response evaluable patients (49 PDAC + 1 acinar cell carcinoma) with RNASeq data, 12 (24%) had basal-like tumors and 38 (76%) had classical tumors. PR was observed in only one basal-like (1/12; 8%) and in 13 classical cases (13/38; 34%; $P = 0.0002$, Fisher's exact test; Fig. 3A). The mean percent change in tumor size with chemotherapy was +17% and −19.5% in basal-like and classical tumors, respectively ($P = 0.004$, Welch two sample t test). The median duration of treatment on first line therapy was 1.5 months (range 1–6.5 months) for basal-like cases and 4.4 months (range 0.5–18 months) for classical cases (Fig. 3B).

All locally advanced cases ($N = 8$; 13%) displayed a classical RNA profile (Supplementary Fig. S4). The more aggressive basal-like RNA subtype (13) was observed exclusively in metastatic PDAC, suggesting that basal-like subtype tumors may present at more advanced stages. Consistent with previous studies in resected PDAC (9, 12, 13, 25), tumors with the basal-like subtype had significantly lower levels of *GATA6* expression compared to classical subtype tumors (Supplementary Fig. S5). Although our *GATA6* expression data were derived from RNASeq results of enriched tumor samples with very low contaminating signals from normal tissues (to exclude false-positive signals from tumor infiltrating immune and stromal cells), *GATA6* expression was explored further in a subset of 39 tumors using an RNA *in situ* hybridization approach (RNAscope ISH). This analysis showed that 28 of 29 classical subtype patients had high *GATA6* signals (scores 2 and 3), compared to 0/10 basal subtype patients ($P < 0.001$, Fisher's exact test; Fig. 4). There was also strong correlation between *GATA6* expression measured by RNASeq and RNA *in situ* hybridization (Supplementary Fig. S6). Our data strongly support that *GATA6* expression in tumor epithelium, measured by RNA in situ hybridization, is a robust surrogate biomarker for differentiating classical and basal-like PDAC subtypes (13). We also found that low *GATA6* expression was associated with high tumor grade ($P = 0.04$, Fisher's exact test). *GATA6* expression scores of 39 tumors measured by RNA *in situ* hybridization are shown in Supplementary Table S2, together with Moffitt RNA subtype, histologic type, and histologic tumor grade data.

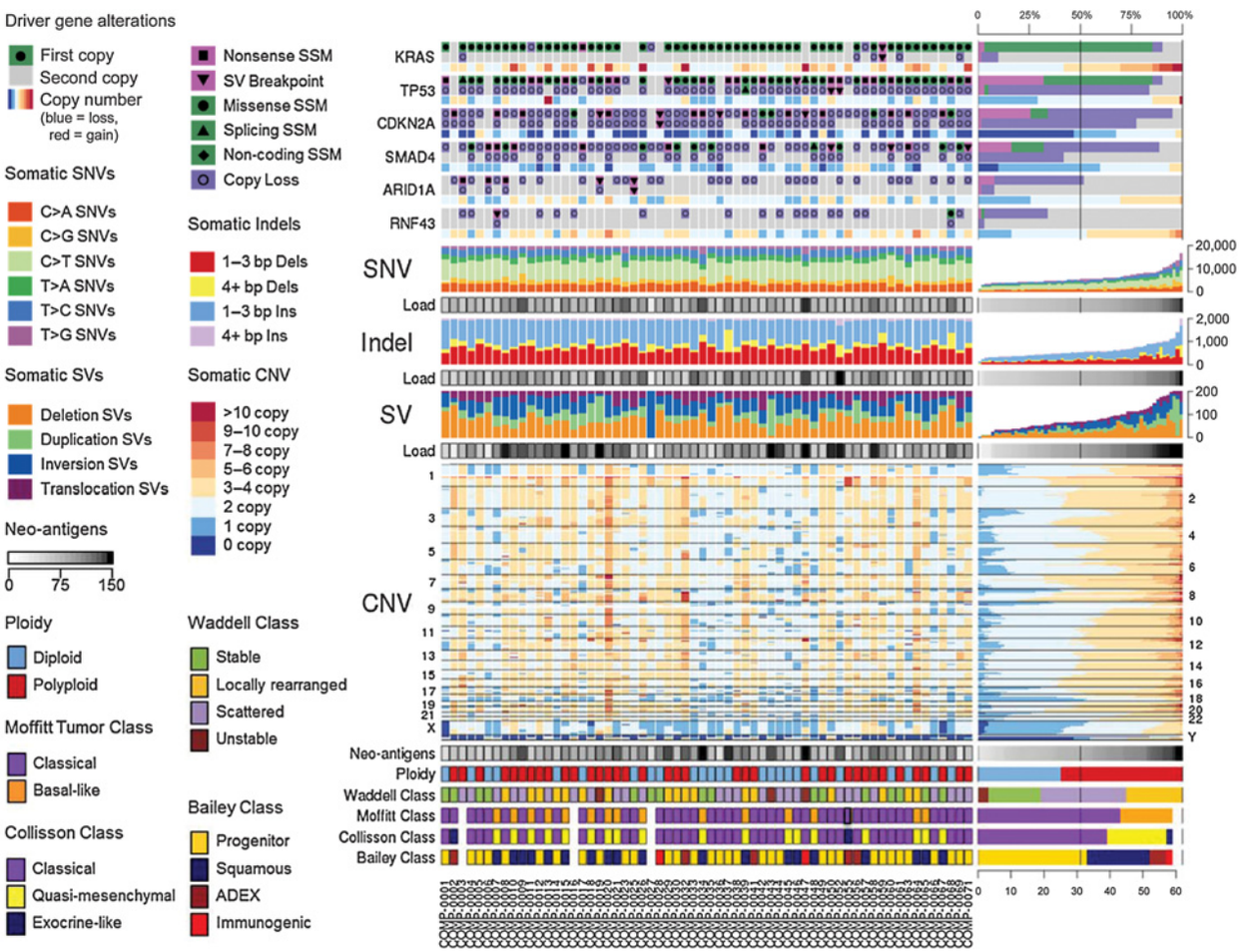

Figure 2.

Summary of WGS and RNASeq results. Oncoprint showing sequencing results from 62 tumors as columns, with summary plots on the right. The top section shows the mutation and copy number status of six PDAC driver genes. Next, the somatic SNV, indel, and SV load and type distribution are displayed. CNVs are shown for each chromosome, followed by neo-antigen load and ploidy status. Finally, Waddell, Moffitt, Collisson, and Bailey classifications are presented for each sample.

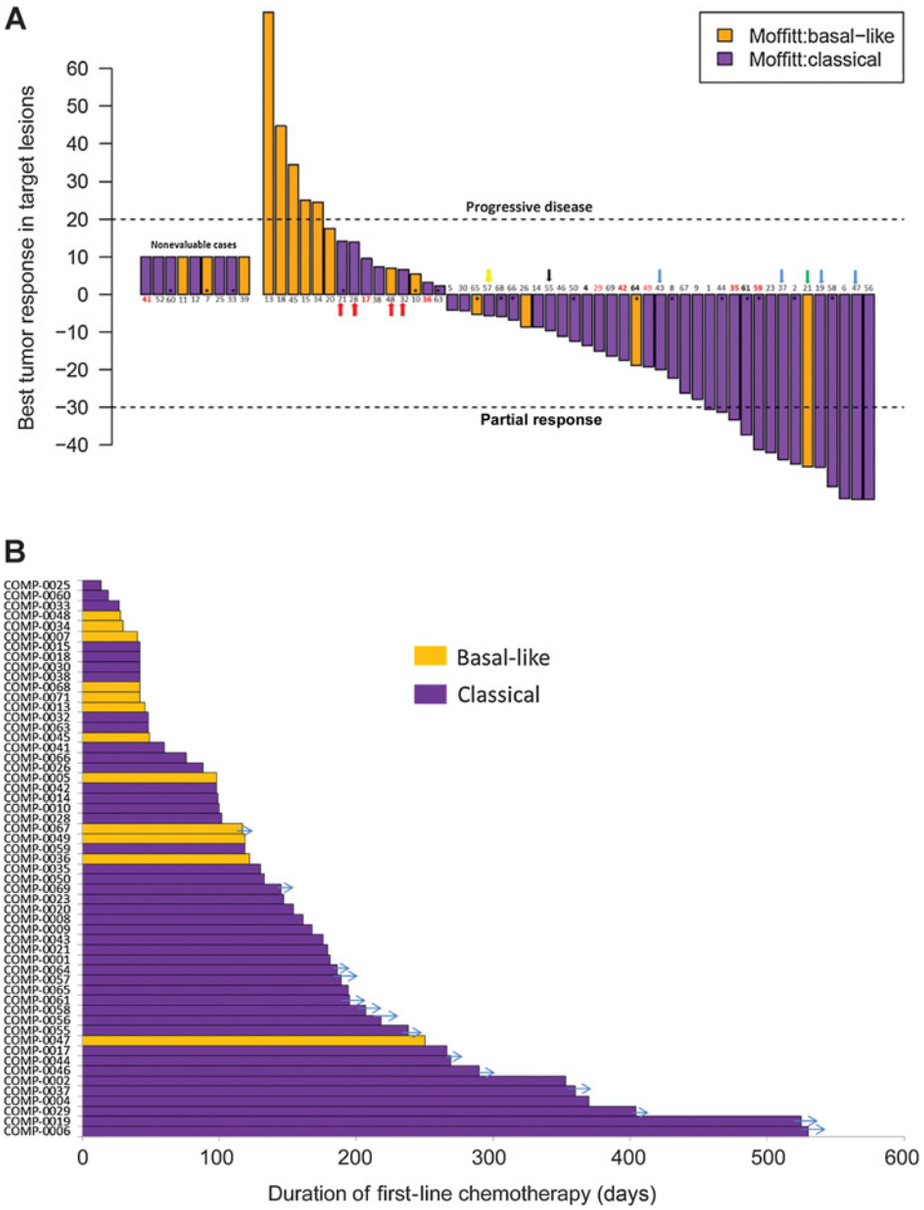

Figure 3.

A, RNA subtypes and chemotherapy response. The best percent change in size of tumor target lesions from baseline (before starting chemotherapy) measured by RECIST 1.1 for 49 chemotherapy response evaluable patients (48 PDAC + 1 acinar cell carcinoma) with RNASeq data are shown. Patients marked with * were treated with gemcitabine/nab-paclitaxel (GP)-based therapy, and the rest were treated with modified FOLFIRINOX (m-FOLFIRINOX). The cases numbered in red were locally advanced disease, the rest had metastatic disease. Four patients highlighted by red arrows had progressive disease by RECIST 1.1 due to unequivocal progression in nontarget lesions despite <20% growth in target lesions measured. The cases highlighted with blue arrows were COMP-0019 and COMP-0043 with duplication signature, COMP-0037 with *BRCA2* germline mutation with somatic LOH, and COMP-0047 with unstable Waddell subtype and a *BRAF* mutation. These patients all had classical subtype tumors and responded (3 PR+ 1 SD) to m-FOLFIRINOX chemotherapy. COMP-0021, highlighted with a green arrow, who achieved partial response to m-FOLFIRINOX, had basal-like RNA subtype and high neo-antigen load. COMP-0057, highlighted with yellow arrow, harbored *BRCA2* germline mutation with no second hit (somatic LOH or mutation). COMP-0055 (black arrow) had acinar cell carcinoma.

B, Duration of treatment on first-line therapy for COMPASS patients with RNASeq data who had at least one cycle of chemotherapy ($N = 55$). The median duration of treatment on first-line therapy in basal-like cases was 1.5 months (range, 1–6 months) and that in classical cases was 4 months (range, 0.5–18 months). The arrow indicates that patient was still on first-line chemotherapy.

RNA pathway analysis using GSEA showed that, in the basal-like subtype, hypoxia and metastasis pathways are upregulated whereas TGFβ receptor signaling and luminal versus basal pathways are downregulated (Supplementary Fig. S7A). In the classical subtype, hepatocyte nuclear factor 4alpha (HNF4A) targets, luminal versus basal, and sensitivity to cisplatin pathways are upregulated (Supplementary Fig. S7B).

PFS and OS

All patients with WGS data ($N = 61$, 60 PDAC + 1 acinar cell carcinoma), with the exception of the pancreaticoblastoma case, were included in survival analysis. At the data cutoff date of November 28, 2017, with a median follow up of 13.9 months, 45 (74%) had disease progression and 36 (59%) had died. Median PFS was 5.8 months (95% CI, 3.6–6.5) and median OS was 8.4 months (95% CI, 7.5–11.6). RNA subtype data were available for 59 patients. Median PFS for patients with classical and basal-like subtype were 6.4 months (95% CI, 5.0–11.0) and 2.3 months (95% CI, 1.8–6.0), respectively (HR 0.28 [0.14–0.57], $P < 0.001$) (Fig. 5A) and median OS was 10.4 months (95% CI, 8.3–not reached) for classical cases and 6.3 months (95% CI, 4.0–not reached) for basal-like cases (HR 0.33 [95% CI, 0.15–0.7], $P = 0.004$; Fig. 5B). For patients with metastatic disease who had at least one cycle of chemotherapy ($N = 51$), median PFS was 5.3 months (95% CI, 3.6–6.5) and median OS was 7.8 months (95% CI, 6.8–10.3). Of those, RNA subtype data were available for 49 patients (two did not have RNA for analysis). PFS for metastatic classical cases who had at least one cycle of chemotherapy ($N = 35$) was 6.2 months (95% CI, 4.1–9.9), and 2.3 months (95% CI, 2.1–6.1) for metastatic basal-like cases who had at least one cycle

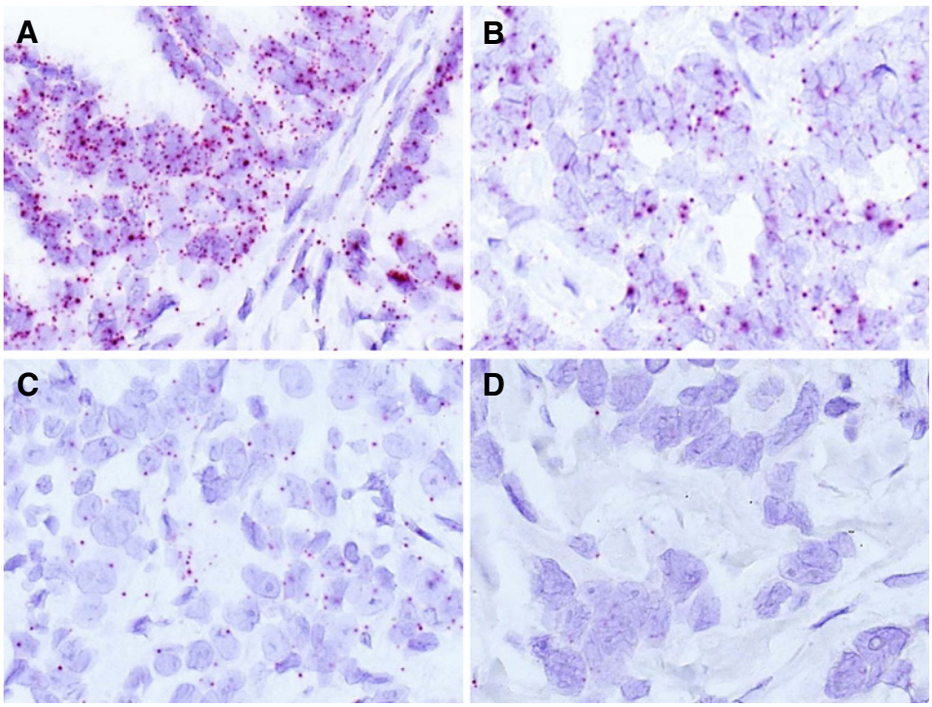

Figure 4.
Detection of *GATA6* by RNA *in situ* hybridization assay (RNAScope) in metastatic PDAC (×400). Most tumor cells depicted in **A** (COMPASS 0042) and **B** (COMPASS 0046) display numerous red punctate signals, scores 3 and 2, respectively. In **C** (COMPASS 0026), many tumor cells contain only one to two dot-like signals each (score 1). Very few dots in rare tumor cells (score 0) are shown in **D** (COMPASS 0039). Scores 2 and 3 were typically seen in tumors with classical subtype Moffitt signature, whereas scores 1 and 0 were uniformly present in the basal-like subtype tumors.

of chemotherapy ($N = 14$; HR 0.31 [95% CI, 0.15–0.65], $P = 0.002$; Fig. 5C). OS for metastatic classical cases who had at least one cycle of chemotherapy was 10.0 months (95% CI, 7.5–14.9) and 6.3 months (95% CI, 4.4–not reached) for metastatic basal-like cases who had at least one cycle of chemotherapy (HR 0.39; 95% CI, 0.18–0.86; $P = 0.02$; Fig. 5D). Subgroup analysis of PFS for those who had at least one cycle of chemotherapy ($N = 55$) showed that those with the classical RNA subtype treated with m-FOLFIRINOX achieved the best PFS (8.5 months; 95% CI, 6.5–not reached) and worst PFS was observed for those with the basal-like subtype treated with m-FOLFIRINOX (2.7 months; 95% CI, 2.1–not reached).

Actionable mutations, ploidy status, and neo-antigens

As expected, the most common recurrent genetic alterations detected were in *KRAS* (85%), *TP53* (85%), *CDKN2A* (75%), and *SMAD4* (43%; Supplementary Table S1). Two patients had a pathogenic germline *BRCA2* mutation as described above, and another patient had a putative pathogenic splice-site germline *ATM* mutation (Supplementary Table S1). Twenty potentially actionable somatic mutations were found in 18 (30%) patients involving *ARID1A* ($N = 5$; 8%), *BRAF* ($N = 1$; 2%), *CDK4/6* ($N = 4$; 7%), *PIK3CA* ($N = 4$; 7%), *PTEN* ($N = 3$; 5%), and *RNF43* ($N = 2$; 3%; Supplementary Table S1). The activating *BRAF* mutation was found in a *KRAS* wild-type tumor, caused by a three base pair deletion spanning both the V600 and K601 positions, resulting in glutamic acid (E) as the 600th residue and codon 601 loss (p. V600_K601delinsE). Highly amplified *CDK4* was found in two patients (32 and 18 copies) and *CDK6* in two patients (24 and 11 copies). The two highest amplifications were confirmed by fluorescence *in situ* hybridization (Supplementary Fig. S8). Sixty percent (31/52) of metastatic PDAC had polyploid genomes whereas 50% (4/8) of locally advanced PDAC in this study and 40% (79/197) of resected PDAC primaries we studied previously were polyploid (15, 16). The difference in frequency of polyploidy between metastatic tumors and resected primaries is statistically significant ($P = 0.04$; Fisher's exact test). A broad range of neo-antigen loads (median 45, range 15–218) was observed across the cohort with elevated antitumor cytolytic activity in ~30% cases. Interestingly, the only patient with a basal-like tumor who responded to m-FOLFIRINOX (COMP-0021; Fig. 3) had both a high neo-antigen load and high expression of antitumor immune transcripts. COMP-0021 displayed a high SNV load and an APOBEC mutational signature in both the baseline and progression biopsy, as described next.

Progression biopsies

In five patients, genomic results from paired baseline and progression tumor biopsies were available for comparison. Of those, four patients were treated with m-FOLFIRINOX and one with GP. Three patients (two treated with m-FOLFIRINOX and one treated with GP) had PD as best response after 2 months of chemotherapy and one patient treated with m-FOLFIRINOX achieved SD after 2 months but progressed after 4 months of treatment. In these four patients, comparison of baseline and progression biopsy genomic results did not reveal significant changes in genomic features, including the RNA subtype. Very few deleterious variants were private to either the original or progression biopsy, and none were in genes of interest, which suggests that the tumors did not gain oncogenic variants that would indicate acquired chemotherapy resistance. It is likely that these tumors were chemotherapy resistant at the outset, and continued to accumulate random mutations during treatment. One patient (COMP-0021) treated with m-FOLFIRINOX achieved PR after 4 months of therapy but experienced disease progression 2 months later. In this patient, the baseline biopsy

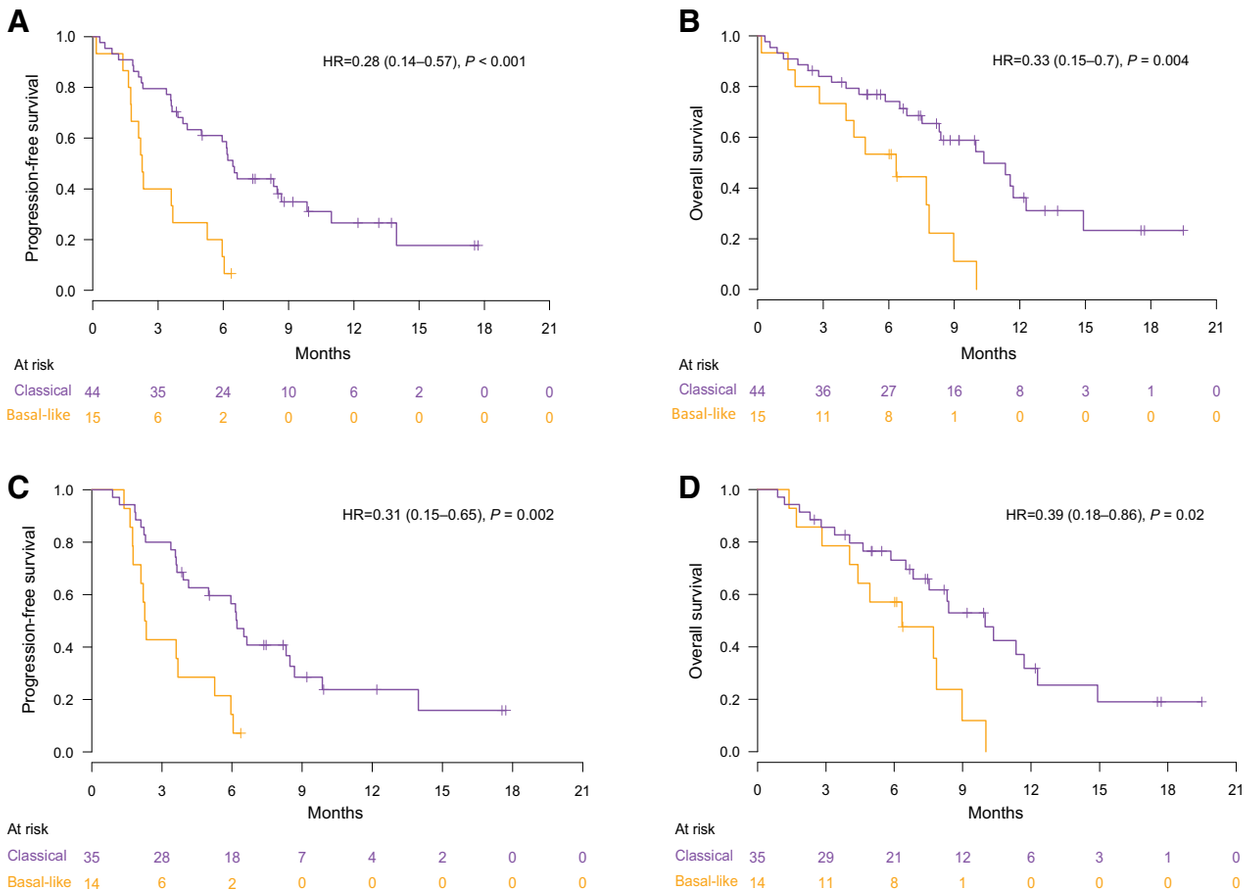

Figure 5.

PFS and OS. Hazard ratios (HR) are shown with 95% confidence intervals and *P* values. **A,** PFS of patients with advanced PDAC with Moffitt tumor classical and basal-like RNA subtypes ($N = 59$). **B,** Overall survival of patients with advanced PDAC with classical and basal-like subtypes ($N = 59$). **C,** PFS of patients with metastatic PDAC with classical and basal-like subtypes who had at least one cycle of chemotherapy ($N = 49$). **D,** OS of patients with metastatic PDAC with classical and basal-like subtypes who had at least one cycle of chemotherapy ($N = 49$).

harbored 9,406 SNVs, of which ∼42% were attributable to APOBEC signatures, whereas the progression biopsy showed 15,840 SNVs (∼56% APOBEC signature). Nearly all of the variants in the baseline biopsy were present in the progression biopsy (∼96%) and, as above, the new variants did not affect genes obviously related to resistance. Further work is necessary to elucidate chemotherapy resistance mechanisms using progression biopsies.

Second-line therapy

Thirty-five of 50 chemotherapy response evaluable patients to date have progressed on first-line chemotherapy. Of those, 19/35 (54%) received second line therapy: 7 gemcitabine, 2 GP, and 10 biologic agents. In 5 of 35 (14%) patients, the choice of second-line therapy was based on COMPASS results: one rapid progressor on m-FOLFIRINOX with a basal-like tumor and copy number amplification of a *KRAS* activating mutation achieved clinical stability for 5 months with combined target inhibition of RAS effector pathways, but 2 patients with *CDK4/6* amplified tumors treated with palbociclib, one patient with high neo-antigen load treated with a PD-L1 inhibitor, and one patient with a polyploid genome treated with a PLK4 (polo like kinase 4) inhibitor on trial did not respond to therapy.

Discussion

As genomics-driven precision medicine expands beyond actionable simple somatic mutations, comprehensive tumor sequencing to identify structural, copy number, and expression biomarkers is becoming increasingly relevant to guide therapy. COMPASS is the first prospective trial that takes this approach in advanced PDAC, in a time-sensitive manner, and with rigorous clinical annotation. We successfully profiled over 95% of cases biopsied by using LCM enrichment to clearly reveal tumor-derived mutational/transcriptomic signatures. We establish that this strategy can be safely integrated into current standards of care with rapid turnaround time.

A promising advance in deciphering PDAC biology has been the identification of expression-based subtypes, akin to those in breast cancer and other malignancies (26–29). We characterize here these subtypes for the first time in advanced PDAC, and demonstrate that Stage III/IV PDAC patients with Moffitt "classical" tumors respond better to first-line chemotherapy compared to those with "basal-like" tumors. Furthermore, the Moffitt tumor RNA subtype was shown to be prognostic despite the fact that our sample size is relatively small at this time. These results should

have implications in designing new PDAC trials involving chemotherapy and for mining existing trial tumor banks, particularly for neoadjuvant and adjuvant therapies where the therapeutic potential of chemotherapy is greatest. Basal-like subtypes may need to be identified early for dedicated studies exploring non-standard chemotherapy approaches and chemotherapy-based trials enriched for `classical' tumors. Our results and others (25) strongly support using *GATA6* expression as a surrogate biomarker to differentiate tumor RNA subtypes, an important translational advance that can be used in the clinic by pathologists using fresh or archival tumors. Our preliminary RNA pathway analysis work also demonstrated differentially regulated pathways in the classical and basal-like tumor RNA subtype and this will have implications on future development of PDAC subtype specific therapies.

COMPASS is the first series to agnostically characterize PDAC metastases independently of paired primary samples (14, 30–33). Although aberrations in *KRAS*, *CDKN2A*, *TP53*, and *SMAD4* are again the key drivers in late-stage PDAC, a significantly higher rate of polyploidization was observed in metastases compared to resected cases with ∼60% of metastatic tumors showing aneuploidy, which is a valid therapeutic target in epithelial malignancies including PDAC (34, 35).

We observed the structural variation phenotype dominated by duplication events in two diploid metastases which, in retrospect, was present in ∼3% of our series of resected primary PDAC (15, 16). Although tandem duplications have been observed in PDAC (8), a predominant duplicator phenotype has been described and studied more extensively in breast cancer especially within the context of HR deficiency (36–38) and platinum sensitivity (39). We observed responses to m-FOLFIRINOX in both duplicator cases, as well as in one patient with a germline *BRCA2* mutation and somatic LOH, and in another with an "unstable" genome. These results support the hypothesis that these unique tumors may respond better to platinum-based chemotherapy. Further studies involving PARP inhibitors in PDAC are underway in a number of centers, including ours, and may influence treatment choices for these patients, who account for ∼10% of all PDAC (40).

Somatic genetic aberrations that might predict benefit from tailored therapies were found in 30% of patients (Supplementary Table S1). Theoretically, five patients with mutations in *ARID1A*, a SWI/SNF-related, actin-dependent chromatin modifier, may respond to EZH2 inhibitors (41), ATR inhibitors (42), and PARP inhibitors (43) and efforts to implement these strategies will be a priority at progression in our study. PI3K pathway activation was observed in seven patients (12%) through *PIK3CA* activating mutations ($N = 4$) or *PTEN* inactivating mutations ($N = 3$). However, all the *PIK3CA* and *PTEN* mutations detected were found to co-occur with *KRAS* mutations indicating that PI3K inhibition alone may not be therapeutically effective in these cases, although they may potentially derive benefit from novel therapeutic strategies such as combination of PI3K and CDK4/6 inhibitors (44). Furthermore, two of three patients with *CDK4* or *CDK6* amplification did not derive measurable benefit from a CDK4/6 inhibitor, a reminder that the presence of a biomarker alone may not predict response to therapy, and that combination therapies may be more effective in advanced stage patients (45).

The main limitation of the COMPASS trial and other PDAC genomic studies (10, 17) is that only a small proportion of patients with potentially actionable genetic aberrations were matched to targeted second-line therapy, mainly due to a lack of biomarker-directed clinical trials. As the number of patients with each biomarker is small, it is challenging to conduct adequately powered relevant trials at a single institution. To address this, COMPASS is expanding to major cancer centers across Canada to more rapidly recruit greater numbers of advanced stage PDAC into biomarker directed trials so that the real potential of genomics driven second-line therapy can be evaluated rigorously.

The COMPASS trial has been successful in meeting its primary feasibility endpoint and has provided the first prospective translational evidence that chemotherapy responses differ among advanced PDAC patients with different tumor RNA subtypes. As per protocol, we will continue to recruit patients with continued emphasis on WGS and RNASeq with enriched cellularity and rigorous clinical annotation. This resource should be combined with other high quality prospective genomic and transcriptomic data sets (http://www.precisionpanc.org; https://www.pancan.org/research/precision-promise/), and linked to comprehensive clinical outcomes, to refine and rapidly expand knowledge of advanced stage PDAC molecular subtypes. A large number of cases will likely be required to understand advanced PDAC biology and to truly impact patient treatment options, our early data highlight the potential to achieve this ultimate outcome. Given the lack of effective standard therapies, our evidence-based and informed approach is required to recruit as many advanced stage patients as possible into biomarker hypothesis-driven clinical trials to improve outcomes for this deadly malignancy.

Disclosure of Potential Conflicts of Interest

S. Moura reports receiving speakers bureau honoraria from Celgene Corporation. J.M.S. Bartlett holds ownership interest (including patents) in 61 Due North, BioNTech, Biotheranostics, Insight Genetics, and Oncology Education, and is a consultant/advisory board member for BioNTech, Biotheranostics, and Insight Genetics. J.J. Yeh and R.A. Moffitt have a pending patent #15/518,900 with United States Patent and Trademark Office on methods and compositions for prognostic and/or diagnostic subtyping of pancreatic cancer. No potential conflicts of interest were disclosed by the other authors.

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