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Association of Distinct Mutational Signatures With Correlates of Increased Immune Activity in Pancreatic Ductal Adenocarcinoma

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IMPORTANCE Outcomes for patients with pancreatic ductal adenocarcinoma (PDAC) remain poor. Advances in next-generation sequencing provide a route to therapeutic approaches, and integrating DNA and RNA analysis with clinicopathologic data may be a crucial step toward personalized treatment strategies for this disease.

OBJECTIVE To classify PDAC according to distinct mutational processes, and explore their clinical significance.

DESIGN, SETTING, AND PARTICIPANTS We performed a retrospective cohort study of resected PDAC, using cases collected between 2008 and 2015 as part of the International Cancer Genome Consortium. The discovery cohort comprised 160 PDAC cases from 154 patients (148 primary; 12 metastases) that underwent tumor enrichment prior to whole-genome and RNA sequencing. The replication cohort comprised 95 primary PDAC cases that underwent whole-genome sequencing and expression microarray on bulk biospecimens.

MAIN OUTCOMES AND MEASURES Somatic mutations accumulate from sequence-specific processes creating signatures detectable by DNA sequencing. Using nonnegative matrix factorization, we measured the contribution of each signature to carcinogenesis, and used hierarchical clustering to subtype each cohort. We examined expression of antitumor immunity genes across subtypes to uncover biomarkers predictive of response to systemic therapies.

RESULTS The discovery cohort was 53% male (n = 79) and had a median age of 67 (interquartile range, 58-74) years. The replication cohort was 50% male (n = 48) and had a median age of 68 (interquartile range, 60-75) years. Five predominant mutational subtypes were identified that clustered PDAC into 4 major subtypes: age related, double-strand break repair, mismatch repair, and 1 with unknown etiology (signature 8). These were replicated and validated. Signatures were faithfully propagated from primaries to matched metastases, implying their stability during carcinogenesis. Twelve of 27 (45%) double-strand break repair cases lacked germline or somatic events in canonical homologous recombination genes—*BRCA1*, *BRCA2*, or *PALB2*. Double-strand break repair and mismatch repair subtypes were associated with increased expression of antitumor immunity, including activation of CD8-positive T lymphocytes (*GZMA* and *PRF1*) and overexpression of regulatory molecules (cytotoxic T-lymphocyte antigen 4, programmed cell death 1, and indolamine 2,3-dioxygenase 1), corresponding to higher frequency of somatic mutations and tumor-specific neoantigens.

CONCLUSIONS AND RELEVANCE Signature-based subtyping may guide personalized therapy of PDAC in the context of biomarker-driven prospective trials.

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Supplemental content

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Pancreatic ductal adenocarcinoma (PDAC) has the lowest 5-year overall survival (OS) of any epithelial carcinoma.[1] Randomized clinical trials[2,3] of adjuvant[4] and palliative[5,6] cytotoxic chemotherapies show modest end point improvements with considerable attendant toxicities. Targeted agents investigated without biomarker selection, including evofosfamide, programmed cell death 1 ligand (PD-L1),[7] cytotoxic T-lymphocyte antigen 4 (CTLA-4),[8] and human epiadermal growth factor receptor 2[9] inhibitors, have not improved OS, except for marginal benefit from erlotinib hydrochloride.[10-12] Outcomes for patients with PDAC will improve with rational molecular subtyping and ensuing directed therapies, as with breast[13] and lung[14] carcinomas. The PDAC exome[15-17] contains 4 driver genes, *KRAS*, *TP53*, *CDKN2A,* and *SMAD4*, and few disturbed pathways that are not translatable into predictive subtypes. Stratification by somatic events, including *MYC* amplification and specific *KRAS* mutant codons,[17] is not consistently prognostic. Structural variation in 100 genomes[18] identified 4 PDAC subtypes, with 1 predictive of platinum chemotherapy response, but progression-free survival and OS were not assessed. Finally, prognostic transcription-based subtypes have been described[19] and refined,[20,21] but with neither relation to genomic features nor therapeutic implications.

Cancer genomes accumulate mutations over cell cycles from DNA damage and repair. Analyses of these processes,[22,23] informative in other tumors,[24-26] have not been comprehensively reported in PDAC. Signatures representative of each process[22] can be quantified per tumor, and the population of tumors subtyped[25] by their relative contributions. Genomic and transcriptomic landscapes of antitumor immunity have been systemically explored in other tumor types[23] and predict response to immunotherapies[26,27]; however, the character of immune infiltration and its association with mutational signatures has not been studied in PDAC.

We integrated genome, transcriptome, and clinicopathologic data from 2 independent data sets to define 4 major signature-based PDAC subtypes. These aligned with known hereditary pancreas cancer predisposition syndromes (HPCSs),[28] were propagated from primary tumors to paired metastases, and differentially expressed antitumor immune markers.

Methods

All studies were approved by local research ethics boards or institutional review boards and written informed consent was obtained for all donors. Whole-genome sequencing (WGS) variant calls, RNA sequencing and microarray expression values, and clinical information and metadata for discovery and replication cohorts are available from the International Cancer Genome Consortium (ICGC) data portal.[29] Discovery cohort samples underwent tumor enrichment prior to sequencing. All reads were processed through the same data workflows. Bioinformatics tool names and versions are provided in the eMethods in Supplement 1.

Key Points

Question Can mutational signatures be used for developing translationally relevant personalized treatment in patients with pancreas cancer?

Findings Using a discovery/validation cohort study of resected pancreas cancer cases from the International Cancer Genome Consortium, distinct somatic mutational signatures in genomic DNA and RNA were identified. Mechanisms of both germline and somatic genomic instability, characteristic of DNA mismatch repair and double-stranded break repair, were found in approximately 12% of cases and were associated with transcriptional and immunohistochemical hallmarks of antitumor immune activation.

Meaning Mutational signatures may guide biomarker development and application of personalized chemo/immunotherapeutic approaches for a subset of patients with pancreas cancer.

Results

Mutational Signatures Define 4 Principal PDAC Subtypes

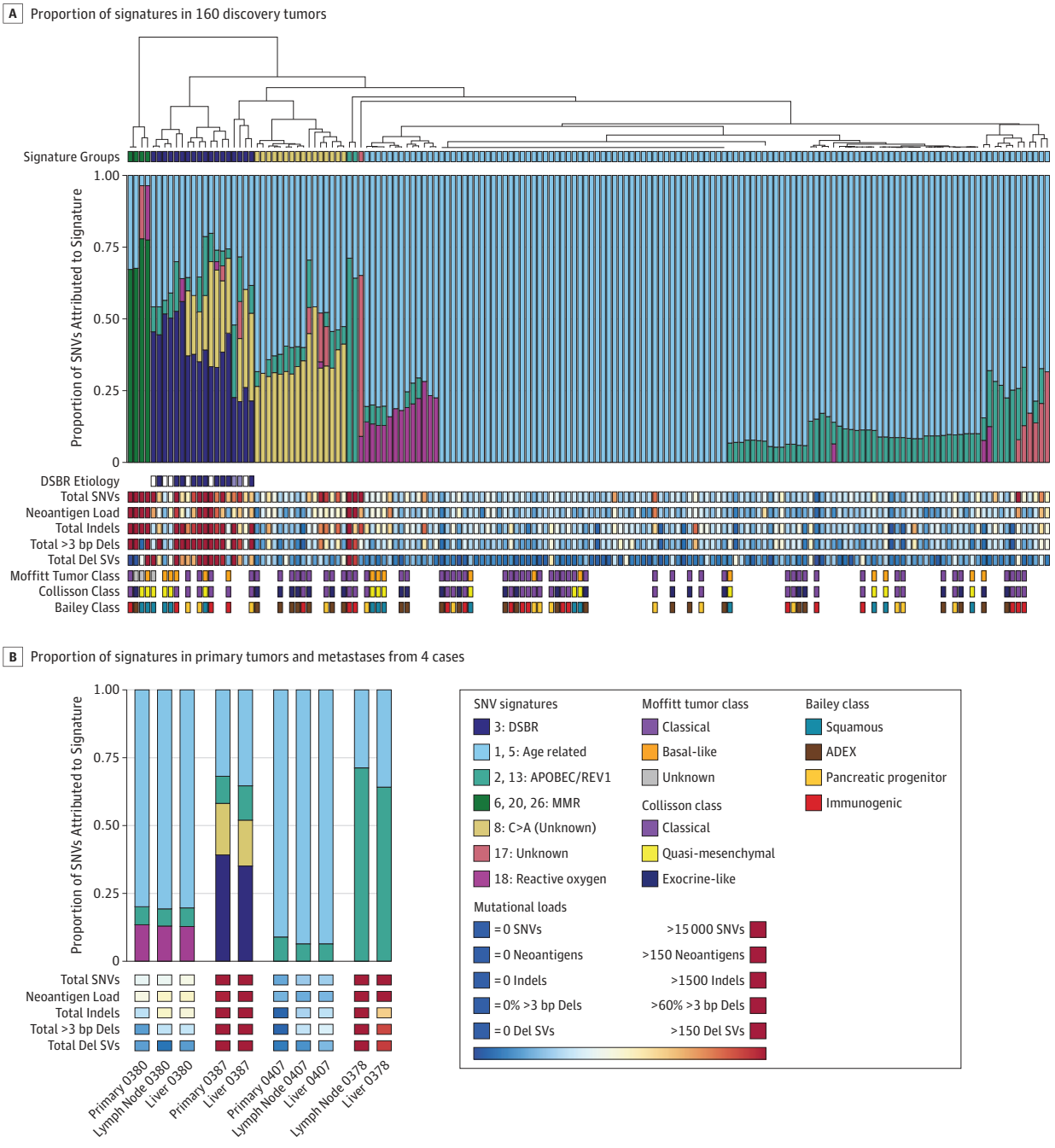
Our discovery cohort consisted of 148 primary PDACs and 12 metastases from 154 patients who underwent WGS (**Figure 1**A and eTable 1 in Supplement 1). For replication, 95 whole PDAC genomes from 95 patients were obtained from the ICGC (eFigure 1 and eTable 1 in Supplement 1).

We identified 11 mutational signatures in our discovery and 12 in our replication genomes using the approach of Alexandrov et al,[30] which were merged by shared etiologies into 7 signatures per cohort. Hierarchical clustering by the proportion of single-nucleotide variants (SNVs) attributable to each signature (eFigure 2A and B in Supplement 1) in each cohort independently confirmed 4 major subtypes: (1) an age-related group dominated by signatures 1 and 5, attributed to clocklike mutational processes accumulated over cell divisions[31]; (2) a double-strand break repair (DSBR) group characterized by signature 3, attributed to deficiencies in homologous recombination repair (HRR) of double-strand breaks; (3) a mismatch repair (MMR) group characterized by signatures 6, 20, and 26, attributed to defects in DNA MMR; and (4) a group characterized by signature 8, of unknown etiology (Figure 1A and eFigure 1 in Supplement 1). There were 2 minor groups in both cohorts, 1 dominated by signature 17, another by APOBEC. Tumor cellularity and coverage were consistent between subtypes (eFigure 3 in Supplement 1). Subtype prevalence was equivalent between cohorts ($P = .08$, χ^2).

We verified that signatures associated with their attributed etiologies. The number of SNVs in signatures 1 and 5 correlated with patient age at diagnosis across all cases (r for discovery = 0.21, P for discovery = .008; r for replication = 0.23, P for replication = .03; Pearson correlation), while total SNVs did not (eFigure 4 in Supplement 1).

Tumors dysfunctional in HRR rely on nonconservative forms of DSBR, namely, single-strand annealing, which creates large structural deletions,[32,33] and nonhomologous end joining and microhomology-mediated end joining, which create short deletions (3-20 base pairs [bp] in length). Consistent

Figure 1. Mutational Signatures in Primary and Metastatic Pancreatic Ductal Adenocarcinoma



A, Bar plot of proportion of 7 merged signatures in each of the 160 discovery tumors, sorted by hierarchical clustering (dendogram at bottom), showing germline (dark blue), somatic (mauve), and occult (white) double-strand break repair (DSBR) etiologies and heat maps for total number of single-nucleotide variants (SNVs), total number of neoantigens, total number of indels, total number of short deletions (dels) greater than 3 base pairs (bp), total number of structural deletions, and transcriptional subtypes (Moffitt tumor class, Collisson class, and Bailey class) in cases for which RNA sequencing is available for the tumor. B, Bar plots of proportion of 7 merged signatures in paired primary tumors and metastases from 4 cases. ADEX indicates aberrantly differentiated endocrine exocrine.

with this, DSBR cases had greater numbers of both large structural and short deletions greater than 3 bp relative to age-related cases (*P* for discovery < .001 for each; *P* for replication < .001; Wilcoxon) (Figure 1A and eFigure 5 in Supplement 1).

The MMR cases had dramatically more SNVs than the age-related cases (*P* for discovery < .001; Wilcoxon) (Figure 1A). Mismatch repair deficiency was verified by immunohistochemical analysis and a polymerase chain reaction (PCR)-based assay (eTable 2 in Supplement 1). Of

the 4 MMR cases, 3 had germline and 1 had only somatic mutations in MMR genes (eTable 3 in Supplement 1). Published frequencies of MMR deficiency in PDAC vary widely.[17,34] Absence of MMR from the replication cohort is likely due to its smaller size. To validate MMR prevalence, we stained a tumor microarray of 370 PDACs from the European Society Group for Pancreatic Cancer (ESPAC)[35-37] for 4 MMR proteins. Of 342 successfully stained, 6 were immunodeficient. Assuming discovery, replication, and ESPAC cohorts to be unbiased samplings of 1 population, we infer MMR deficiency prevalence in PDAC to be 1.7% (95% CI, 0.65%-2.7%), nearly equal to that of Lynch syndrome in PDAC[38] (eTable 4 in Supplement 1). Somatic MMR deficiency thus contributes little to PDAC, unlike colorectal[39] and endometrial[40] cancers.

The discovery cohort included 12 metastases: 10 age related, 1 DSBR, and 1 MMR. Five of these were matched with 3 primaries and showed faithful propagation of signatures (Figure 1B), including a DSBR pair with a germline *PALB2* mutation. This implies that mutational processes are established early in carcinogenesis and is important for trials in which PDAC metastases are more safely biopsied. Paired primaries and metastases were obtained at autopsy from patients who received palliative chemotherapy (eTable 5 in Supplement 1).

Tiers of DSBR Deficiency

Clinical interest in HRR deficiency is increasing, with tailored treatment strategies for breast[41] and ovarian[42] cancer. Of 17 discovery DSBR cases, 11 are explained by biallelic inactivation of *BRCA1*, *BRCA2,* or *PALB2*. Nine had pathogenic germline mutations with somatic inactivations of the second allele, and 2 had biallelic somatic inactivations (eTable 6 in Supplement 1). The remaining 6 were occult, lacking germline or somatic inactivation of canonical HRR genes, referred to as "BRCAness" in the literature.[33] In the replication cohort, DSBR etiology was similar, with 2 germline, 2 somatic, and 6 BRCAness. We inferred DSBR prevalence in PDAC to be 10.8% (95% CI, 7.0%-14.7%), comprising 4.4% (95% CI, 1.9%-7.0%) germline deficiency, 1.6% (95% CI, 0.04%-3.2%) somatic, and 4.8% (95% CI, 2.2%-7.5%) BRCAness. This germline frequency is nearly equal to the prevalence of germline *BRCA1* or *BRCA2* deficiency in PDAC,[43] implying that *PALB2* contributes minimally to PDAC predisposition.

In the amalgamated discovery and replication DSBR cases, the proportion of SNVs attributed to signature 3 was greater in germline than somatic cases, with BRCAness cases intermediate (**Figure 2**). The number of SNVs attributed to a mutational process likely increases with its duration in tumorigenesis.[30] Thus, germline cases may become HRR deficient earlier, while somatic cases become deficient later or subclonally, with BRCAness an admixture of both etiologies. This may have implications for therapies targeting HRR deficiency. BRCAness cases also have relatively low numbers of structural variants (SVs) (Figure 2) and may alternatively harbor a mutational process distinct from classical HRR deficiency.

Assuming that 1 or a few genes with "2 hits" explain the 12 BRCAness cases, we agnostically compared frequencies of biallelic inactivation of genes in the DSBR and age-related tumors of our amalgamated cohorts (**Figure 3**). We considered only primary tumors because metastasis-specific events were reported in PDAC.[44] *BRCA2* was the only gene preferentially inactivated in the DSBR group (false discovery rate, 0.004%).

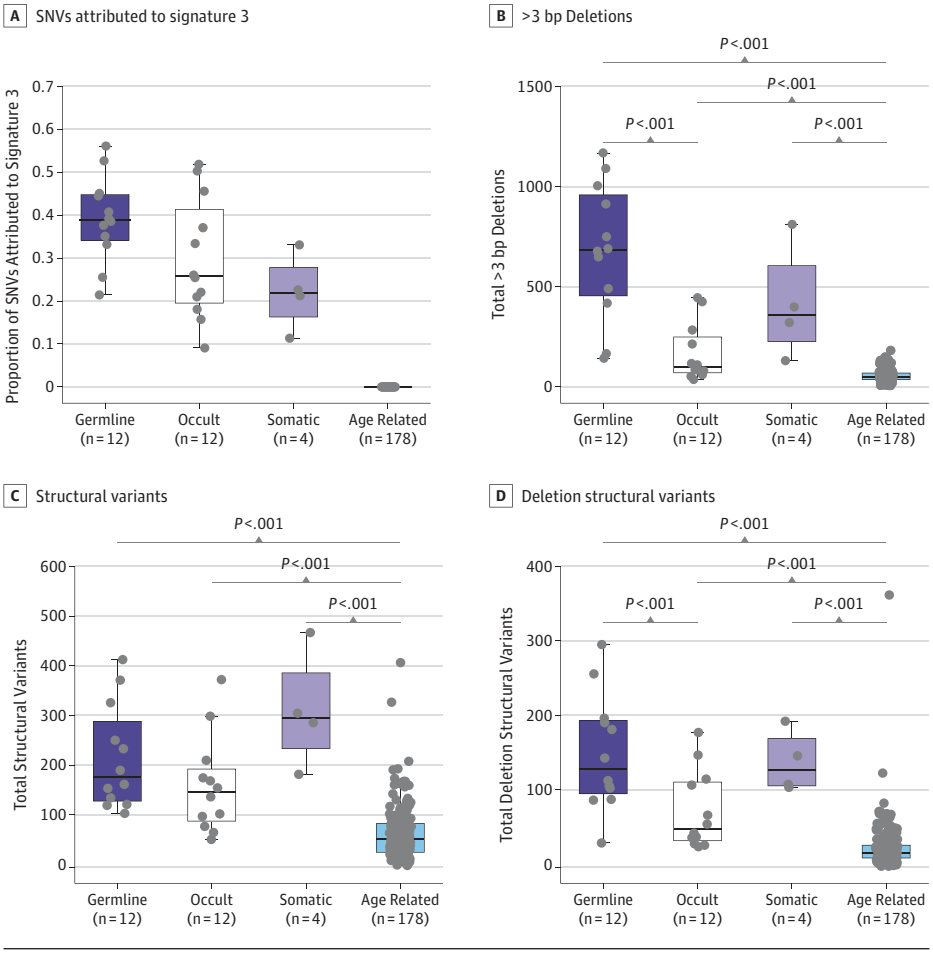
The idiopathic signature 8 is similar to signature 3, with the additional feature of strand bias for C>A substitutions. The latter was reported in PDAC exomes[17] and attributed to smoking, a PDAC risk factor,[45] although our data do not support this epidemiologic association (eFigure 6 in Supplement 1). Signature 8 is also found in breast cancer,[30,46] suggested as due either to past activity of transcription-coupled nucleotide excision repair or to HRR deficiency. Comparison of frequencies of biallelic inactivation per gene in signature 8 with either DSBR or age-related primary cases revealed no associations (eFigure 7A and B in Supplement 1). One signature 8 case bore a germline missense mutation (rs141465583) of uncertain significance in *BRCA1* with somatic loss of the wild-type allele. This variant is unlikely to impair HRR because overexpression of green fluorescent protein (GFP)-fused *BRCA1* p.P977L restored the ability of RAD51 to form ionizing radiation–induced foci in U2OS Flp-In cells depleted of endogenous *BRCA1* to a similar extent as wild-type GFP-*BRCA1* (eFigure 8 in Supplement 1). Thus, occult drivers of BRCAness and signature 8 either were so heterogeneous that each affected few cases or were not assayed—for example, noncoding or epigenetic changes or haploinsufficiency of an HRR-pathway gene or exogenous carcinogens.

Mutational Signatures Are Linked to Predisposition Syndromes

Truncating germline mutations of HPCS genes were found in 16 cases in our discovery cohort, including *BRCA1*, *BRCA2,* and *PALB2* mutations in 10, *MSH2* and *MSH6* in 3, *ATM* in 2, and *CDKN2A* in 1. There were 7 HPCS carriers in the replication cohort, including 4 *BRCA2*, 1 *PALB2*, 1 *ATM*, and 1 *PMS2* (eTable 7 in Supplement 1). Age at diagnosis differed in discovery but not replication donors with vs without HPCS (P for discovery = .002, P for replication = .32, t test) (eFigure 9 in Supplement 1).

Most patients with HPCS developed tumors driven by processes linked to their predispositions, demonstrating the importance of recognizing HPCS, including genetic counseling and germline testing. A minority developed tumors with processes unrelated to their predisposition. The somatic MMR discovery case had a germline *BRCA2* frameshift. Another discovery donor had a germline *MSH6* frameshift, but a tumor that was microsatellite stable and strongly positive for signature 17, of unknown etiology. One replication case had a germline stop-gain in *PMS2* (not long-range PCR verified) that was microsatellite stable, and 2 cases had germline *BRCA2* truncations without somatic "second hits" that lacked signature 3. The latter agrees with a mouse model heterozygous for *BRCA2* that retained the second, functional allele in PDAC and was not sensitive to mitomycin C and PARP1 (poly [ADP-ribose] polymerase 1) inhibitors.[47]

Figure 2. Etiologic Stratification of Double-Strand Break Repair Genomes



Boxplots of proportion of single-nucleotide variants (SNVs) attributed to signature 3, number of short deletions greater than 3 base pairs (bp) in length, number of structural variants, and number of large (structural) deletions in the double-strand break repair subtype divided by etiology—germline, BRCAness/occult, or somatic—and in the age-related subtype, for amalgamated discovery and replication cohorts. All values are significantly greater in both double-strand break repair germline and BRCAness/occult groups relative to the age-related subtype ($P < .001$ for each, Wilcoxon test). The horizontal line in the middle of each box indicates the median, while the top and bottom borders of the box mark the 75th and 25th percentiles, respectively. The top whisker marks the 75th percentile plus 1.5 times the interquartile range. The bottom whisker marks the 25th percentile minus 1.5 times the interquartile range. Each point indicates a tumor genome.

Nine discovery and 7 replication cases had biallelic events in *ATM*. Only 1 bore signature 3, the replication germline *ATM* carrier who lacked inactivation of another canonical HRR gene (eFigure 10 in Supplement 1).

Integration of Mutational Signatures With Gene Expression

We performed RNA sequencing on 76 discovery tumors. Our replication cohort had array expression data for 91 cases. We classified these by the methodologies of Collisson et al,[19] Moffitt et al,[20] and Bailey et al.[21] As with other cancers, including melanoma[24] and colorectal cancer,[48] mutational and transcriptional subtypes did not overlap (eFigure 11 in Supplement 1). Survival analyses had a nonsignificant finding of worse prognosis in the Moffitt basal subtype (eFigure 12 in Supplement 1).

We used gene sets[23] representative of 16 categories of immune function to characterize local immune activity. Adaptive immunity and co-inhibition genes were more highly expressed in DSBR and MMR cases (**Figure 4**A and eFigure 13A in Supplement 1). Cytolytic activity of infiltrating CD8-positive T lymphocytes, measured by the geometric mean of *GZMA* and *PRF1* expression, and co-regulatory molecules, namely, CTLA-4, PD-L1, PD-L2, and indolamine 2,3-dioxygenase 1 (IDO-1), were increased in DSBR and MMR relative to age-related cases (eFigure 14 in Supplement 1), reminiscent of expression patterns in melanoma responsive to checkpoint blockade.[26] Clustering of cases by differential expression of the genes in these sets[23] identified most DSBR (discovery, 6 of 6 DSBR; replication, 5 of 8) and all MMR cases as "immunogenic" (eFigures 15 and 16 in Supplement 1). The DSBR primary and metastasis pair both had high cytolytic activity, implying that antitumor responses are driven intrinsically.

To relate signatures to elevated cytolytic activity, we enumerated tumor neoantigens in discovery and replication cases. These paralleled SNV counts (r for discovery = 0.98, P for discovery < .001; r for replication = 0.85, P for replication < .001; Pearson) (Figure 4B and eFigure 13B in Supplement 1) and were elevated in DSBR and MMR cases (P for discovery < .001; P for replication < .001; DSBR vs age related; Wilcoxon) (eFigure 17 in Supplement 1). The number of neoantigens per SNV did not differ by subtype, implying that no signature was inherently immunogenic. Neither neoantigen nor SNV counts were associated with OS (eFigure 18 in Supplement 1). We found no other drivers of antitumor immunity, including incorporation of exogenous viruses or expression of endogenous retroviruses or of cancer testes antigens.

Equal frequencies of biallelic mutations in genes in the DSBR and age-related cases (Figure 3) imply that neither tumor suppressor, nor HLA class 1, nor extrinsic apoptosis gene inactivation is an immune resistance strategy in PDAC.

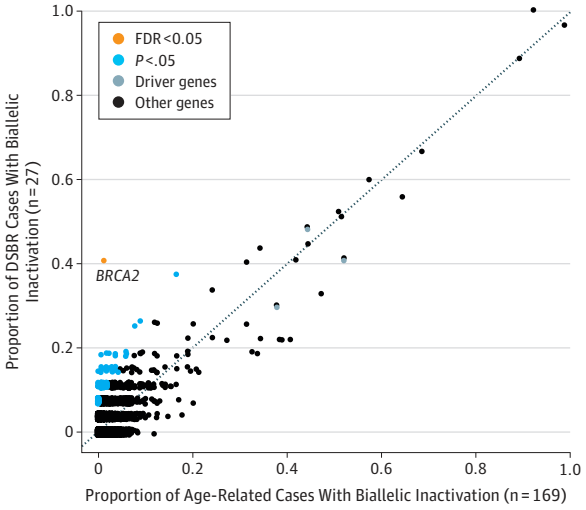
Cytolytic activity and CD8A and PD-L1 expression strongly correlated with CD8 and PD-L1 immunohistochemistry on a tumor microarray of 33 separate PDAC cases, validating our RNA sequencing results (**Figure 5**). Histologic analysis from 81 discovery cases showed no difference in the degree of peritumoral and intratumoral inflammation across signature classes, implying that microscopy alone cannot accurately measure local antitumor immunity (eFigure 19 in Supplement 1).

Prognostic and Predictive Value of Mutational Signatures

Signature groups were neither prognostic nor associated with tumor grade and stage (eFigures 20 and 21 in Supplement 1). Favorable outcomes are anecdotally reported for MMR-deficient PDAC.[49-51] The 4 discovery MMR patients had median OS of 1281 (interquartile range [IQR], 1248-1457) days compared with 461 (IQR, 254-1165) days for age-related cases. The patient with the stage IV MMR tumor is alive 24 months from diagnosis, responding to immunotherapy. In contrast, the 6 MMR immunodeficient ESPAC cases had worse survival than immunointact cases (P = .03, log-rank test) (eFigure 22 in Supplement 1). Rarity of MMR deficiency precludes definitive conclusions.

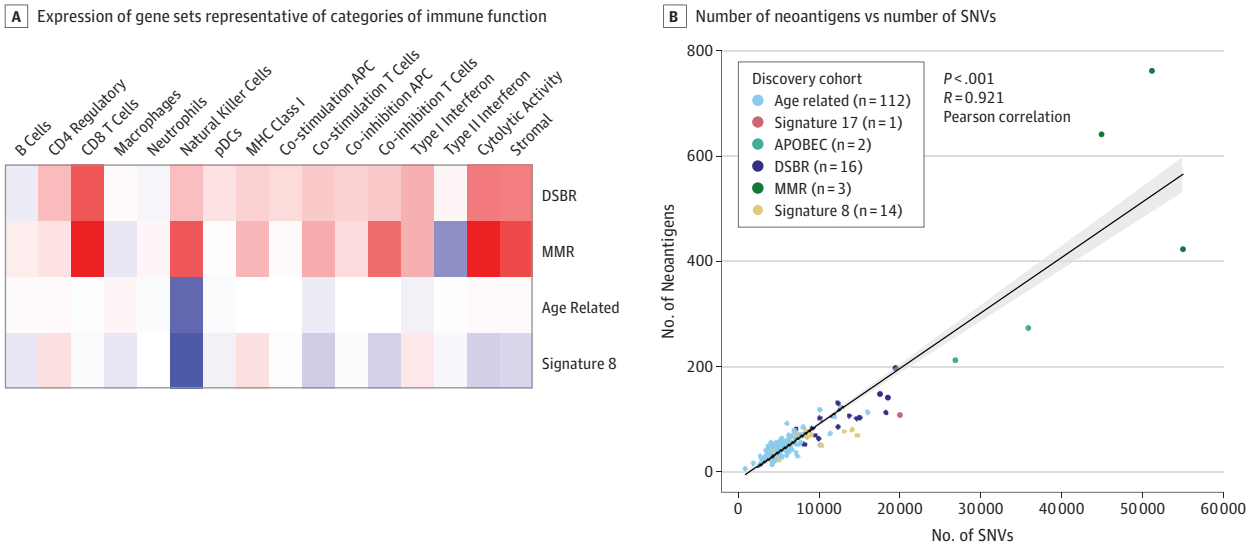
Roughly 1 in 10 cases in both cohorts have the DSBR signature. As HRR-deficient PDAC,[18] breast,[42] and ovarian[41] cancers may be sensitive to platinum-based therapy, we compared outcomes in 18 cases treated with either cisplatin or oxaliplatin (eTable 8 in Supplement 1 and eWorksheet in Supplement 2). In the palliative setting, median progression-free survival was not significantly longer in DSBR than in age-related cases (253 [IQR, 148.5-452] vs 108 [IQR, 82-194] days) (eFigure 23 in Supplement 1). Platinum responders were observed in both groups, suggesting that platinum-based therapy may also benefit non-DSBR cases.

Figure 3. Association of Genetic Inactivations With Double-Strand Break Repair (DSBR) Signature



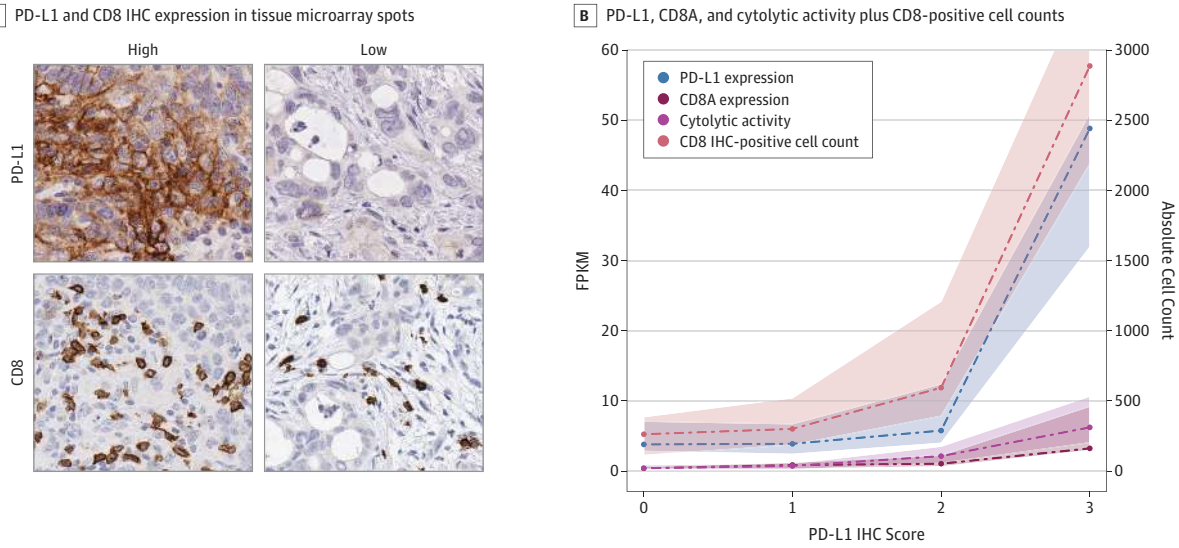
Scatterplot of proportions of cases with biallelic inactivation of every gene in the DSBR subtype primary tumors (n = 27) vs those in the age-related subtype primary tumors (n = 169) for the amalgamated discovery and replication cohorts. Driver genes include *CDKN2A*, *SMAD4*, and *TP53*. FDR indicates false discovery rate.

Figure 4. Integrated Genomic and Transcriptomic Features of Antitumor Immunity in Pancreatic Ductal Adenocarcinoma



A, Heat map of median expression of gene sets representative of categories of immune function by signature group for discovery cohort cases with tumor cellularities between 20% and 80%. B, Scatterplot of number of neoantigens vs number of somatic single-nucleotide variants (SNVs) per tumor, colored by signature-based subtype, for 137 discovery cohort cases to which we confidently assigned HLA class 1 genotypes. The regression line from the linear model (y ~ x) is shown in black with areas between confidence bands shaded in gray. APC indicates antigen-presenting cell; DSBR, double-strand break repair; MHC, major histocompatibility complex; MMR, mismatch repair; pDC, plasmacytoid dendritic cell.

Figure 5. Correlation of Immunohistochemistry With RNA Sequencing



A, Programmed cell death 1 ligand (PD-L1) and CD8 immunohistochemical (IHC) expression in representative cancer tissue microarray spots showing high and low expression of PD-L1 and CD8 counts. B, Median (dotted lines) and interquartile ranges (shaded regions) of expression of PD-L1, CD8A, and cytolytic activity (left-hand y axis) and absolute counts of cells with IHC staining for CD8 (right-hand y axis) at each level of PD-L1 IHC staining (0-3) (see Methods). CD8 staining cell counts and CD8A expression were strongly correlated ($P < .001$, $r = 0.744$, Pearson correlation). Programmed cell death 1 ligand and cytolytic activity expression were significantly higher across PD-L1 staining levels (P for PD-L1 = .006, P for cytolytic activity = .01, PD-L1 0-1 vs 2-3 staining, Wilcoxon test). FPKM indicates fragments per kilobase of exon per million fragments mapped.

Sample size limitations preclude determining whether susceptibility varies with proportion of DSBR.

Discussion

Mutational signatures in WGS defined 4 major PDAC classes, namely age related, DSBR, MMR, and signature 8. These were verified, replicated in independent cohorts, associated with predisposition syndromes, and propagated from primary to metastatic lesions. Cases of PDAC bearing DSBR and MMR signatures have elevated local antitumor immunity, driven by high levels of tumor neoantigens and evaded by expression of regulatory genes. This has implications for personalized management of PDAC.

Approximately 10% of PDAC is categorized as DSBR. Slightly more than half of these have biallelic inactivation of HRR genes; the rest are occult. The latter have lower numbers of large and small deletions greater than 3 bp relative to DSBR cases with known causal variants. These BRCAness tumors may have milder HRR deficiency or may represent a novel process that generates DSBR-like nucleotide substitutions but is distinct from classical HRR deficiency at the SV level. We might not expect platinum- or PARP inhibitor-based therapies directed at HRR deficiencies to be as effective in the BRCAness group, nor perhaps in the somatic DSBR cases that have a lower proportion of signature 3 attributed SNVs. Similarly, ovarian cancers with *BRCA1* promoter hypermethylation are less sensitive to chemotherapy than those with *BRCA1* mutations,[52,53] despite both being HRR deficient. This may explain why exceptional responses to platinum-based chemotherapy are not seen in 10% of patients with PDAC in clinical trials. Our failure to retrospectively detect significant improvement in progression-free survival in a palliative setting in DSBR cases is also consistent with heterogeneous mechanisms of HRR deficiency and secondary platinum resistance. Biomarker-driven prospective trials of PARP inhibitors[54] and platinum-based therapies should clarify this controversy.

Although BRCAness genomes do not appear to be driven by 1 or a few genes, multiple lines of evidence support the distinction of these cases. At the nucleotide level, the analogous mutational processes acting in germline, somatic, and occult DSBR cases give rise to tumor-specific neoantigens that in turn drive antitumor cytolytic activity, a prerequisite to successful immunotherapy.[23] A recent study found that metastatic melanomas responding to anti-programmed cell death 1 (PD-1) therapy are enriched for mutations in *BRCA2*.[55] The rate of neoantigen formation per SNV was equal across signature types, implying that increased mutation rate alone may predict checkpoint inhibitor response, as shown in colorectal cancer,[27] and platinum-based chemotherapy response, as shown in ovarian cancer.[56] While it has been hypothesized that sequestration protects PDAC cells from adaptive immunity,[57-59] our data suggest that resistance occurs through increased expression of *PD-1*, *CTLA-4*, and *IDO-1*. The potential for immunotherapy in PDAC has recently been demonstrated in a mouse model that recapitulates its fibrotic stroma using T cells engineered to recognize PDAC-specific antigen.[60] The progressive dysfunction of these T cells in vivo is compatible with our RNA expression findings, implying a role for immune checkpoint inhibition. Also, high expression of IDO-1 in both DSBR and MMR cases argues for trials of IDO-1 inhibitors in PDAC,

as in other cancers.[61,62] Current limited success of immunotherapy in PDAC[7,8] may be because only a minority of cases have significant local antitumor activity. Nonetheless, our data do not prove responsiveness to immunotherapies in subtypes of PDAC. Other important factors, such as host immunocompetence and tumor microenvironment, must be better understood to facilitate use of immunotherapeutics in clinical settings.

The nature of our complementary DNA-based RNA capture did not allow assessment of expression of all endogenous retroviruses or cancer testes antigens, nor quantification of tumor cellularity from RNA sequencing. Tumor cellularity estimates of the same fresh tissue from sections used for WGS were not significantly different between subtypes (eFigure 3 in Supplement 1). Our outcome analyses are limited by the retrospective nature of this work, including nonrandomized patient treatment selection and possible confounding factors not balanced between subtypes. Also, biallelic inactivation of other genes important to both DNA damage response and PDAC predisposition, such as *ATM*,[63] was not associated with signatures, implying that either our whole genome sample size was too small to detect all mutational processes or that the contributions of mutations produced by some processes were too few to be detected.[30] Nonetheless, that genomic and transcriptomic data generated separately with different platforms agree in all aspects validates our findings.

Conclusions

Our and other sequencing efforts have focused on resectable PDAC, constituting one-fifth of cases. Improving outcomes for the majority of patients with metastatic disease is needed. Our analysis provides a framework for integrating genomics and transcriptomics to suggest translatable differences between tumor subtypes. We are now applying this to whole-genome and transcriptome sequences from tumor biopsies to understand resistance to conventional treatment and to select second-line strategies for patients with advanced disease within the context of a prospective clinical trial (NCT02750657).

ARTICLE INFORMATION

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REFERENCES

1. National Cancer Institute, Surveillance, Epidemiology, and End Results Program. SEER Stat Fact Sheets: Pancreas Cancer. http://seer.cancer.gov/statfacts/html/pancreas.html. Accessed December 12, 2015.

2. Kalser MH, Ellenberg SS. Pancreatic cancer: adjuvant combined radiation and chemotherapy following curative resection. *Arch Surg*. 1985;120(8):899-903.

3. Oettle H, Neuhaus P, Hochhaus A, et al. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. *JAMA*. 2013;310(14):1473-1481.

4. Garrido-Laguna I, Hidalgo M. Pancreatic cancer: from state-of-the-art treatments to promising novel therapies. *Nat Rev Clin Oncol*. 2015;12(6):319-334.

5. Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med*. 2013;369(18):1691-1703.

6. Conroy T, Desseigne F, Ychou M, et al; Groupe Tumeurs Digestives of Unicancer; PRODIGE Intergroup. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med*. 2011;364(19):1817-1825.

7. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366(26):2455-2465.

8. Royal RE, Levy C, Turner K, et al. Phase 2 trial of single agent ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother*. 2010;33(8):828-833.

9. Harder J, Ihorst G, Heinemann V, et al. Multicentre phase II trial of trastuzumab and capecitabine in patients with HER2 overexpressing metastatic pancreatic cancer. *Br J Cancer*. 2012;106(6):1033-1038.

10. Moore MJ, Goldstein D, Hamm J, et al; National Cancer Institute of Canada Clinical Trials Group. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol*. 2007;25(15):1960-1966.

11. da Cunha Santos G, Dhani N, Tu D, et al. Molecular predictors of outcome in a phase 3 study of gemcitabine and erlotinib therapy in patients with advanced pancreatic cancer: National Cancer Institute of Canada Clinical Trials Group Study PA.3. *Cancer*. 2010;116(24):5599-5607.

12. Kim ST, Lim DH, Jang KT, et al. Impact of KRAS mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. *Mol Cancer Ther*. 2011;10(10):1993-1999.

13. Prat A, Pineda E, Adamo B, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. *Breast*. 2015;24(suppl 2):S26-S35.

14. Devarakonda S, Morgensztern D, Govindan R. Genomic alterations in lung adenocarcinoma. *Lancet Oncol*. 2015;16(7):e342-e351.

15. Biankin AV, Waddell N, Kassahn KS, et al; Australian Pancreatic Cancer Genome Initiative. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature*. 2012;491(7424):399-405.

16. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*. 2008;321(5897):1801-1806.

17. Witkiewicz AK, McMillan EA, Balaji U, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun*. 2015;6:6744.

18. Waddell N, Pajic M, Patch AM, et al; Australian Pancreatic Cancer Genome Initiative. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature*. 2015;518(7540):495-501.

19. Collisson EA, Sadanandam A, Olson P, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med*. 2011;17(4):500-503.

20. Moffitt RA, Marayati R, Flate EL, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. *Nat Genet*. 2015;47(10):1168-1178.

21. Bailey P, Chang DK, Nones K, et al; Australian Pancreatic Cancer Genome Initiative. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature*. 2016;531(7592):47-52.

22. Alexandrov LB, Nik-Zainal S, Wedge DC, et al; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain. Signatures of mutational processes in human cancer. *Nature*. 2013;500(7463):415-421.

23. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell*. 2015;160(1-2):48-61.

24. Cancer Genome Atlas Network. Genomic classification of cutaneous melanoma. *Cell*. 2015;161(7):1681-1696.

25. Schulze K, Imbeaud S, Letouzé E, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet*. 2015;47(5):505-511.

26. Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science*. 2015;350(6257):207-211.

27. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509-2520.

28. Connor AA, Gallinger S. Hereditary pancreatic cancer syndromes. *Surg Oncol Clin N Am*. 2015;24(4):733-764.

29. International Cancer Genome Consortium. ICGC Data Portal. https://dcc.icgc.org/. Accessed November 1, 2015.

30. Alexandrov LB, Nik-Zainal S, Wedge DC, Campbell PJ, Stratton MR. Deciphering signatures of mutational processes operative in human cancer. *Cell Rep*. 2013;3(1):246-259.

31. Alexandrov LB, Jones PH, Wedge DC, et al. Clock-like mutational processes in human somatic cells. *Nat Genet*. 2015;47(12):1402-1407.

32. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. *Mol Cell*. 2010;40(2):179-204.

33. Lord CJ, Ashworth A. BRCAness revisited. *Nat Rev Cancer*. 2016;16(2):110-120.

34. Riazy M, Kalloger SE, Sheffield BS, et al. Mismatch repair status may predict response to adjuvant chemotherapy in resectable pancreatic ductal adenocarcinoma. *Mod Pathol*. 2015;28(10):1383-1389.

35. Neoptolemos JP, Dunn JA, Stocken DD, et al; European Study Group for Pancreatic Cancer. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. *Lancet*. 2001;358(9293):1576-1585.

36. Neoptolemos JP, Stocken DD, Friess H, et al; European Study Group for Pancreatic Cancer. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med*. 2004;350(12):1200-1210.

37. Neoptolemos JP, Stocken DD, Bassi C, et al; European Study Group for Pancreatic Cancer. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. *JAMA*. 2010;304(10):1073-1081.

38. Grant RC, Selander I, Connor AA, et al. Prevalence of germline mutations in cancer predisposition genes in patients with pancreatic cancer. *Gastroenterology*. 2015;148(3):556-564.

39. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487(7407):330-337.

40. Kandoth C, Schultz N, Cherniack AD, et al; Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497(7447):67-73.

41. Muggia F, Safra T. 'BRCAness' and its implications for platinum action in gynecologic cancer. *Anticancer Res*. 2014;34(2):551-556.

42. Bayraktar S, Glück S. Systemic therapy options in BRCA mutation-associated breast cancer. *Breast Cancer Res Treat*. 2012;135(2):355-366.

43. Holter S, Borgida A, Dodd A, et al. Germline BRCA mutations in a large clinic-based cohort of patients with pancreatic adenocarcinoma. *J Clin Oncol*. 2015;33(28):3124-3129.

44. Campbell PJ, Yachida S, Mudie LJ, et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature*. 2010;467(7319):1109-1113.

45. Bosetti C, Lucenteforte E, Silverman DT, et al. Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer

Case-Control Consortium (Panc4). *Ann Oncol*. 2012;23(7):1880-1888.

46. Nik-Zainal S, Davies H, Staaf J, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*. 2016;534(7605):47-54.

47. Skoulidis F, Cassidy LD, Pisupati V, et al. Germline Brca2 heterozygosity promotes $Kras^{G12D}$-driven carcinogenesis in a murine model of familial pancreatic cancer. *Cancer Cell*. 2010;18(5):499-509.

48. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med*. 2015;21(11):1350-1356.

49. Banville N, Geraghty R, Fox E, et al. Medullary carcinoma of the pancreas in a man with hereditary nonpolyposis colorectal cancer due to a mutation of the MSH2 mismatch repair gene. *Hum Pathol*. 2006;37(11):1498-1502.

50. Goggins M, Offerhaus GJ, Hilgers W, et al. Pancreatic adenocarcinomas with DNA replication errors (RER+) are associated with wild-type K-ras and characteristic histopathology. Poor differentiation, a syncytial growth pattern, and pushing borders suggest RER+. *Am J Pathol*. 1998;152(6):1501-1507.

51. Wilentz RE, Goggins M, Redston M, et al. Genetic, immunohistochemical, and clinical features of medullary carcinoma of the pancreas: a newly described and characterized entity. *Am J Pathol*. 2000;156(5):1641-1651.

52. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011;474(7353):609-615.

53. Ruscito I, Dimitrova D, Vasconcelos I, et al. BRCA1 gene promoter methylation status in high-grade serous ovarian cancer patients—a study of the Tumour Bank Ovarian Cancer (TOC) and Ovarian Cancer Diagnosis Consortium (OVCAD). *Eur J Cancer*. 2014;50(12):2090-2098.

54. Kindler HL, Locker GY, Mann H, Golan T. POLO: a randomized phase III trial of olaparib tablets in patients with metastatic pancreatic cancer (mPC) and a germline BRCA1/2mutation (gBRCAm) who have not progressed following first-line chemotherapy. *J Clin Oncol*. 2015;33(suppl):abstr TPS4149.

55. Hugo W, Zaretsky JM, Sun L, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell*. 2016;165(1):35-44.

56. Birkbak NJ, Kochupurakkal B, Izarzugaza JM, et al. Tumor mutation burden forecasts outcome in ovarian cancer with BRCA1 or BRCA2 mutations. *PLoS One*. 2013;8(11):e80023.

57. Jacobetz MA, Chan DS, Neesse A, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. *Gut*. 2013;62(1):112-120.

58. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science*. 2009;324(5933):1457-1461.

59. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell*. 2012;21(3):418-429.

60. Stromnes IM, Schmitt TM, Hulbert A, et al. T cells engineered against a native antigen can surmount immunologic and physical barriers to treat pancreatic ductal adenocarcinoma. *Cancer Cell*. 2015;28(5):638-652.

61. Jiang T, Sun Y, Yin Z, Feng S, Sun L, Li Z. Research progress of indolamine 2,3-dioxygenase inhibitors. *Future Med Chem*. 2015;7(2):185-201.

62. Zhai L, Spranger S, Binder DC, et al. Molecular pathways: targeting IDO1 and other tryptophan dioxygenases for cancer immunotherapy. *Clin Cancer Res*. 2015;21(24):5427-5433.

63. Roberts NJ, Jiao Y, Yu J, et al. ATM mutations in patients with hereditary pancreatic cancer. *Cancer Discov*. 2012;2(1):41-46.