Genomic Methods Identify Homologous Recombination Deficiency in Pancreas Adenocarcinoma and Optimize Treatment Selection

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ABSTRACT

Purpose: Genomic methods can identify homologous recombination deficiency (HRD). Rigorous evaluation of their outcome association to DNA damage response–targeted therapies like platinum in pancreatic ductal adenocarcinoma (PDAC) is essential in maximizing therapeutic outcome.

Experimental Design: We evaluated progression-free survival (PFS) and overall survival (OS) of patients with advanced-stage PDAC, who had both germline- and somatic-targeted gene sequencing. Homologous recombination gene mutations (HRm) were evaluated: *BRCA1, BRCA2, PALB2, ATM, BAP1, BARD1, BLM, BRIP1, CHEK2, FAM175A, FANCA, FANCC, NBN, RAD50, RAD51, RAD51C*, and *RTEL1* HRm status was grouped as: (i) germline versus somatic; (ii) core (*BRCAs* and *PALB2)* versus non-core (other HRm); and (iii) monoallelic versus biallelic. Genomic instability was compared using large-scale state transition, signature 3, and tumor mutation burden.

Results: Among 262 patients, 50 (19%) had HRD (15% germline and 4% somatic). Both groups were analyzed together due to lack of difference in their genomic instability and outcome. Median [95% confidence interval (CI)] follow-up was 21.9 (1.4–57.0) months. Median OS and PFS were 15.5 (14.6–19) and 7 (6.1–8.1) months, respectively. Patients with HRD had improved PFS compared with no HRD when treated with first-line (1L) platinum [HR, 0.44 (95% CI: 0.29–0.67); $P < 0.01$], but not with 1L-non-platinum. Multivariate analysis showed HRD patients had improved OS regardless of their first-line treatment, but most had platinum exposure during their course. Biallelic HRm (11%) and core HRm (12%) had higher genomic instability, which translated to improved PFS on first-line platinum (1L-platinum) versus 1L-non-platinum.

Conclusions: Pathogenic HRm identifies HRD in patients with PDAC with the best outcome when treated with 1L-platinum. Biallelic HRm and core HRm further enriched benefit from 1L-platinum from HRD.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy projected to become the second leading cause of cancer-related deaths in the United States by 2030 (1). Most patients with PDAC present with advanced stage and the median overall survival (mOS) for all-comers is less than a year (2). Choosing the most effective treatment for each patient can enhance survival. Nevertheless, to date, treatment decisions have typically relied on performance status, comorbidities, and patient preference. Two randomized phase III studies have defined FOLFIRINOX or gemcitabine plus nab-paclitaxel as superior to single-agent gemcitabine and both represent first-line standard therapies; however, neither has validated biomarkers for treatment decision making (3, 4).

A recent randomized phase III trial (POLO) has highlighted a new era for precision oncology in PDAC by evaluating olaparib, the PARP inhibitor (PARPi), in germline *BRCA*-mutated metastatic PDAC (5). Among 154 enrolled patients, the progression-free survival (PFS) was significantly longer in the olaparib group compared with the placebo group [7.4 vs. 3.8 months; hazard ratio HR, 0.53; 95% confidence interval (CI), 0.35–0.82; $P = 0.004$; ref. 5). Further, a recent randomized phase II trial in patients with germline BRCA1/2-or PALB2-mutated PDAC yielded very high response rates (64%–75%) and OS (15–16 months) on first-line platinum (1L-platinum) chemotherapy regimen (6). These results support that homologous recombination

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Translational Relevance

The primary objective of this study was to evaluate the biological and clinical significance of homologous recombination deficiency (HRD) determined by several genomic methods in canonical *BRCA* germline mutations and other putative HR gene mutations (HRm) in pancreas cancer (PDAC). We systematically evaluated these methods and their associated clinical outcome in PDAC from a large prospectively maintained database. We interrogated the mutational status of HR genes and HRD genetic signatures. We identified that biallelic HRm was associated with higher level genomic instability and with better outcome on platinum. Somatic HRm was rarer than germline HRm and no significant difference in genomic instability and clinical outcome was observed between them. Higher degree genomic instability and superior progression-free survival was observed in core HRm (*BRCA1, BRCA2*, and *PALB2*) compared with wild-type (no HRD). These translational findings are significant for future application of DNA damage response–targeted treatment and immunotherapy clinical trials in broader population with HRD in PDAC.

deficiency (HRD) can be effectively targeted in PDAC and underpins the need for a comprehensive evaluation of homologous recombination gene mutations (HRm) beyond germline *BRCA* mutations for their sensitivity to DNA damage response (DDR)-targeted therapies including platinum.

Recent investigation of genomic profiling in large cohorts of PDAC have reported the significance of HRD/DDR in predicting sensitivity to platinum and PARPi (7–9). In addition to mutations in canonical HR genes, multiple groups have identified HR-deficient tumors by mutational and/or copy-number methods, which may capture a broader group of patients sensitive to DDR-targeted therapies (7, 8, 10). To date, neither a consensus on the definition of HRD has been reached nor a systematic evaluation for different methods to determine HRD and their association in predicting treatment sensitivity in PDAC has been performed (7, 8, 10–14).

Herein, we sought to evaluate the mutational status of HR genes and HRD genetic signatures to determine their correlation with each other and their benefit to platinum therapy. We report on a systematic evaluation of these methodologies to identify HRD using an FDA-approved targeted-sequencing assay. The HRD subgroups defined by each method were compared for clinical outcomes (OS and PFS) when treated with or without 1L-platinum–based therapies.

Materials and Methods

Study cohort

Written informed consent for genomic profiling was obtained (institutional review board # 12–245, NCT01775072) from all the patients with pathologically confirmed locally advanced or metastatic pancreatic exocrine cancers who received care at Memorial Sloan Kettering Cancer Center (New York, NY) prior to this genomic analysis. The protocol was approved by the Institutional Review Board at Memorial Sloan Kettering Cancer Center (New York, NY), and the study was conducted in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. Written consent was obtained from every patient. We excluded patients without germline testing (15, 16). Clinical, demographic, pathologic, genomic, treatment details, and outcome data were abstracted from the prospectively maintained database and the electronic medical record.

Determination of germline and somatic mutations

DNA was isolated from formalin-fixed, paraffin-embedded tumors and matched with normal blood, then sequenced for gene sets by MSK-IMPACT, a hybridization capture–based next-generation sequencing assay. Sequenced data were analyzed to identify nucleotide variants, small insertions and deletions, copy-number alterations (CNA), and structural rearrangements (15, 17). Both germline and somatic variants were called using previous algorithms (13). The FACETS algorithm was used to evaluate CNA, the fraction in genome, and to identify regions with LOH.

Mutational status of HR genes

Labeling priority was in the order of hotspot and pathogenic alteration, and missense variants of unknown significance (VUS). Pathogenic alterations included frameshift, truncating, splice-site, or pathogenic missense mutations, annotated by OncoKB (18–20). HRD was defined as germline or somatic pathogenic alterations of the following 17 HRm: *ATM, BAP1, BARD1, BLM, BRCA1, BRCA2, BRIP1, CHEK2, FAM175A, FANCA, FANCC, NBN, PALB2, RAD50, RAD51, RAD51C*, and *RTEL1* (Supplementary Data S1). These 17 candidate HR genes were selected as they are included in both germline and somatic gene sets by MSK-IMPACT (12, 14, 21). Emerging candidate DDR genes such as *ARID1A, ATR, ATRX, CHEK1, RAD51L1*, and *RAD51L3* were excluded (22, 23). Pathogenic HRm were interpreted as somatic HRm (sHRm) if identified only in tumor, whereas HRm found in blood sample were named germline HRm (gHRm). Core HRm (cHRm) was defined as either somatic or gHRm

Table 1. Patient characteristics at baseline.

Characteristic	Total $n = 262$ (%)
Age, median (range), years	64 (56–73)
Gender, male, no. (%)	144 (55)
Ethnicity, no. (%)	
White	216 (82)
Black	16 (6.1)
Asian	15 (5.7)
Unknown	15 (5.7)
Pathology, no. (%)	
Adenocarcinoma	258 (98)
Adenosquamous carcinoma	2 (1)
Carcinoma, NOS	2 (1)
Location of primary	
Head	96 (37)
Body	60 (23)
Tail	53 (20)
Overlap and NOS	53 (20)
Stage at presentation	
Metastatic	230 (88)
Locally advanced	32 (12)
First-line treatment no. (%)	
1L-platinum	160 (61)
FOLFIRINOX	138 (53)
Gemcitabine plus nab-paclitaxel	92 (35)
FOLFOX	8 (3)
Gemcitabine only	8 (3)
Gemcitabine plus cisplatin	5 (2)
Others	11 (4)

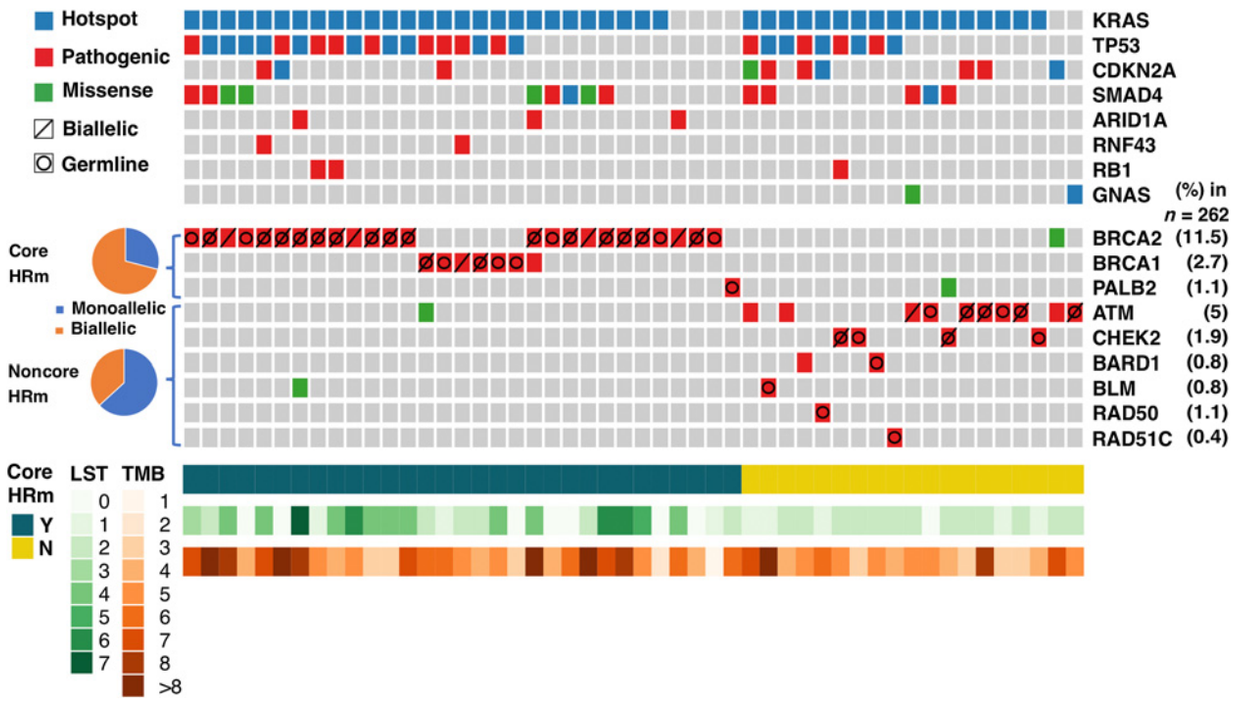

Figure 1.

OncoPrint: pathogenic alterations of HR genes and recurrent gene alterations from 50 patients with HRD are depicted (1 patient had both germline and somatic BRCA variants). For each sample, mutation profile is shown in a column including eight recurrently mutated genes (top) and nine candidate HR genes (bottom). Pathogenic alterations included frameshift, truncating, splice site, or known pathogenic missense mutations, annotated by OncoKB. The labeling priority was in the order of hotspot alteration, pathogenic alteration, and missense VUS. cHRm (BRCA1, BRCA2, and PALB2) is shown as a track with green and yellow color bar. The level of genomic instability of each sample is depicted by LST score and TMB (mutation/MB) in the bars below. Biallelic HRm is annotated with a diagonal line, whereas germline mutations are annotated with circles inside the square. Abbreviations: HRD, homologous recombination deficiency; LST, large-scale state transition; TMB, tumor mutation burden; HRm, HR mutation(s).

of the canonical genes: *BRCA1, BRCA2*, or *PALB2*. And noncore HRm (ncHRm) was defined as HRm of the other 14 HR genes. The allelic status of HR genes was assessed using FACETS, manual review of the copy-number plots, and evaluation of somatic mutation allele frequency to confirm loss of the wild-type (WT) allele (13, 24). On the basis of the mutation and LOH calling, a tumor was considered to have biallelic HRm if HRm was coupled with loss of the WT allele, or two pathogenic mutations on one HR gene.

HRD genomic signatures

Two signature-based methods were evaluated to identify HRD: large-scale state transition (LST) and mutational signature 3 (Sig3; refs. 12, 25, 26). Copy number change profile produced by FACETS was analyzed with a custom R script to count transition break points across the genome, which were summed for LST scores. For Sig3, we first called single-nucleotide variants (SNV) with MuTect2 on both IMPACT-targeted and off-target regions, filtered SNV loci with 150× coverage depth in the tumor sequence and 80× in the blood sequence, then performed signature multivariate analysis (SigMA) for cases with at least four somatic synonymous or nonsynonymous SNV (26). The SigMA R library with parameters "data = msk, tumor type = panc_ad" was used.

Statistical and survival analysis

We measured OS and PFS from the first treatment date until death (for OS) or until progression or death (for PFS). Disease progression was determined by imaging as adjudicated by the treating physician and radiologist. Patients were censored on the last follow-up date or on July 15, 2019. OS and PFS were estimated using the Kaplan–Meier method and compared between subgroups using log-rank test. Kruskal–Wallis test was used to compare mutational signatures among HRm subgroups. Pairwise two-sided multiple comparison was performed using Dwass–Steel–Critchlow–Fligner method, and Spearman correlation was used to assess correlation between continuous mutational signatures. Cox proportional hazards model was used for multivariate survival analyses. To evaluate whether 1L-platinum significantly affected the outcome differentially by HRD status, an interaction product term between binary covariates of HRD and 1L-platinum was included. Multivariate OS and PFS models were constructed by including known risk factors such as age and stage at diagnosis. GraphPad Prism (GraphPad Software), SAS Version 9.4 (SAS Institute, Inc.), and R statistical environment (v3.6.1, http://www.r-project.org) were used. All P values were two-sided with $P < 0.05$ indicating statistical significance.

Results

Study population

From October 2013 to July 2019, $n = 411$ patients with advanced PDAC were screened and a total of $n = 262$ were identified for the final analysis who had undergone both germline and somatic MSK-

IMPACT analysis. (Supplementary Data S2) The median age was 64 years (interquartile range: 56–73). There were $n = 145$ (55%) males. Majority were whites ($n = 216$, 82%). Fifty-two self-reported as Ashkenazi Jewish. The majority had adenocarcinoma ($n = 258$, 98%). Most had *de novo* stage IV ($n = 230$, 88%). 1L-platinum was administered in 61% ($n = 160$; **Table 1**).

Genetic landscape of cohort and frequency of different HRms

From 262 patients, we identified 1,193 high confidence nonsynonymous somatic coding mutations including 949 SNVs, 81 insertions, and 163 deletions. The median SNVs per case were four on the 468-gene MSK-IMPACT. The genes most frequently altered were *KRAS, TP53, CDKN2A, SMAD4, ARID1A, RNF43, RB1*, and *GNAS* (refs. 27–29; **Fig. 1**) Then, we evaluated the presence of pathogenic mutations affecting the 17 HR gene set, and identified $n = 50$ patients (19%) with HRm (**Table 2**). Notably, 1 patient with initially identified somatic *BRCA2* loss from outside testing later developed a reversion mutation, therefore intact *BRCA2* from reversion, which was not counted toward sHRm group. There were more gHRm ($n = 40$, 15%) than sHRm ($n = 10$, 4%). One patient had both germline *BRCA2*, and somatic *BRCA1* mutation. There were more cHRm (*BRCA1, BRCA2*, and *PALB2*, $n = 31$, 12%) than ncHRm ($n = 19$, 7%). Biallelic HRm ($n = 29$, 58%) was more common than monoallelic HRm ($n = 21$, 42%).

Table 2. Frequencies of different HR gene mutational status and genetic signatures.

WT (no HRD; %)	Total: 212 (81)
Zygosity of HR genes (%)	Total: 50 (19)
Biallelic loss (biallelic HRm)	29 (11)
Monoallelic loss (monoallelic HRm)	21 (8)
Mutated HR genes (%)	Total: 50 (19)
cHRm (BRCA1, BRCA2, and PALB2)	31 (12)
ncHRm (other 14 genes)[a]	19 (7)
HRD (%)	Total: 50 (19)[b]
Germline HRms	40 (15)[b]
Somatic HRms	10 (4)[c]
Monoallelic HRm (%)	$N = 21$ (8)
Monoallelic ncHRm	12 (5)
Monoallelic germline ncHRm	8 (3)
Monoallelic somatic ncHRm	4 (2)
Monoallelic cHRm	9 (3)
Monoallelic germline cHRm	9 (3)
Monoallelic somatic cHRm	0 (0)
Biallelic HRm (%)	$N = 29$ (11)[b]
Biallelic ncHRm	7 (3)
Biallelic germline ncHRm	6 (2)
Biallelic somatic ncHRm	1 (0.4)
Biallelic cHRm	22 (8)[b]
Biallelic germline cHRm	17 (6)[b]
Biallelic somatic cHRm	5 (2)
Genetic signatures beyond HRms, no. (%)	
LST available: 262 (100%)	Range: 0–27
Sig3	Positive 49 (19%),
Available: 151 (58%), unavailable: 111 (42%)	Negative 102 (39%)

Abbreviations: BRCA, breast cancer gene; PALB2, partner and localizer of BRCA2.

[a]Noncore HR genes: *ATM, BAP1, BARD1, BLM, BRIP1, CHEK2, FAM175A, FANCA, FANCC, NBN, RAD50, RAD51, RAD51C*, and *RTEL1*.

[b]One individual had both germline and somatic BRCA variants, thus included in both but the count for the total number of HRD, it was counted as one individual in germline.

[c]One individual was excluded from HRD group because only negative results for BRCA2 mutation from germline and somatic MSK-IMPACT. Initially reported BRCA2 loss from outside report.

Survival outcomes

The median follow-up among surviving patients ($n = 75$) was 21.9 (range: 1.4–57) months. The median OS of the entire cohort was 15.5 months (95% CI, 14.6–19.0). Patients with gHRm and sHRm had similar outcomes and were combined as HRD (Supplementary Data S3). HRD patients treated with 1L-platinum ($n = 35$) had a superior median OS compared with patients with no HRD treated with or without 1L-platinum [25.1 (21.6–NR) vs. 15.3 (14.2–20.3) or 13 (10.1–16.9) months, respectively; (**Fig. 2A**). Association of HRD on OS was independent of 1L-platinum and in multivariate model, patients with HRD had significantly reduced risk of all-cause mortality compared with patients with no HRD (hazard ratio, 0.50; 95% CI, 0.33–0.75; $P < 0.01$) after adjusting for age, 1L-platinum, and stage at diagnosis (**Table 3A**).

The median PFS (mPFS) of the entire cohort was 7.1 (95% CI, 6.1–8.1) months. Patients with HRD had a significantly improved mPFS when treated with 1L-platinum compared with 1L-non-platinum [12.6 (95% CI, 9.6–24.9) vs. 4.4 (3.0–10.0) months; **Fig. 2B**]. In multivariate interaction model, HRD patients treated with 1L-platinum had a significantly reduced risk of a disease progression or death (PFS) compared with non-HRD patients (hazard ratio, 0.44; 95% CI, 0.28–0.66; $P < 0.01$; **Table 3B**). In contrast, although statistically not significant, an increased risk for progression or death was observed among patients with HRD treated with 1L-non-platinum (hazard ratio, 1.42; 95% CI, 0.80–2.50).

Although 15 HRD patients did not receive 1L-platinum, 7 received platinum at second-line (2L), two had platinum exposure as a later line, and six did not have any platinum exposure. The mPFS for these 15 patients in the first-line setting was 4.4 months. Notably, for those 6 patients who received 2L-platinum, the second-line mPFS (from the 2L-platinum start date until progression or death) was 8.3 months and mOS was markedly longer than the median of entire cohort at 24.2 months. More cHRm patients received platinum compared with ncHRm patients beyond first-line as part of their treatment course; 6 of 8 (75%) cHRm patients compared with 3 of 7 (42%) ncHRm patients.

Correlation of HR gene mutational status with genetic signatures

We compared the magnitude of genomic instability among different mutational status of HRm (HRD: $n = 50$ and WT: $n = 212$) by assessing HRD mutational signatures, namely LST, Sig3, and tumor mutation burden (TMB). In view of SigMA requiring four mutations or higher, Sig3 score could only be derived from a subset of patients ($n = 151$; 58%). As such, we first focused on LST and TMB, which were universally derivable.

We observed a significantly diverse level of genomic instability between different zygosity groups. Biallelic HRm ($n = 29$) was associated with higher LST scores both when compared with WT ($n = 212$, $P = 0.0006$) and compared with monoallelic HRm ($n = 21$, $P = 0.005$; **Fig. 3**) Both cHRm ($n = 31$) and biallelic cHRm ($n = 22$) had higher LST scores than WT ($P = 0.04$ and $P = 0.0004$, respectively). Biallelic gHRm ($n = 23$), sHRm ($n = 10$), and biallelic sHRm ($n = 6$) all individually had higher LST scores than WT (all $P < 0.05$). However, no statistical difference was seen when compared with each other.

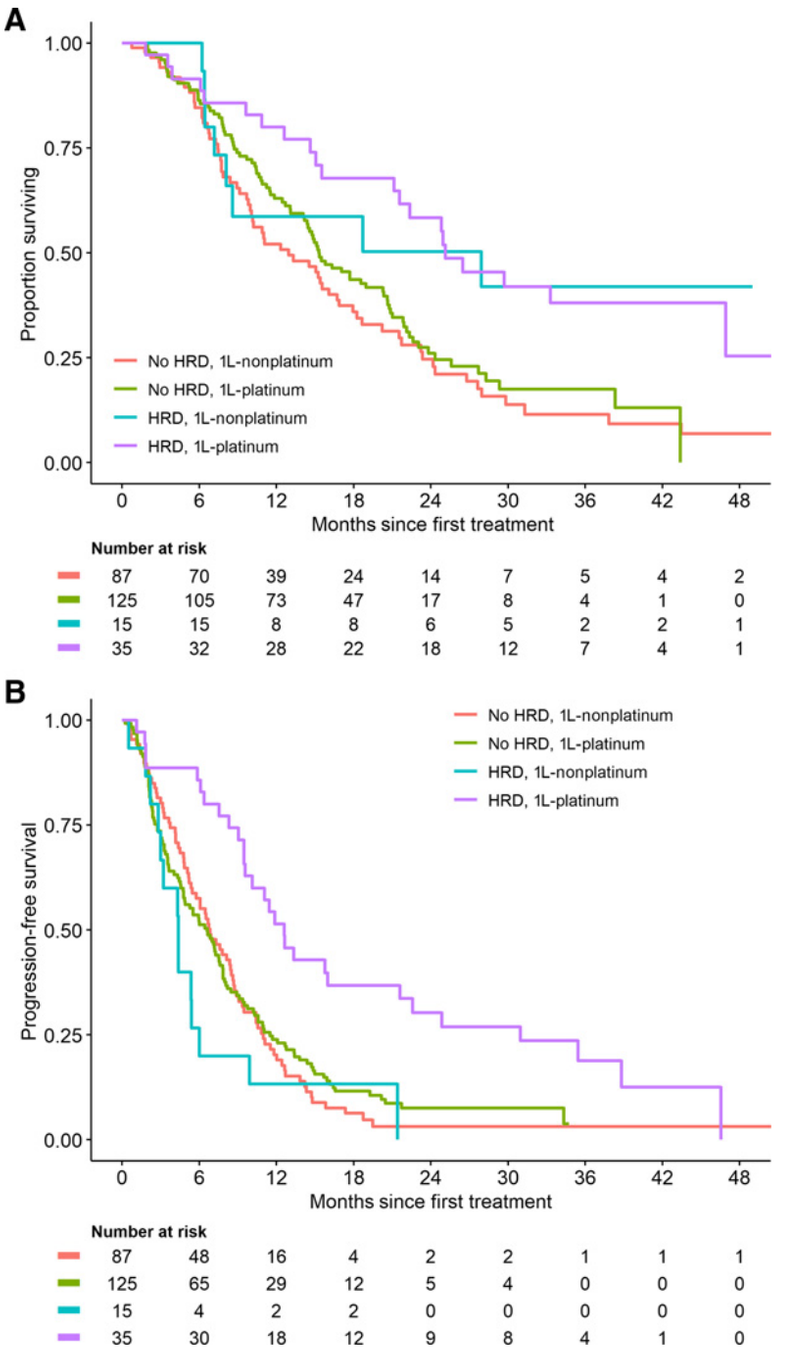

Figure 2.

Outcome of patients with pancreatic ductal adenocarcinoma with HRD depending on 1L-platinum or 1L-non-platinum (**A** and **B**). Abbreviations: HRD, homologous recombination deficiency; HRm, homologous recombination gene mutation(s); 1Lplatinum, first-line platinum; 1L-non-platinum, first-line non-platinum.

Sig3 was derivable from 35 of 50 HRm tumors because of low mutation numbers. Biallelic HRm had higher Sig3 score compared with WT ($P = 0.01$), and cHRm ($n = 25$) had higher Sig3 score compared with WT ($P = 0.0047$), but there was no difference when compared with ncHRm (Supplementary Data S4). Biallelic cHRm ($n = 18$, shaded) continued to have higher Sig3 score when compared with WT ($P = 0.001$). There were limited Sig3-positive tumors among sHRm tumors ($n = 8$). sHRm had higher Sig3 compared with WT ($P = 0.026$), but there was no difference in Sig3 between gHRm and WT. Higher Sig3 trend continued when the mutation was biallelic ($n = 5$), but it was not statistically significant in our analysis.

Biallelic HRm also had higher TMB compared with WT ($P = 0.028$), and cHRm had a trend of higher TMB but not significant ($P = 0.076$; Supplementary Data S5) However, among biallelic HRm, this difference was observed (biallelic cHRm vs. WT; $P = 0.0095$). Similar to LST and Sig3, there was no difference of TMB among gHRm, sHRm, and WT.

Overall, biallelic HRm had the strongest association with genomic instability, and this trend was maintained in biallelic cHRm and both

Table 3. Multivariate analysis for OS (A) and PFS (B).

A. OS			
Variables	**Comparisons (*n*)**	**HR (95% CI)**	***P***
HRD	HRD (50) vs. No HRD (212)	0.50 (0.33–0.75)	<0.01
1L-platinum	Yes (160) vs. No (102)	0.93 (0.68–1.26)	0.64
Stage	IV (232) vs. III (32)	2.26 (1.32–3.85)	<0.01
Age at diagnosis	Per 10-year increase	1.05 (0.91–1.21)	0.5

B. PFS			
Variables	**Comparisons**	**HR (95% CI)**	***P***
1L-platinum	HRD vs. No HRD	0.44 (0.28–0.66)	<0.01
1L-nonplatinum	HRD vs. No HRD	1.42 (0.80–2.50)	0.23
Stage	IV vs. III	1.85 (1.22–2.80)	<0.01

in biallelic gHRm and sHRm. These genetic signatures capturing different characteristics of HRD did not have significant association with each other except for LST and TMB (Spearman coefficient, 0.19; $P = 0.0012$; Supplementary Data S6).

Platinum affects PFS of patients with different HR gene mutational status and genetic signatures

Biallelic HRm patients ($n = 29$) had significantly improved mPFS on 1L-platnium compared with 1L-non-platinum [13.3 (95% CI, 9.57–NR) vs. 3.8 (95% CI, 2.79–NR) months; $P < 0.0001$]. However, this was not observed in monoallelic HRm patients ($n = 21$; $P = 0.22$; Supplementary Data S7). Furthermore, a sensitivity multivariate PFS analysis showed that compared with WT (= no HRD) patients, both cHRm patients (hazard ratio, 0.43; 95% CI, 0.26–0.70) as well as ncHRm (hazard ratio, 0.45; 95% CI, 0.23–0.87) had better PFS when treated with 1L-platinum (Supplementary Data S8). Also, in univariate analysis, among patients with gHRm ($n = 40$), patients treated with 1L-platinum also had significantly improved PFS compared with those who received 1L-non-platinum (hazard ratio, 0.21; 95% CI, 0.10–0.50). Sig3-positive patients also had better mPFS compared with Sig3-negative patients [11.0 (95% CI, 8.5–15.7) vs. 7.2 (95% CI, 6.0–9.1) months; $P = 0.03$] when treated with 1L-platinum compared with 1L-non-platinum. Although there was a reduced risk of event between patients with 1L-platinum compared with 1L-non-platinum among Sig3-positive patients ($n = 49$), it was not statistically significant (hazard ratio, 0.57; 95% CI, 0.29–1.10). Unexpectedly, no association between LST groups in tertiles and PFS was detected (data not shown).

Concordance and discordance of genetic methods for HRD identification

Patients with concordant HRD results had significant outcome differences depending on their first-line therapy. Sig3 was computable in 151 (58%) and 49 (19%) were Sig3 positive. There were 15 patients who had concordance for HRm and Sig3. Eleven received 1L-platinum and their mPFS was 12.6 (95% CI, 11.87–NR) months and mOS was 53.7 (95% CI, 24.8–NR) months, whereas the other four received 1L-non-platinum and had mPFS of 4.9 (95% CI, 2.8–NR) months and mOS of 18.2 (95% CI, 8.1–NR) months. In addition, 82 patients (43%) had tumors that were concordantly negative for HRm and Sig3. OS and PFS distributions were not significantly different between patients treated with 1L-platinum or 1L-non-platinum (data not shown). Patients with discordant results had fewer apparent outcome differences depending on platinum treatment exposure. Interestingly, there were 33 patients who had exceptional OS over 24 months with HRm-negative tumors who had received 1L-platinum and 8 (24%) of them were Sig3 positive.

Case study of platinum resistance and development of a reversion mutation

We emphasize the importance of understanding resistance mechanisms by presenting an exceptional case notable for extraordinary response to platinum, however, eventually progressed after reversion mutation. Sixty-one-year-old male with Ashkenazi Jewish heritage and metastatic PDAC harboring a somatic *BRCA2* loss responded durably to several platinum-based therapies for almost 3 years. Furthermore, the patient had a prior diagnosis of early-stage colon cancer, but germline testing was negative for Lynch syndrome or germline *BRCA* mutation. A liver biopsy from a progressing liver metastasis following platinum revealed subclonal

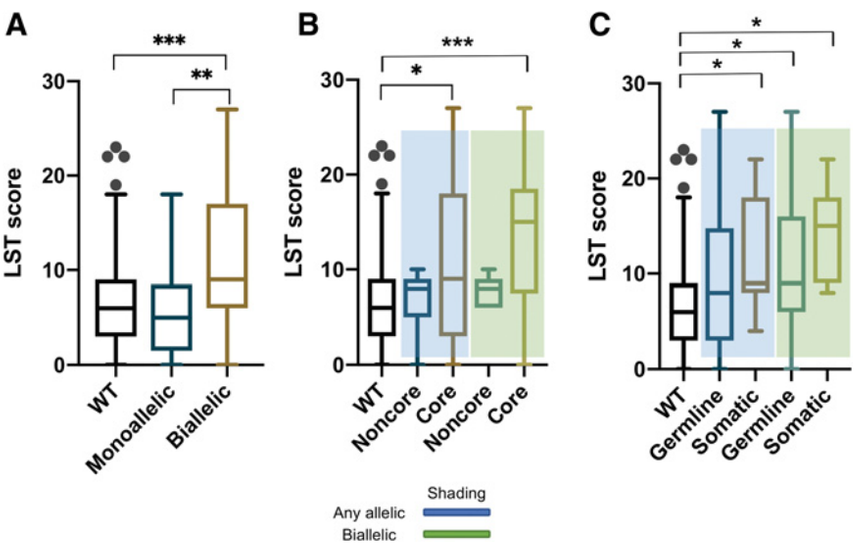

Figure 3.
Correlation of HR gene mutational status with LST. **A,** LST and zygosity: biallelic HRm ($n = 29$) had higher LST than monoallelic HRm ($n = 21$) and WT ($n = 212$). **B,** LST with cHRm ($n = 31$) and biallelic cHRm ($n = 22$) had higher LST than WT ($n = 212$). **C,** LST with germline and sHRm, biallelic gHRm, and biallelic sHRm all had higher LST than WT ($n = 212$), however, no statistical difference was seen among gHRm, sHRm, biallelic gHRm, and sHRm. *P* values are annotated with *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$. Abbreviations: LST, large-scale state transition; WT, wild type; HRm, homologous recombination gene mutation(s); cHRm, core HRm; ncHRm, non-core HRm; gHRm, germline HRm; sHRm, somatic HRm.

branching mutations of *ERBB3* G1271C and an *ATR–AGTR1* fusion and loss of *CDKN2A* and absent *BRCA2* loss (i.e., reversion mutation) in addition to truncal mutations in *KRAS* G12D and *TP53* E180K, which were shared with the original biopsy.

Discussion

Genetic alterations associated with HRD/DDR pathways have been shown to correlate with response to PARPi and platinum (7, 8, 30). Herein, we report on a comprehensive analysis of HRD detection methodologies in a cohort of patients with advanced PDAC.

The prevalence of HRD was 19%, primarily of germline origin (15%) similar to previous reports (7, 8, 31). A key aim was to determine whether outcome differed by germline versus sHRm, as most of the data in PDAC to date is based on germline findings (POLO; ref. 5). We observed neither differences in genomic instability nor clinical outcome between germline and somatic HRD, although the number of patients with somatic HRD was small, suggesting that both may predict for benefit to platinum-based therapies. Among mutational status of HR genes (germline vs. somatic, core vs. noncore, and monoallelic vs. biallelic) and HRD genetic signatures (LST and Sig3) evaluated, HRD identification using a targeted-sequencing approach, a current standard for pathogenic HRm determination, appears to be the most predictive methodology evaluated herein.

We observed that patients with HRD had significantly improved PFS when treated with 1L-platinum compared with those who received 1L-non-platinum. Subgroup analyses in PFS further confirmed that biallelic HRm, cHRm, and Sig3-positive patients mostly benefited from 1L-platinum. These data suggest that patients with either pathogenic somatic or germline *BRCA1, BRCA2*, or *PALB2* mutations as well as biallelic loss of other rarer HR genes such as *ATM, CHEK2*, and perhaps Sig3 positivity, should be recommended for DDR-targeted therapies like platinum and PARPi. Collectively, each method has limitations and may complement each other in identifying platinum-sensitive HRD patients.

It is important to note that most patients with HRD who did not receive 1L-platinum, ultimately did receive platinum therapy during their treatment course and had benefit, which may explain the lack of the predictive effect of HRD to 1L-platinum on OS. Interestingly, 3 of 6 patients with no platinum exposure had very long mOS (47.2 months). This may be due to HRD being a prognostic, as well as predictive biomarker or perhaps HR-mutant tumors have a broader sensitivity to other cytotoxic agents that induce double-strand DNA breaks, such as irinotecan (31–33).

Although the benefit of 1L-platinum in patients with PDAC with HRD is apparent, these considerations need to be weighed against the turn-around time of obtaining sequencing results. Specifically, tissue-based genomic sequencing for first-line treatment decision making in PDAC remains challenging. Typically, the timeframe for these results is 3–6 weeks, outside the timeframe to determine first-line treatment.

The subgroup analyses described here are limited by relatively small numbers, are of an exploratory nature, are hypothesis-generating, and survival outcomes are retrospectively correlated. Biallelic cHRm showed higher LST, Sig3, and TMB, indicating more significant genomic instability. These data support that these patients can benefit from DDR-targeted therapies and immunotherapy. The hypothesis that synthetic lethality with PARPi can render HRm tumors immunogenic is being investigated (34, 35).

Furthermore, MSK is a high-volume referral-based cancer center and has a population enriched for certain ethnic backgrounds. There were 52 patients self-reported to be Ashkenazi Jewish and 15 of them were HRD (29%), and this study defined HR genes as those that are included in both germline and somatic gene sets from MSK-IMPACT. Rarer candidate HR genes (e.g., *ATR, ATRX, CHEK1, RAD51L1*, and *RAD51L3*) were excluded from the analysis. Computing mutational signatures or structural variants associated with HRD from a targeted-sequencing approach is known to have limitations and may influence our findings. Evaluating the association of Sig3 and LST with HRms was difficult due to generally low mutation numbers in PDAC, thus limiting the clinical applicability of targeted-sequencing approach for genomic signatures. We further acknowledge there maybe additional survivorship bias in the cohort pertaining to the timepoint of consenting to MSK-IMPACT, related to the early part of this cohort (patients enrolled in 2013–2014).

Clinical outcomes vary due to the development of acquired resistance mechanisms such as drug efflux, reversion mutations, or compensatory changes in DDR (36–40). Initially sensitive tumors develop clonal evolution and develop resistance. Identifying and delaying emergence of resistance mechanisms and developing novel strategies to overcome resistance will meaningfully improve the outcome of future patients with PDAC. Broader sequencing methods (whole exome or whole genome) and serial genomic, transcriptomic, and functional analyses from patients with HRD are warranted to better understand the biology of HRD in PDAC.

Conclusion

HRD status defined as germline or somatic pathogenic HR gene alterations are associated with superior survival outcome to 1L-platinum in advanced PDAC. Biallelic mutation of HR genes showed the best association with this genomic characteristic independent of germline versus somatic origin. Determining the HRD status is ideally needed at the time of diagnosis to optimize survival and refine treatment selection in advanced PDAC. Short-term goals are to make this clinically realizable.

Disclosure of Potential Conflicts of Interest

W. Park reports receiving other remuneration from Ipsen. K.H. Yu reports receiving other commercial research support from BMS, Ipsen, and Halozyme. J.J. Harding is an employee/paid consultant for Bristol-Myers Squibb, Eli Lilly, Exelixis, Eisai, QED, and Imvax and reports receiving commercial research grants from Bristol-Myers Squibb. V. Makarov is an inventor on patent EP3090066A2, Determinants of cancer response to immunotherapy, current assignee Sloan-Kettering Institute for Cancer Research. M.E. Robson is an employee/paid consultant for Change HealthCare, reports receiving commercial research grants from Invitae, is an advisory board member/unpaid consultant for Pfizer, Merck, Daiichi-Sankyo, AstraZeneca, and Epic Sciences, and reports receiving other remuneration from Pfizer - editorial services. L. Zhang reports receiving speakers bureau honoraria from Future Technology Research LLC, BGI, Illumina, and Roche Diagnostics Asia Pacific, and holds ownership interest (including patents) in Shanghai Genome Center. M.F. Berger is an employee/paid consultant for Roche and reports receiving commercial research grants from Grail. T.A. Chan is an employee/paid consultant for Illumina, AstraZeneca, NysnBio, BMS, Merck, and Gritstone Oncology, reports receiving commercial research grants from AstraZeneca, Illumina, and BMS, and holds ownership interest (including patents) in Gritstone Oncology. J.S. Reis-Filho is an employee/paid consultant for Goldman Sachs, Paig.AI, REPARE Therapeutics, Roche Tissue Diagnostics, Ventana Medical Systems, Genentech, Novartis, and InVicro. C.A. Iacobuzio-Donahue reports receiving commercial research grants from Bristol-Myers Squibb. N. Riaz is an employee/paid consultant for REPARE Therapeutics and reports receiving commercial research grants from BMS, Pfizer, AZ, and REPARE

Therapeutics. E.M. O'Reilly is an employee/paid consultant for Ipsen, Merck, Sobi, Cytomx Therapeutics, Celgene, Polaris, and Rafael and reports receiving commercial research grants from Genentech, BMS, Celgene, MabVax Therapeutics, AstraZeneca, Silenseed, and ActaBiologica. No potential conflicts of interest were disclosed by the other authors.

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