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Homologous Recombination Repair pathway alterations and their relationship to Homologous Recombination Deficiency in advanced pancreatic cancer patients

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BACKGROUND

Homologous recombination repair (HRR) is a critical mechanism for limiting DNA damage arising from double strand breaks. Targeting homologous recombination deficient (HRD) tumors via synthetic lethality has emerged as a therapeutic strategy in pancreatic cancer given the recent approvals of PARP inhibitors in tumors harboring alterations in *BRCA1/2* which are the canonical alterations associated with HRD tumors. The molecular signature of HRD has yet to be established clinically or biologically, with varying profiling methodologies yielding a wide range of HRD positivity from 3-26% (Nguyen et al, Zhuang et al). In the current study, we set out to describe the relationship between *BRCA1/2* status, other HRR pathway alterations, and gwLOH, with HRD positivity via an RNA based HRD signature in a real-world cohort of advanced pancreatic cancer patients.

METHODS

We retrospectively analyzed de-identified records from 628 patients with advanced pancreatic cancer that underwent next generation sequencing (NGS) with the Tempus xT assay (DNA-seq of 648 genes at 500x coverage, matched normal, full transcriptome RNA-seq). 368/628 patients had RNA available for analysis. Gene expression values from RNA-seq were used as inputs to determine HRD status using a logistic regression model (Leibowitz et al). HRD-positivity and genome-wide loss of heterozygosity (LOH) were compared across groups, which were defined based on alterations to *BRCA1/2* or other HRR pathway genes.

Description of HRR Evaluation Groups:

- **BRCA biallelic loss:** double alteration detected in BRCA
- **BRCA monoallelic loss:** single alteration detected in BRCA, but no double alteration
- **Other HRR biallelic loss:** double alteration detected in at least one other HRR gene, no BRCA alterations
- **Other HRR monoallelic loss:** single alteration detected in at least one other HRR gene, no BRCA alterations and no double alterations in other HRR genes
- **No alterations, either BRCA or other HRR**: no LOH

Biallelic alteration criteria: deep deletions, a somatic + germline mutation, or either a germline + somatic mutation + overlapping LOH.

Single alteration criteria: any mutation (germline or somatic pathogenic mutation)

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SIGNIFICANCE

- Median gwLOH and HRD-RNA positivity represent distinct measurements that vary by alterations in BRCA and/or other HRR genes
- Using an RNA based algorithm to predict biallelic BRCA loss, we identified approximately 6% of patient who were predicted to be HRD positive
- HRD-RNA complements DNA-based HRD detection methods, especially for indications with low prevalence of BRCA alterations
- Characterization of pathogenic alterations in non-BRCA HRR genes provides a more comprehensive assessment of the HRD phenotype

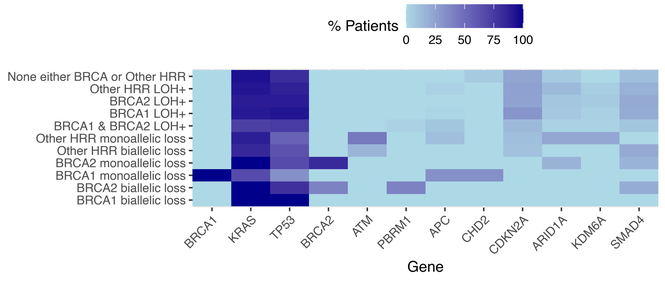
RESULTS

Table 1: Patient Demographic Characteristics

Characteristic	Overall, N = 628[1]	BRCA biallelic loss, N = 23[1]	BRCA monoallelic loss, N = 11[1]	Other HRR biallelic loss, N = 32[1]	Other HRR monoallelic loss, N = 16[1]	No alterations either BRCA or other HRR, N = 546[1]
Gender						
Male	347.0 (55.3%)	11.0 (47.8%)	9.0 (81.8%)	17.0 (53.1%)	9.0 (56.2%)	301.0 (55.1%)
Female	278.0 (44.3%)	12.0 (52.2%)	2.0 (18.2%)	15.0 (46.9%)	6.0 (37.5%)	243.0 (44.5%)
Age at Diagnosis	67 (59, 73)	65 (58, 67)	70 (64, 78)	67 (59, 72)	63 (58, 76)	67 (59, 74)
Race						
White	278.0 (44.3%)	9.0 (39.1%)	7.0 (63.6%)	11.0 (34.4%)	9.0 (56.2%)	242.0 (44.3%)
Black or African American	47.0 (7.5%)	1.0 (4.3%)	1.0 (9.1%)	1.0 (3.1%)	1.0 (6.2%)	43.0 (7.9%)
Asian	7.0 (1.1%)	2.0 (8.7%)	0	1.0 (3.1%)	0	4.0 (0.7%)
Native Hawaiian or Other Pacific Islander	1.0 (0.2%)	0	0	0	0	1.0 (0.2%)
American Indian or Alaska Native	4.0 (0.6%)	0	0	0	0	4.0 (0.7%)
Other Race	14.0 (2.2%)	2.0 (8.7%)	0	1.0 (3.1%)	1.0 (6.2%)	10.0 (1.8%)
Unknown	277.0 (44.1%)	9.0 (39.1%)	3.0 (27.3%)	18.0 (56.2%)	5.0 (31.2%)	242.0 (44.3%)
Stage						
Stage 3	30.0 (4.8%)	0	1.0 (9.1%)	1.0 (3.1%)	1.0 (6.2%)	27.0 (4.9%)
Stage 4	598.0 (95.2%)	23.0 (100.0%)	10.0 (90.9%)	31.0 (96.9%)	15.0 (93.8%)	519.0 (95.1%)
MSI Status						
Not detected	0	0	0	0	0	0
Low	0	0	0	0	0	0
Stable	626.0 (99.8%)	23.0 (100.0%)	11.0 (100.0%)	31.0 (96.9%)	16.0 (100.0%)	545.0 (100.0%)
High	1.0 (0.2%)	0	0	1.0 (3.1%)	0	0
Number of pathogenic variables reported						
0	558.0 (88.9%)	0	0	16.0 (50.0%)	0	542.0 (99.3%)
1	62.0 (9.9%)	20.0 (87.0%)	10.0 (90.9%)	14.0 (43.8%)	14.0 (87.5%)	4.0 (0.7%)
2	8.0 (1.3%)	3.0 (13.0%)	1.0 (9.1%)	2.0 (6.2%)	2.0 (12.5%)	0
TMB (m/MB)	2.63 (2.10, 3.68)	3.70 (2.90, 5.53)	3.16 (2.63, 3.69)	2.11 (1.58, 3.29)	2.63 (1.58, 3.29)	2.63 (2.10, 3.20)
Genome-wide LOH proportion	0.26 (0.17, 0.34)	0.32 (0.25, 0.39)	0.27 (0.18, 0.30)	0.29 (0.18, 0.38)	0.24 (0.05, 0.32)	0.25 (0.17, 0.34)

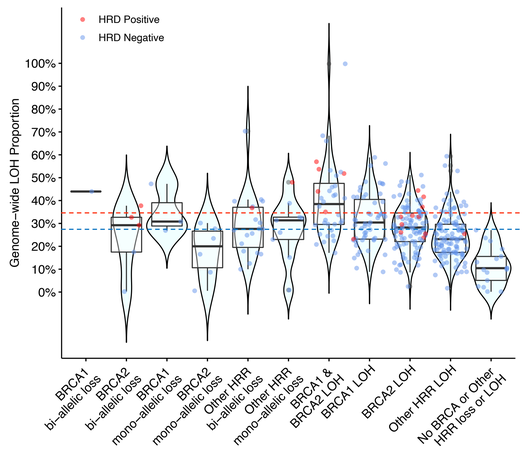
[1]n (%); Median (IQR)
[2]Fisher's exact test; Kruskal-Wallis rank sum test

Figure 2: Genomic landscape of advanced pancreatic cancer cohort



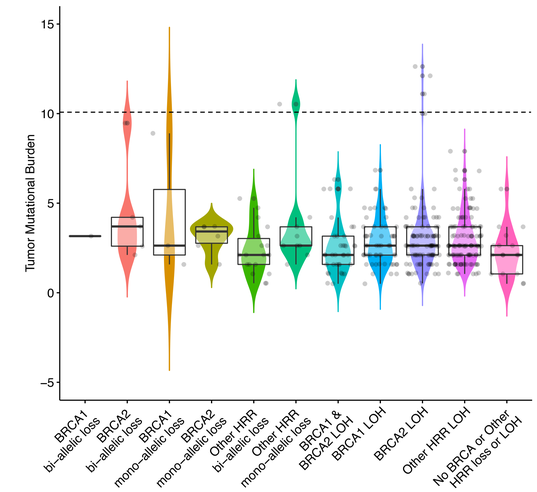
Mutation plot shows pathogenic alterations in genes where at least 20% of patients had a positive finding. Evaluation groups are separated by BRCA or Other HRR biallelic or monoallelic alterations status.

Figure 1: Characterization of HRD-RNA as a function of gwLOH



Distribution of HRD-RNA scores across different HRR genotypes in the evaluation cohort of 368 patients with RNA sequencing available. Evaluation groups are further stratified by either monoallelic or biallelic loss in a select number of HRR genes. Values in the box represent the median gwLOH percentage within each evaluation group. Overall median gwLOH values for HRD positive and negative groups are represented by red (34%) and blue (27%) dotted lines, respectively.

Figure 3: Distribution of tumor mutational burden by HRR genotype



Tumor mutational burden (mutations/megabase) represented by HRR genotype. Dotted line indicates a TMB of 10 m/MB.

CONCLUSIONS

Molecular profiling with the xT assay and an RNA based HRD signature identified approximately 6% (22/368) of pancreatic cancer patients as HRD positive. Of this subset of patients, 17/22 (77%) exhibited HRD positivity without *BRCA1/2* or other HRR bi-allelic loss and without pathogenic variants in HRR; however, these cases did exhibit LOH in HRR genes. Additionally, median gwLOH values varied between HRD positive and HRD negative groups. Collectively, these data supports the potential value of an RNA based HRD signature to identify additional patients who may be candidates for therapeutic strategies that target the HRD phenotype. Future studies will be needed to further establish the biological and clinical validity of HRD signatures for use in guiding therapeutic decision making.

References
(1) Nguyen, Luan, et al. "Pan-cancer landscape of homologous recombination deficiency." Nature communications 11.1 (2020): 1-12.
(2) Zhuang, Shuping, et al. "A transcriptional signature detects homologous recombination deficiency in pancreatic cancer at the individual level." Molecular Therapy-Nucleic Acids 26 (2021): 1014-1026