

Germline and Somatic DNA Damage Repair Gene Mutations and Overall Survival in Metastatic Pancreatic Adenocarcinoma Patients Treated with FOLFIRINOX

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Abstract

Purpose: Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer with lack of predictive biomarkers. We conducted a study to assess DNA damage repair (DDR) gene mutations as a predictive biomarker in PDAC patients treated with FOLFIRINOX.

Experimental Design: Indiana University Simon Cancer Center pancreatic cancer database was used to identify patients with metastatic PDAC, treated with FOLFIRINOX and had tissue available for DNA sequencing. Baseline demographic, clinical, and pathologic information was gathered. DNA isolation and targeted sequencing was performed using the Ion AmpliSeq protocol. Overall survival (OS) analysis was conducted using Kaplan–Meier, logistic regression and Cox proportional hazard methods. Multivariate models were adjusted for age, gender, margin status, CA 19-9, adjuvant chemotherapy, tumor and nodal stage.

Results: Overall, 36 patients were sequenced. DDR gene mutations were found in 12 patients. Mutations were seen in BRCA1 ($N = 7$), BRCA2 ($N = 5$), PALB2 ($N = 3$), MSH2 ($N = 1$), and FANCF ($N = 1$) of all the DDR genes sequenced. Median age was 65.5 years, 58% were male, 97.2% were Caucasian and 51.4% had any family history of cancer. The median OS was near significantly superior in those with DDR gene mutations present vs. absent [14 vs. 5 months; HR, 0.58; 95% confidence interval (CI), 0.29–1.14; log-rank $P = 0.08$]. Multivariate logistic (OR, 1.47; 95% CI, 1.04–2.06; $P = 0.04$) and Cox regression (HR, 0.37; 95% CI, 0.15–0.94; $P = 0.04$) showed presence of DDR gene mutations was associated with improved OS.

Conclusions: In a single institution, retrospective study, we found that the presence of DDR gene mutations are associated with improved OS in PDAC patients treated with FOLFIRINOX. *Clin Cancer Res*; 24(24); 6204–11. ©2018 AACR.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains a lethal cancer with a 5-year survival rate of less than 5% overall (1) and 15% to 20% for early-stage resectable disease (2). Moreover, PDAC is projected to become the second leading cause of cancer-related mortality in the United States within a decade (3). Genome stability is compromised in all cancers, including PDAC and can be broadly categorized into chromosomal instability (CIN) and microsatellite instability (MIN; refs. 4, 5). The spectrum of altered genes, as well as the types of

alterations, makes each PDAC rather distinctive. These alterations can be divided into required (nearly universally seen; such as, KRAS, and CDKN2A), frequently mutated (occurring in more than 25% of tumors; such as, SMAD4, TP53, and NCOA3) and low frequency mutations (occurring in less than 25% of tumors; such as RB1, BRCA2 and ERBB2; refs. 5, 6). Low-frequency mutations, including mutations in DNA damage repair (DDR) pathways, provide a very heterogeneous mutational background that gives each PDAC a unique molecular signature.

DDR pathways play an important role in the cellular response to platinum chemotherapy. FOLFIRINOX (5-Fluorouracil, leucovorin, irinotecan and oxaliplatin) is a platinum based chemotherapeutic regimen that has shown an unprecedented improvement in median overall survival (OS) of PDAC to 11.1 months (7). However, not all PDAC patients respond to FOLFIRINOX chemotherapy; objective response rate is around 32% (7). Chemotherapy resistance mechanisms are multifactorial, but alterations in DDR pathways such as nucleotide excision repair (NER), mismatch repair (MMR), and BRCA pathways (8) likely contribute significantly. For example, the excision repair cross-complementation group 1 (ERCC1) protein is a key component of nucleotide excision repair pathway. High expression of ERCC1 has been associated in multiple studies with acquired or intrinsic resistance to platinum drugs (8, 9). Similarly, loss of

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Translational Relevance

Emerging data suggest that DNA damage repair (DDR) gene mutations may be predictive of response to platinum-based chemotherapy in some cancers; however, the role of these mutations in pancreatic adenocarcinoma (PDAC) is unknown. FOLFIRINOX (5-Fluorouracil, leucovorin, irinotecan and oxaliplatin) is a platinum-based chemotherapeutic regimen that is commonly used as first-line treatment of patients with advanced PDAC. However, the efficacy of FOLFIRINOX is limited by its high toxicities and low objective response rate. Currently, there is no predictive biomarker to select patients for treatment with FOLFIRINOX. In this study, we report that the presence of DDR gene mutations is associated with improved OS in metastatic PDAC patients treated with FOLFIRINOX. These results will need to be further tested in a larger study and may serve to select PDAC patients for treatment with FOLFIRINOX.

function of MMR genes (mainly MLH1 and MSH2) has been associated with resistance to platinum compounds (10). Finally, BRCA1 and BRCA2 genes have established roles in DNA repair and chemotherapy sensitivity (11, 12). However, these data come from either preclinical studies or clinical studies done in other cancer types and the role of DDR pathway gene alterations on the efficacy of FOLFIRINOX in PDAC is not well known.

The primary objective of this study was to assess the effect of mutations in DDR genes (*BRCA1*, *BRCA2*, *PALB2*, *CHEK1*, *CHEK2*, *RAD51*, *MLH1*, *MSH2*, *ERCC1*, *ERCC4*, *PARP1*, *FANCF*, *ATR* and *MDC1*) on the OS of patients with metastatic PDAC treated with FOLFIRINOX.

Materials and Methods

Study population and patient selection

Patients were selected from the Indiana University Simon Cancer Center pancreatic cancer database, which is a large Institutional review board (IRB) approved high quality prospective and retrospective data collection repository (13). The main inclusion criterion (to allow for adequate available tissue) for the study was histologic diagnosis of recurrent pancreatic adenocarcinoma. Patients must have been treated after recurrence with FOLFIRINOX (in the first-line setting) and had available formalin-fixed paraffin embedded tissue for DNA sequencing. The individuals who met the inclusion criteria formed the final cohort for the study and underwent DNA sequencing. The study was approved by the institutional IRB with a waiver for informed consent. This was mainly because of the retrospective nature of the study and the fact that most patients had died at the time of study conception. The study was conducted in accordance with recognized ethical guidelines (Declaration of Helsinki, Belmont Report and U.S. Common Rule).

Data collection

Baseline demographic, clinical and pathologic information including age at diagnosis, gender, race, family history of any cancer, location of primary tumor, Eastern Cooperative Oncology Group (ECOG) performance status, body mass index (BMI),

baseline carbohydrate antigen 19-9 (CA 19-9), history of surgical resection, type of surgical resection (R0 or R1), adjuvant chemotherapy, pathological tumor and nodal stage were collected. The start date of FOLFIRINOX and date of death were collected for OS calculation.

DNA-extraction

Matched tumor-normal paired tissue sections with $\geq 20\%$ tumor cellularity were selected for DNA isolation. The sections were scraped from glass slides using microtome blades and the QIAamp DNA FFPE Tissue Kit (56404, Qiagen) was used for DNA isolation.

Next-generation sequencing for germline and somatic mutations

Germline mutation testing was done using adjacent normal tissue whereas somatic mutation testing was done using tumor tissue. Sequencing libraries were constructed using the Ion AmpliSeq Kit for Chef DL8 (A29024, Thermo Scientific) in conjunction with the Ion Comprehensive Cancer Panel Primer Pool (4477685, Thermo Scientific). Templates were prepared on the Ion Chef using the Ion P1 Hi-Q Chef Kit (A27198, Thermo Scientific). Templates were sequenced to at least 500X mean coverage on the Ion Proton sequencer using a P1 Chip v3 (A26771, Thermo Scientific). Genomic data (BAM files) were uploaded to Ion Reporter (Thermo Scientific) for somatic variant and copy-number calling. Ingenuity Variant Analysis (Qiagen) was also used for second-level annotation.

Statistical analyses

Descriptive statistics were used to summarize the baseline characteristics and mutations in DDR genes (*BRCA1*, *BRCA2*, *PALB2*, *CHEK1*, *CHEK2*, *RAD51*, *MLH1*, *MSH2*, *ERCC1*, *ERCC4*, *PARP1*, *FANCF*, *ATR* and *MDC1*). The study cohort was then divided into those with and without germline and somatic mutations in DDR genes. Baseline characteristics were compared between the two groups (DDR gene mutations present vs. absent) using χ^2 test for categorical variables and Student *t* test for continuous variables.

Two statistical approaches were used for data analyses. First, we analyzed the data using logistic regression analysis as in a case-control study to investigate the association between DDR gene mutations and OS (categorized as above and below 3rd quartile). Our goal here was to see whether PDAC patients with DDR gene mutations had higher odds of being in the highest quartile for OS. The cases were defined as PDAC patients with mutations in these genes and the controls were those without mutations. The multivariate logistic regression analysis was adjusted for age at diagnosis, gender, margin status at surgery, CA 19-9 level, adjuvant chemotherapy, family history of cancer, pathological T and N stage. Adjusted odds ratios (AOR) were reported along with 95% confidence intervals (CI).

Second, we conducted time-to-event analysis with the event being OS. OS was defined as the time from the start of chemotherapy with FOLFIRINOX to the time of death or last follow up. The Kaplan-Meier method was used for estimating OS. OS between those with and without mutations in DDR genes was compared by log-rank test. A Cox proportional hazard regression model was used to calculate hazard ratios (HR) and 95% confidence interval (CI) while adjusting for other covariates (same variables as for multivariate logistic

Sehdev et al.

regression analysis) between the two groups (DDR gene mutation present or absent). We also conducted an analysis with "OS from surgery" to understand whether the differences of OS in patients with and without DDR gene mutations were irrespective of the treatment with FOLFIRINOX. The "OS from surgery" was defined as the time interval from the date of surgery to the date of death or last follow up.

Finally, we also conducted a sensitivity analysis where we assessed the role of only *BRCA1* and *BRCA2* (*BRCA1/2*) mutations (instead of DDR gene mutations) on OS using the logistic and Cox regression models while adjusting for other covariates. For this study, $P \leq 0.05$ was considered statistically significant and $0.05 < P \leq 0.10$ was considered near significant. Data management and statistical analysis were performed with R 1.1.423 (14).

Results

Patient characteristics

There were 47 PDAC patients in the entire cohort who received platinum-based chemotherapy with FOLFIRINOX in the first-line metastatic setting. Of these 47 patients, 11 patients were excluded either due to inability to locate the tissue blocks ($N = 9$) or due to low tumor cellularity ($N = 2$). Thus, there were 36 PDAC patients in the final cohort that received FOLFIRINOX. All the patients had previously undergone curative intent resection of their PDAC. Baseline characteristics are summarized in Table 1. The median age for the entire cohort was 65.5 years (range, 45.1–82.7 years), 58% were male, and the majority (97.2%) were Caucasian. Family history of any cancer was noted in 51.4% of the patients. Most patients

Table 1. Baseline characteristics of patients

Variable	Overall cohort $N = 36$	Germline and somatic DDR genes		P^a
		Mutation absent $N = 24$ (66.7%)	Mutation present $N = 12$ (33.3%)	
Age, y				0.002
Median (range)	65.5 (45.1–82.7)	66.0 (45.1–82.7)	64.6 (45.7–74.8)	
Gender				0.28
Male	21 (58.3)	16 (66.7)	5 (41.7)	
Female	15 (41.7)	8 (33.3)	7 (58.3)	
Race, N (%)				0.72
Caucasian	35 (97.2)	24 (100)	11 (91.7)	
African American	1 (2.8)	0	1 (8.3)	
CA 19–9, U/mL				0.68
Normal	9 (25.0)	5 (20.8)	4 (33.3)	
Elevated	27 (75.0)	19 (79.2)	8 (66.7)	
Family history of cancer, N (%)				1
No	17 (48.6)	12 (50.0)	5 (45.5)	
Yes	18 (51.4)	12 (50.0)	6 (54.5)	
Location of primary tumor (%)				0.85
Head	32 (88.9)	22 (91.7)	10 (83.3)	
Body and Tail	4 (11.1)	2 (8.3)	2 (16.7)	
Tobacco use (%)				0.28
No	15 (48.7)	12 (50.0)	3 (25.0)	
Yes	21 (58.3)	12 (50.0)	9 (75.0)	
BMI (kg/m ²)				0.39
≤ 24.9	18 (50.0)	13 (54.2)	5 (41.7)	
24.9–29.9	13 (36.1)	9 (37.5)	4 (33.3)	
≥ 30	5 (13.9)	2 (8.3)	3 (25.0)	
Jaundice at presentation (%)				0.90
No	14 (38.8)	10 (41.7)	4 (33.3)	
Yes	22 (61.1)	14 (58.3)	8 (66.7)	
Diabetes at presentation (%)				1
No	23 (63.9)	15 (62.5)	8 (66.7)	
Yes	13 (36.1)	9 (37.5)	4 (33.3)	
Adjuvant gemcitabine, N (%)				0.86
No	5 (13.9)	4 (16.7)	1 (8.3)	
Yes	31 (86.1)	20 (83.3)	11 (91.7)	
Pathological T stage, N (%)				0.35
T1 and T2	4 (11.1)	3 (12.5)	1 (8.3)	
T3	32 (88.9)	21 (87.5)	11 (91.7)	
Pathological N stage, N (%)				0.39
N0	5 (13.9)	2 (8.3)	3 (25.0)	
N1	31 (86.1)	22 (91.7)	9 (75.0)	
Surgery, N (%)				1
R0 (Negative margin)	29 (80.5)	19 (79.2)	10 (83.3)	
R1 (Positive margin)	7 (19.5)	5 (20.8)	2 (16.7)	

Abbreviations: BMI, body mass index; CA, carbohydrate antigen; DDR, DNA damage repair; N, node; N, number; T, tumor.

^aCalculated by χ^2 except t test for age.

Table 2. Mutations seen in DNA damage repair genes in 12 patients (ID) of our cohort and their predicted functional significance

ID	Gene	Chromosome position	dbSNP ID	Function	Amino acid change	SIFT function prediction	PolyPhen-2 function prediction
1	BRCA1	17:41246481	rs1799950	Missense	p.Q356R; p.Q309R	Damaging	Probably damaging
2	BRCA1	17:41199716	rs80356920	Missense; Stop gain	p.C675*; p.V700D; p.V1757D; p.V1825D; p.V1804D	Tolerated	Benign
3	BRCA1	17:41246481	rs1799950	Missense	p.Q356R; p.Q309R	Damaging	Probably damaging
	BRCA1	17:41222975	rs1799967	Missense	p.M548I; p.M1673I; p.M1652I; p.M1605I	Tolerated	Benign
4	BRCA2	13:32914755	rs767567428	Missense	p.T2088I	Damaging	Benign
	BRCA2	13:32911295	rs28897716	Missense	p.D935N	Tolerated	Benign
5	BRCA1	17:41246061	rs28897677	Missense	p.R496H; p.R449H	Tolerated	Benign
6	BRCA1	17:41244429	rs4986852	Missense	p.S993N; p.S1040N	Tolerated	Benign
	BRCA2	13:32972626	rs11571833	Stop gain	p.K3326*	N/A	N/A
7	BRCA1	17:41246481	rs1799950	Missense	p.Q356R; p.Q309R	Damaging	Probably damaging
	PALB2	16:23646857	rs45494092	Stop gain	p.L337S	Tolerated	Benign
8	BRCA1	17:41222975	rs1799967	Missense	p.M548I; p.M1673I; p.M1652I; p.M1605I	Tolerated	Benign
	BRCA2	13:32912750	rs28897727	Missense	p.D1420Y	Damaging	Benign
9	BRCA2	13:32945172	rs11571747	Missense	p.E2856A	Tolerated	Probably damaging
10	PALB2	16:23646857	rs45494092	Missense	p.L337S	Tolerated	Benign
11	PALB2	16:23634293	rs45551636	Missense	p.G998E	Damaging	Probably damaging
	PALB2	16:23641461	rs45532440	Missense	p.E672Q	Tolerated	Benign
12	MSH2	2:47403319	rs17217723	Missense	Y43C	Intolerant	Probably damaging

(86.1%) had received postoperative adjuvant chemotherapy with gemcitabine.

DNA damage repair gene mutations

DDR gene mutations were found in 12 (33.3%) patients in our cohort. *BRCA1*, *BRCA2*, *PALB2*, *MSH2* and *FANCF* genes were mutated in 7 (19.4%), 5 (13.9%), 3 (8.3%), 1 (2.8%), and 1 (2.8%) patients, respectively (Table 2). There were no mutations (germline or somatic) seen in other DDR genes (*CHEK1*, *CHEK2*, *RAD51*, *MLH1*, *ERCC1*, *ERCC4*, *PARP1*, *ATR* and *MDC1*). Nine (25%) patients had one or more *BRCA1/2* mutation. Both *BRCA1* and *BRCA2* were mutated in 3 (8.3%) of these patients. The details of specific mutations seen in DDR genes are summarized in Table 2. Besides relatively younger age at diagnosis in those with DDR gene mutations (64.6 vs. 66.0 years respectively, $P = 0.002$), there were no significant demographic or clinical differences between those with and without mutations in DDR genes (Table 1).

DNA damage repair gene mutations and overall survival

The median OS for the entire cohort was 6 months (range, 1–24 months). The first and third quartile were 3 and 14 months, respectively. Eight (25%) patients had OS above the third quartile. Bivariate logistic regression showed that the patients with mutations in DDR genes had higher odds of being in the highest quartile of OS (OR, 1.34; 95% CI 1.01–1.77; $P = 0.05$). This association remained significant after adjusting for other potential prognostic variables in a multivariate logistic regression model (AOR, 1.47; 95% CI, 1.04–2.06; $P = 0.04$; Table 3). Interestingly, multivariate analysis did not reveal any other variables that were significantly associated with the highest quartile of OS (Table 3).

The time to event OS analysis included all 36 patients, 34 died and 2 were censored. The median OS was superior in those with presence of DDR gene mutations as compared with those without these mutations (14 vs. 5 months, respectively) although this association failed to reach statistical significance (HR, 0.58; 95% CI, 0.29–1.14, $P = 0.08$; Fig. 1). However, the

multivariable Cox regression model showed a significant improvement in OS in PDAC patients with DDR gene mutations as compared with those without these mutations (adjusted HR, 0.37; 95% CI, 0.15–0.94; $P = 0.04$; Table 3). None of the other covariates were significantly associated with OS although body/tail pancreatic tumors had a near significant lower hazard of OS compared with head of pancreas tumors (adjusted HR, 0.24; 95% CI, 0.05–1.22; $P = 0.08$; Table 3).

The median "OS from surgery" was not significantly different between those with presence of DDR gene mutations as compared with those without these mutations (23 vs. 18.5 months, respectively; Log-Rank $P = 0.94$).

BRCA1/2 gene mutations and overall survival

Bivariate logistic regression showed significantly higher odds of being in the highest quartile of OS with the presence of *BRCA1/2* mutations (OR, 1.56; 95% CI, 1.17–2.08; $P = 0.004$). This association remained significant after adjusting for other potential prognostic variables in a multivariate logistic regression model (AOR, 1.77; 95% CI, 1.26–2.46; $P = 0.003$; Table 4). None of the other covariates were significantly associated with highest quartile of OS in the multivariate analysis (Table 4).

Similarly, Kaplan–Meier analysis showed a superior median OS in those with *BRCA1/2* mutations as compared with those without these mutations (15 vs. 5 months), however this association was not statistically significant (HR, 0.64; 95% CI, 0.32–1.29; $P = 0.17$; Fig. 2). Interestingly, the multivariable Cox regression model showed a significant improvement in OS with the presence of *BRCA1/2* mutations (adjusted HR, 0.32; 95% CI, 0.11–0.94; $P = 0.04$; Table 4). Pathological nodal involvement (adjusted HR, 3.48; 95% CI, 1.06–11.39; $P = 0.04$) was significantly associated with higher hazard of dying from PDAC, although male gender (adjusted HR, 0.42; 95% CI, 0.15–1.20; $P = 0.10$), location of primary tumor in body/tail (adjusted HR, 0.21; 95% CI, 0.04–1.03; $P = 0.05$) were nearly significantly associated with lower hazard of dying from PDAC (Table 4).

Sehdev et al.

Table 3. Multivariate logistic and Cox regression analyses of Somatic and Germline DDR Mutations genes

Variable	Logistic regression		Cox regression	
	AOR (95% CI)	P	Adjusted HR	P
Somatic and germline DDR mutations				
Absent	1 (reference)		1 (reference)	
Present	1.47 (1.04–2.06)	0.05	0.37 (0.15–0.94)	0.04
Gender				
Female	1 (reference)		1 (reference)	
Male	1.27 (0.85–1.89)	0.26	0.50 (0.18–1.37)	0.18
Age at diagnosis	0.99 (0.97–1.00)	0.18	1.04 (0.99–1.10)	0.15
Primary tumor site				
Head	1 (reference)		1 (reference)	
Body/Tail	1.29 (0.78–2.11)	0.33	0.24 (0.05–1.22)	0.08
Family history of cancer, N (%)				
No	1 (reference)		1 (reference)	
Yes	1.03 (0.74–1.42)	0.87	1.29 (0.51–3.25)	0.59
Pathological T Stage				
T1/T2	1 (reference)		1 (reference)	
T3	1.10 (0.65–1.86)	0.72	0.71 (0.13–3.75)	0.69
Pathological N Stage				
N0	1 (reference)		1 (reference)	
N1	1.00 (0.63–1.58)	0.99	2.21 (0.67–7.25)	0.19
Margin involvement at surgery				
No	1 (reference)		1 (reference)	
Yes	0.88 (0.58–1.30)	0.52	1.73 (0.59–5.04)	0.32
CA 19–9, U/mL				
Normal	1 (reference)		1 (reference)	
Elevated	1.05 (0.72–1.52)	0.82	1.95 (0.64–5.93)	0.24
Adjuvant chemotherapy				
No	1 (reference)		1 (reference)	
Yes	1.04 (0.66–1.63)	0.87	0.53 (0.14–2.05)	0.47

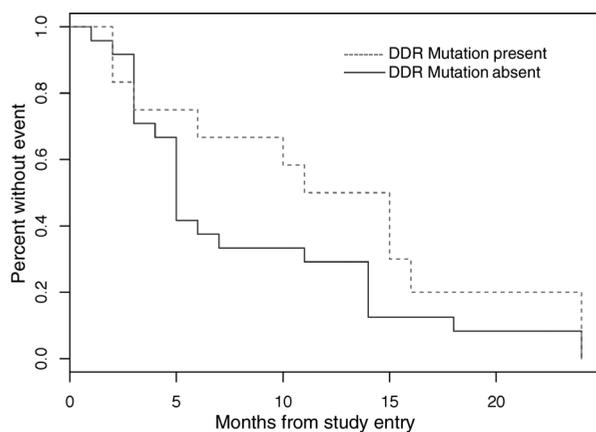
Abbreviations: AOR, adjusted odds ratio; CA, carbohydrate antigen; HR, hazard ratio; N, node; T, tumor.

Discussion

In this study, we found that within a cohort of recurrent PDAC patients treated with FOLFIRINOX, those with presence of DDR gene mutations (germline and somatic) have significantly longer OS compared with those without mutations in DDR genes. We also found that the presence of germline BRCA1/2 mutations was associated with longer OS in PDAC patients treated with FOLFIRINOX. Deleterious germline

mutations in the absence of significant family history has been established as a risk factor and potential therapeutic target for PDAC (15); however, the effect of both germline and somatic DDR gene mutations on the OS in PDAC patients treated with FOLFIRINOX has not been well known. In addition, our study also validates the association of germline BRCA1/2 mutations and sensitivity to platinum-based chemotherapy reported by others in PDAC patients (16, 17) and expands the hypothesis beyond germline BRCA1/2 mutations to both somatic and germline mutation in DDR genes. Notably, our results of improved OS with platinum-based chemotherapy in patients with germline BRCA1/2 mutations are similar to those reported by other large multi-institutional studies (16).

DDR gene mutations play an important role in double stranded DNA damage repair via homologous recombination, and defects in these genes may predict response to platinum-based chemotherapy (16, 18, 19). Platinum compounds cause intercalation of the DNA and distortion of the DNA-helix (20), which requires an intact dsDNA repair apparatus for restoration. DDR-impaired tumors are therefore sensitive to platinum-based chemotherapy. Specifically, germline BRCA mutations have been shown to predict response to platinum-based chemotherapy in breast and ovarian cancers (18, 21). Precision treatment strategies are an attempt to exploit tumor-specific mutations such as those seen in DDR genes (22). However, this is a developing area and not much is known about molecular subtypes in PDAC as opposed to other cancers such as breast, colon, gastric, bladder, and lung where molecular subtypes have been well defined (23–27). Notably, these

**Figure 1.**

Kaplan-Meier curve in patients with and without mutations in Germline and Somatic DNA Damage Repair (DDR) genes.

Table 4. Multivariate logistic and Cox regression analyses of BRCA1/2 genes

Variable	Logistic regression		Cox regression	
	AOR (95% CI)	P	Adjusted HR	P
BRCA1/2 Mutations				
Absent	1 (reference)		1 (reference)	
Present	1.77 (1.26-2.46)	0.003	0.32 (0.11-0.94)	0.04
Gender				
Female	1 (reference)		1 (reference)	
Male	1.36 (0.95-1.97)	0.11	0.42 (0.15-1.20)	0.10
Age at diagnosis	0.99 (0.97-1.00)	0.20	1.04 (0.99-1.10)	0.12
Primary tumor site				
Head	1 (reference)		1 (reference)	
Body/Tail	1.39 (0.89-2.17)	0.16	0.21 (0.04-1.03)	0.05
Family history of cancer, N (%)				
No	1 (reference)		1 (reference)	
Yes	1.07 (0.79-1.44)	0.66	1.10 (0.44-2.75)	0.84
Pathological T Stage				
T1/T2	1 (reference)		1 (reference)	
T3	0.98 (0.61-1.59)	0.95	0.85 (0.18-4.09)	0.84
Pathological N Stage				
N0	1 (reference)		1 (reference)	
N1	0.86 (0.58-1.29)	0.47	3.48 (1.06-11.39)	0.04
Margin Involvement at surgery				
No	1 (reference)		1 (reference)	
Yes	0.90 (0.63-1.29)	0.58	1.66 (0.58-4.75)	0.34
CA 19-9, U/mL				
Normal	1 (reference)		1 (reference)	
Elevated	0.96 (0.68-1.35)	0.82	2.15 (0.71-6.48)	0.17
Adjuvant chemotherapy				
No	1 (reference)		1 (reference)	
Yes	1.04 (0.69-1.57)	0.85	0.63 (0.17-2.31)	0.49

Abbreviations: AOR, adjusted odds ratio; CA, carbohydrate antigen; HR, hazard ratio; N, node; T, tumor.

cancer subtypes have been found to have specific therapeutic targets and have shown to respond differently to anti-cancer drugs (25, 28-30). To our knowledge there is only one large study that has classified PDAC into 4 subtypes based on the genomic alterations: stable, locally rearranged, scattered, and unstable, and has shown good response to platinum-based chemotherapy in patients with unstable subtype and/or high BRCA mutational signature (17). Our study is notable for having a significantly larger number of patients to better explore this association.

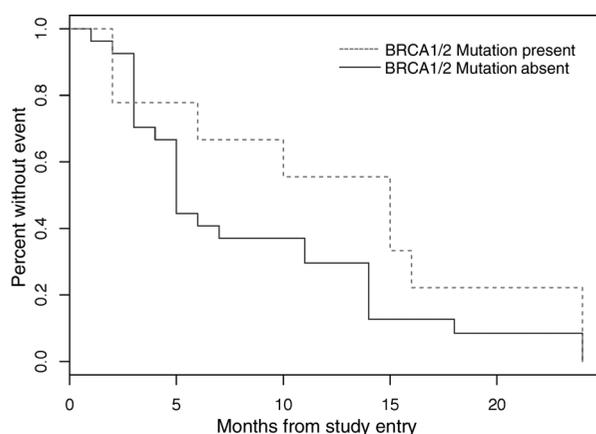


Figure 2. Kaplan-Meier curve in patients with and without mutations in BRCA1/2 genes.

The overall DDR gene mutation frequency in our cohort was 33.3%, which is clearly higher than what was found in other next-generation sequencing studies (31). For instance, Petersen and colleagues (32) found germline DDR gene mutations in 12% of patients with familial PDAC. In high-risk groups (Ashkenazi Jewish), mutation prevalence of up to 17% has been reported (33). This difference in DDR gene mutation prevalence might be due to the selection of different gene panels in different studies and lack of inclusion of both somatic and germline mutation in other studies. Despite the higher prevalence of DDR gene mutations, the genes that were found to be mutated in our study (*BRCA1*, *BRCA2*, *PALB2*, *MSH2*, and *FANCF*) were not different from those reported in other studies (*BRCA1*, *BRCA2*, *PALB2*, *MSH2*, *MLH1* and *MSH6*). Of note, we excluded *ATM* gene mutations (seen in 2 patients) in our analysis as the emerging data suggests that *ATM* gene mutations does not contribute to homologous recombination DNA repair (34).

The results of our study are not definitive, but are hypothesis generating. Clinical application of these results will require prospective validation of our results where specific DDR gene mutations are targeted with specific compounds. Interestingly, immunotherapy with anti-programmed death 1 antibody is one such strategy that is now approved for the treatment of PDAC patients with microsatellite instability (35). Similarly, clinical trials of PARP inhibitors have shown positive results in ovarian cancer patients with non-germline BRCA-mutated tumors (36, 37) and the studies are ongoing in PDAC. In a recent phase I clinical trial in PDAC patients, olaparib (PARP inhibitor) added to irinotecan, cisplatin, and mitomycin C showed durable clinical responses however due to

substantial toxicity the combination was considered unacceptable for further development (38). In another phase II trial, rucaparib (PARP inhibitor) as a single agent has shown to have 11% objective response rate in patients with advanced PDAC who have received 1 to 2 prior chemotherapies. There are several other clinical trials (NCT01585805, NCT01489865, NCT01989546) including a phase III, randomized controlled trial (NCT02184195) that are currently ongoing to assess the role of PARP inhibitors in PDAC patients with germline or somatic BRCA mutations. In addition, trials are also ongoing to assess the effect of ATR inhibitors alone (NCT02223923, NCT02264678, NCT02630199) or in combination with PARP inhibitors (NCT02723864) in solid tumors.

There are several strengths of our study. First, we restricted testing to patients who had previously undergone surgery. This allowed for adequate tissue for analysis in the vast majority of patients. Lack of available tissue is a major barrier to carrying out translational research in PDAC. Second, we used a high-quality, well-annotated clinical database. Third, we tested the hypothesis of association between germline and somatic DDR gene mutations and OS as well as BRCA1/2 gene mutations and OS in PDAC patients.

Together with the strengths of our study, there are several potential limitations. First, although we used a well-annotated and manually curated database and controlled for multiple potential confounders, our results might have been affected by residual confounding due to the retrospective nature, relatively small and single institution cohort. Second, our cohort was limited to only patients with initially localized disease who underwent surgical resection excluding a large proportion of PDAC patients with initially locally advanced or metastatic disease potentially having an aggressive biology thereby raising a possibility of selection bias. Third, we were unable to control for comorbidities and other competing risks of mortality that might have affected our results. However, because FOLFIRINOX is indicated for relatively healthier PDAC patients with good performance status, we do not think that the difference in comorbidities are the only explanation of our results. Fourth, we were unable to gather detailed family history of cancer due to the retrospective nature of this study. However, positive family history of cancer was noted in 51.4% of patients and we controlled for family history of cancer in our multivariate models. Fifth, because we could not analyze for progression-free survival, we cannot say for sure that our results are due to finding a better prognostic group rather than being more sensitive to FOLFIRINOX per se. However, we did not notice any significant difference between the patients with and without DDR gene mutation while analyzing for "OS from surgery."

Finally, we acknowledge that the median OS of our cohort is relatively lower than expected for a *de novo* metastatic population, but there are relatively few reports of survival in the post-

surgical recurrence setting. Our own internal data suggest that it is shorter than that seen in studies of mostly newly diagnosed patients. Another possible reason could be lack of second-line chemotherapy. In our cohort, 15 patients did not receive any second-line chemotherapy. Of those who received second-line chemotherapy, 8 patients received gemcitabine and nab-paclitaxel and the rest received mostly single agent chemotherapy with gemcitabine or capecitabine alone. In fact, many patients who were started on FOLFIRINOX were later reduced to FOLFOX or XELOX. Impaired tolerance to FOLFIRINOX and lack of or ineffective second-line chemotherapy probably explains the relatively lower poor median OS.

In conclusion, in a small, single institution, retrospective study, we found that the presence of DDR gene mutations (germline and/or somatic) as well as germline BRCA1/2 mutations are associated with improved OS in PDAC patients treated with FOLFIRINOX. These results validate the association of germline BRCA1/2 mutations and platinum sensitivity reported by others in PDAC patients (16, 17), and expands the hypothesis beyond germline BRCA1/2 mutations to both somatic and germline mutation in DDR genes that needs to be further tested in a larger study.

Disclosure of Potential Conflicts of Interest

A. Sehdev is a consultant/advisory board member for Eisai Inc. No potential conflicts of interest were disclosed by the other authors.

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Development of methodology: A. Sehdev

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Sehdev, O. Gbolahan, J. Wan, M. Radovich, B.H. O'Neil

Writing, review, and/or revision of the manuscript: A. Sehdev, O. Gbolahan, S. Shahda, H.H. Wu, M. Radovich, B.H. O'Neil

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