Supplementary Online Content

Jameson GS, Borazanci E, Babiker HM, et al. Response rate following albumin-bound paclitaxel plus gemcitabine plus cisplatin treatment among patients with advanced pancreatic cancer: a phase 1b/2 pilot clinical trial. *JAMA Oncol*. Published online October 3, 2019. doi:10.1001/jamaoncol.2019.3394

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. Methods

Genomic Analysis

Enrolled patients were invited to participate in an optional biomarker sub-study for comparative genomic hybridization (CGH) and next generation sequencing (NGS). This participation required a separate written informed consent for the collection and use of tissue samples from previous diagnostic biopsy or surgery.

DNA content flow cytometry of the tissue samples was performed as follows:
For FFPE samples excess paraffin was removed with a scalpel from either side of 40-60 μm scrolls then processed for removal of protein crosslinks and recovery of nuclei, while frozen tissue biopsies were minced in the presence of NST buffer and DAPI according to our published protocols. [1-3] Nuclei from each sample were disaggregated then filtered through a 40 μm mesh prior to flow sorting with an Influx cytometer (Becton-Dickinson, San Jose, CA) with ultraviolet excitation and DAPI emission collected at >450 nm. DNA content and cell cycle were analyzed using the software program MultiCycle (Phoenix Flow Systems, San Diego, CA). Tissue from patient 10140202 had a small (<10% of tissue content) aneuploid (3.2N) peak in the presence of much larger diploid peak, while the remaining two samples had only diploid (2N G_0/G_1) and corresponding tetraploid (4N G_2/M) peaks in their flow cytometry profiles. The frozen biopsy from patient 10140206 also had a small aneuploid (3.8N) peak in the presence of a much larger diploid fraction in the flow sorting profile. DNA was extracted from the nuclei of each sorted peak then processed for genomic analyses. For FFPE samples we used the Agilent SureSelect$^{XT\ HS}$ kit (Agilent Technologies Santa Clara CA) to make libraries from the sorted diploid and aneuploid fractions. Libraries for fresh frozen samples were prepared directly from aliquots of DNA that were also used for CNV analysis using Agilent 400k oligonucleotide CGH arrays. In all cases whole exome sequencing was done using Agilent_V6_PlusUTR_hs37d5_Baits through the Mayo Clinic Medical Genome Facility (MGF) according to established protocols.

Germline Testing

Germline testing was not required of all patients but was done as part of routine medical care and results were collected as part of this study. Germline testing was performed through Ambry Genetics (Aliso Viejo, CA), GenDX (Genome Diagnostics B.V., Netherlands), Myriad Genetics (Salt Lake City, UT), or Invitae (San Francisco, CA). All germline testing was done through CLIA certified clinical diagnostic laboratories.

eTable 1. Patient Detail

Subject ID	Sex	Age	Primary Tumor Location	Prior Surgery	Mets	Prior Adjuvant Therapy	Germline Mutation or VUS	Dose	Total No. Cycles	Best Response	OS (days) As of 12/5/18	Reason for DC
10140201	F	60	Head	Aborted Whipple	Liver	No	Negative	25	12	CR	1815+	Maximum benefit achieved
10140202	F	65	Tail	Aborted Whipple	Liver , omental	No	Not Done	25	8	PR	374	Toxicity
10140203	F	62	Head	Whipple	Lung, liver	Yes Gem/5FU,RT	Not Done	25	7	PR	498	Pt choice to discontinue treatment
10140204	M	62	Tail	No	Omentum, small intestine	No	Negative	50	7	PR	310	Clinical progression
10140205	F	47	Body	No	Lung (bilateral), bone	No	VUS-RAD51C	25	9	PR	1102	Maximum benefit achieved
10140206	M	57	Head	Aborted Whipple	Liver	No	VUS-MUTYH, ATM likely benign	25	8	CR	1312	Maximum benefit achieved
10140209	M	75	Head	Whipple	Mesenteric, peritoneal	Yes FOLFIRNO, RT	Not Done	37.5 25	2	PD	104	Clinical progression
10140210	M	73	Tail	No	Liver	No	Not Done	37.5	3	PD	450	Confirmed disease progression
10140211	F	66	Body	No	Liver	No	Negative	37.5	12	PR	733	Maximum benefit achieved
10140212	F	68	Body	Aborted Whipple	Liver	No	BRCA2	25	14	PR	1378+	Maximum benefit achieved
10140213	F	61	Tail	No	Liver	No	MUTYH	25	9	SD	479	Maximum benefit achieved
10140214	F	56	Body	No	Liver	No	BRCA2	25	4.5	PR	907	Toxicity
10140215	M	75	Body	No	Liver	No	Not Done	25	9	PR	510	Maximum benefit achieved
10140216	M	68	Head	No	Liver	No	Negative	25	2	PD	235	Confirmed disease progression
10140217	F	66	Head	Whipple	Lung (bilateral)	Yes Gem/Abraxane	Not Done	25	5	SD	732	Adverse event-stroke
10140218	M	68	Body	No	Liver	Yes Gem/Abraxane	BRCA2; VUS-POLD1, MSH6	25	8	PR	1099+	Maximum benefit achieved
10140219	M	60	Tail	No	Mesenteric	No	Negative	25	14	PR	771	Maximum benefit achieved
10140220	F	54	Tail	No	Liver, lungs	No	Negative	25	10.5	PR	501	Confirmed disease progression
10140221	M	71	Body	No	Liver	No		25	8	PR	305	Confirmed disease progression
10140223	F	79	Head	Whipple	Lung	Yes Gem/Abraxane	VUS- ATM	25	7.5	SD	211	Clinical progression
10140224	M	53	Body	No	Liver	No	Not Done	25	8	PR	351	Confirmed disease progression
10140225	M	62	Head	No	Liver	No	VUS- POLE	25	4	SD	96	Grade 5 SAE: CVA
10140226	M	75	Head	No	Liver	No	Not Done	25	1	NA	31	Grade 5 SAE: cryptosporidium infection (Pt chose to discontinue treatment for infection and cancer)
10340202	M	63	Body	No	Liver	No	Not Done	25	7	PR	154	Grade 5 SAE: unwitnessed cardiac arrest
10390201	M	56	Head	Whipple	Liver	Chemo/RT	Not Done	25	11	PR	747	Adverse event-sepsis C1, D4 unrelated

eTable 2. Grade 3-5 Treatment-related adverse events

Grade 3-5 Treatment-related adverse events (worse grade ever by patient)	Grade 3		Grade 4		Grade 5	
System Organ Class/Preferred Term	N=25 n (%)		N=25 n (%)		N=25 n (%)	
Total Patients with AEs by Maximum Grade	12 (48.0)		9 (36.0)		2 (8.0)	
Platelet count decreased	8	(32.0)	9	(36.0)	0	
Neutrophil count decreased	5	(20.0)	1	(4.0)	0	
White blood cell decreased	1	(4.0)	1	(4.0)	0	
Lymphocyte count decreased	1	(4.0)	0		0	
Lymphocyte count increased	1	(4.0)	0		0	
Anemia	8	(32.0)	0		0	
Febrile neutropenia	0		1	(4.0)	0	
Diarrhea	2	(8.0)	0		0	
Nausea	1	(4.0)	0		0	
Vomiting	1	(4.0)	0		0	
Fatigue	1	(4.0)	0		0	
Fever	1	(4.0)	0		0	
Dehydration	2	(8.0)	0		0	
Hypokalemia	1	(4.0)	0		0	
Anorectal infection	1	(4.0)	0		0	
Acute cryptosporidiosis	0		0		1	(4.0)
Stroke	0		0		1	(4.0)
Peripheral motor neuropathy	1	(4.0)	0		0	
Epistaxis	1	(4.0)	0		0	

Note: Percentages are based on the total number of patients in the analysis population.

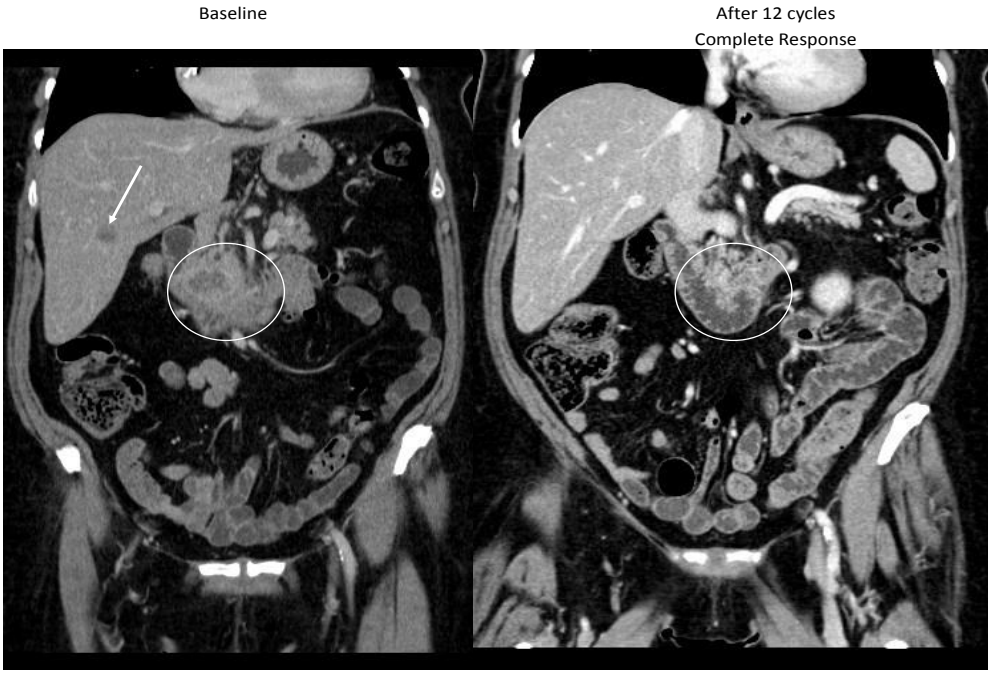
Note: A patient who experienced multiple events within a system organ class (SOC) or preferred term was counted once for that class and once for the preferred term at the maximum observed grade.

eTable 3. Germline testing results and overall survival

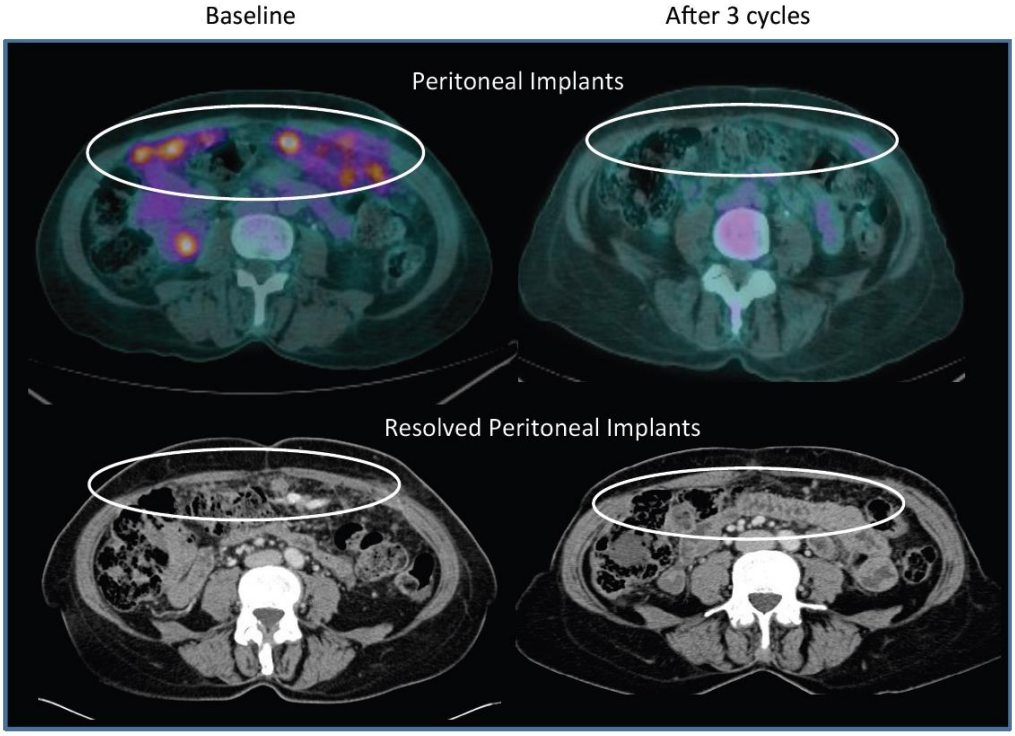
Pt #	Sex	Age at time of consent	Germ Line Mutation or Variance of Unknown Significance (VUS)	OS mos as of 12/05/18
201	F	60	Negative	59.7+
204	M	62	Negative	10.2
205	F	47	VUS-RAD51C	36.2
206	M	57	VUS-MUTYH, ATM likely benign	43.1
211	F	66	Negative	24.1
212	F	68	BRCA2	45.3+
213	F	61	MUTYH	15.7
214	F	56	BRCA2	29.8
216	M	68	Negative	7.7
218	M	68	BRCA2; VUS-POLD1, MSH6	36.1+
219	M	60	Negative	25.3
220	F	54	Negative	16.5
223	F	79	VUS- ATM	6.9
225	M	62	VUS- POLE	3.2

eFigure. Representative Patient Responses. (A) Patient 10140201 Images- Resolved Pancreas Mass and Liver Metastasis, (B) Patient 10140202 Images- Resolved Peripancreatic Mass

A. Patient 10140201- Resolved Pancreas Mass and Liver Metastasis



B. Patient 10140202- Resolved Peripancreatic Mass



eAppendix 2. Results

Genomic Analysis

We obtained diagnostic formalin-fixed paraffin-embedded (FFPE) tissue samples for 13 patients. Intact tissue was not detected in 4/13 FFPE samples, and only 3/9 of the remaining samples had sufficient yield of nuclei (>20,000) after sorting of tumor cells from normal cells for analysis. Unfortunately, only one (patient 202) had tumor present based on the detection of somatic mutations in exome sequencing performed on that specimen. We also obtained a post study treatment frozen tumor tissue sample on patient 206 who underwent a Whipple procedure, of a lesion that appeared on the pancreas after 18 months of response to chemotherapy. The results of these genomic analyses are summarized below for each of these two patients.

Genomic analysis of the baseline specimen for patient 202: In addition to pathogenic mutations in *KRAS*^{G12R}, *TP53*^{Q144K}, and *EGFR*^{S645C}, and a homozygous deletion of *CDKN2A*, patient 202 had a somatic homozygous *BRCA2*^{F3090L} variant. This variant has been described as a rare germ line variant of unknown significance (VUS) associated with familial breast and ovarian cancer .[4] This patient experienced resolution of peritoneal metastases within 9 weeks, eFig 1b.

Genomic analysis of post treatment specimen for patient 206: This patient experienced a durable CR after 8 cycles of chemotherapy, underwent a Whipple resection 20 months after start of treatment, and had an OS of 43.7 months. The tumor tissue obtained in the resection showed the presence of a homozygous deletion of *CDKN2A*, and somatic *KRAS*^{G12D} and *TP53*^{G244D} mutations confirming the tumor nature of the 3.8N population. Strikingly we also detected somatic mutations in *RNF43*^{R132X} and *CDK13*^{R986C} in the 3.8N genome. RNF43 is an E3 ubiquitin-protein ligase that negatively regulates WNT signaling. CDK13 is required for RNA splicing and is a key regulator of transcription elongation. Of note, there was no tumor tissue available from initial diagnostic liver biopsy from this patient for comparison.

Genomic analysis, while limited, yielded interesting insights. In patient 202, tumor genomic analysis yielded a variant of unknown significance (VUS) in the BRCA2 gene, a gene frequently linked to both familial pancreatic cancer and to treatment response in cancers with DNA repair abnormalities.[4] Although classified as a VUS the somatic and homozygous nature of BRCA2^{F3090L} variant in patient 202 suggests a role in the rapid initial response to addition of cisplatin given established efficacy in tumors with DNA damage repair alterations. In contrast a known nonpathogenic heterozygous single nucleotide BRCA1^{K1183R} polymorphism was present at the same allele frequency in both the germ line (2.0N) and tumor (3.2N) genomes.

As mentioned in the analysis of patient 206, the patient's tumor genome had a homozygous deletion of *CDKN2A*, and *KRAS*^{G12D} and *TP53*^{G244D} somatic mutations. In addition to these prevalent lesions, we also detected somatic mutations in RNF43 and CDK13. Mutations in RNF43 have been reported in a subset (7%) of pancreatic cancers and in almost 20% of colorectal cancers, notably frameshift mutations in MSI+ tumors.[5,6] However there was no evidence for MSI in this tumor. CDK13 is a member of the cyclin dependent kinase family most closely related by sequence to the RNA polymerase regulator CDK12.[7] Notably inactivating mutations in CDK12 are associated with genomic instability in ovarian cancer and sensitize cells to agents that induce lesions repaired by HR, and to poly(ADP-ribose) polymerase (PARP) inhibitors.[8] The pathogenic CDK13 R986C variant has been reported in chronic myelogenous leukemia.[9] However the exact function of CDK13 remains to be determined. The aforementioned alterations correlated with responses, albeit low powered, and hint towards potential efficacy of this regimen not only in DNA damage repair alterations but also in other pathways such as cell cycle and Wnt pathways which can be investigated in future studies.

Germline Testing

Germline testing was not a part of this study. However, 14 patients underwent germline testing as part of routine medical care. Of those, 8 patients had germ line mutations and/or variants detailed in eTable 3). Previous work has suggested a greater efficacy of platinum agents in individuals with germline mutations in BRCA1/2.[3,10] Three patients with germ line BRCA mutations survived > 30 months (patients 212, 214, and 218). In the case of the longest survivor to date, (59·7+ months) who achieved a CR there was no identifiable germline mutation or variant suggestive of DNA repair deficiency.

eReferences

1. Holley T, Lenkiewicz E, Evers L, Tembe W, Ruiz C, Gsponer JR, et al. Deep clonal profiling of formalin fixed paraffin embedded clinical samples. *PLoS One*. 2012; **7**(11):e50586. doi: 10.1371/journal.pone.0050586

2. Rabinovitch PS, et al., Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol.* 2001 Nov; **96**(11):3071–83. doi: 10.1111/j.1572-0241.2001.05261.x

3. Barrett, MT, Deiotte R, Lenkiewicz E, Malasi S, Holley T, Evers L, et al. Clinical study of genomic drivers in pancreatic ductal adenocarcinoma. *Br J Cancer.* 2017 Aug 8;117(4): p. 572–582.

4. Hakansson S, Johannsson O, Johansson U, Sellberg G, Loman N, et al. Moderate frequency of BRCA1 and BRCA2 germ-line mutations in Scandinavian familial breast cancer. *Am J Hum Genet.* 1997 May; **60**(5):1068–78.

5. Giannakis M, Hodis E, Jasmine Mu X, Yamauchi M, Rosenbluh J, Cibulskis K, et al. RNF43 is frequently mutated in colorectal and endometrial cancers. *Nat Genet*. 2014 Dec; **46**(12):1264–6. doi: 10.1038/ng.3127

6. Cancer Genome Atlas Research Network. Integrated genomic characterization of pancreatic ductal adenocarcinoma. *Cancer Cell.* 2017 Aug 14; **32**(2):185–203.e13. doi: 10.1016/j.ccell.2017.07.007

7 Even Y, Escande ML, Fayet C, Genevière AM. CDK13, a kinase involved in pre-mRNA splicing, is a component of the perinucleolar compartment. *PLoS One*. 2016 Feb 17; **11**(2):e0149184. doi: 10.1371/journal.pone.0149184

8. Popova T, Manié E, Boeva V, Battistella A, Goundiam O, Smith NK, et al. Ovarian cancers harboring onactivating mutations in CDK12 display a distinct genomic instability pattern characterized by large tandem duplications. *Cancer Res*. 2016 Apr 1; **76**(7):1882–91. doi: 10.1158/0008-5472.CAN-15-2128

9. Mason CC, Khorashad JS, Tantravahi SK, Kelley TW, Zabriskie MS, Yan D, et al. Age-related mutations and chronic myelomonocytic leukemia. *Leukemia*. 2016 Apr; **30**(4):906–13. doi: 10.1038/leu.2015.337

10. Golan T, Kanji ZS, Epelbaum R, Devaud N, Dagan E, Holter S, et al. Overall survival and clinical characteristics of pancreatic cancer in BRCA mutation carriers. *Br J Cancer*. 2014 Sep; **111**(6):1132–8. doi: 10.1038/bjc.2014.418

