Identification Of Novel Protein Biomarkers For FOLFIRINOX-Based Chemotherapy Response In Advanced Pancreatic Adenocarcinoma Using Patient Omics And Bayesian AI

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BACKGROUND

Background: FOLFIRINOX-based chemotherapy regimens are considered standard of care for advanced pancreatic cancer and treatment options become limited once patients are refractory to this initial treatment. The overwhelming risk of standard of care treatment failure for advanced pancreatic cancer highlights the need to identify biomarkers predictive of treatment-failure to improve clinical outcomes.

Methods: Project Survival® (PS), NCT02781012, is a longitudinal (7 yrs) prospective clinical trial of 452 patients, in which 34 patients with pancreatic ductal adenocarcinoma (PDAC) treated with FOLFIRINOX-based regimen were identified. Median overall survival (OS) was 266 days. Multi-omic (global quantitative proteomics, metabolomics, structural lipidomics, and signaling lipidomics) analysis of plasma and buffy coat (proteomics only) were assessed for quantitative differences. Differential analysis of patients surviving less than or greater than 266 days identified 260 analytes (p < 0.05) between OS < 266 days to cohort with OS > 266 days respectively.

Results: To investigate the utility of these markers, we evaluated multi-omic analysis in a completed clinical trial investigating BPM 31510-IV (NCT# 02650804), which would represent an independent cohort of 42 PDAC subjects refractory to FOLFIRINOX at baseline of the Phase 2 clinical trial. BPM 31510 is an ubidecarenone-lipid conjugate nanodispersion formulation that was being investigated with GEMCITABINE for the treatment of PDAC patients. Of the 260 analytes identified in the FOLFIRINOX PDAC group from PS, 4 proteins in buffy coat demonstrated utility in stratifying response to treatment based on length of survival when the markers were measured at baseline prior to treatment. These candidate markers are involved in Keratin regulated processes, WNT signaling, and cell cycle regulation.

Conclusions: The expression of these candidate markers in additional cohorts will be further assessed to demonstrate the utility of the panel for early prediction of therapeutic response in the highly refractive PDAC population.
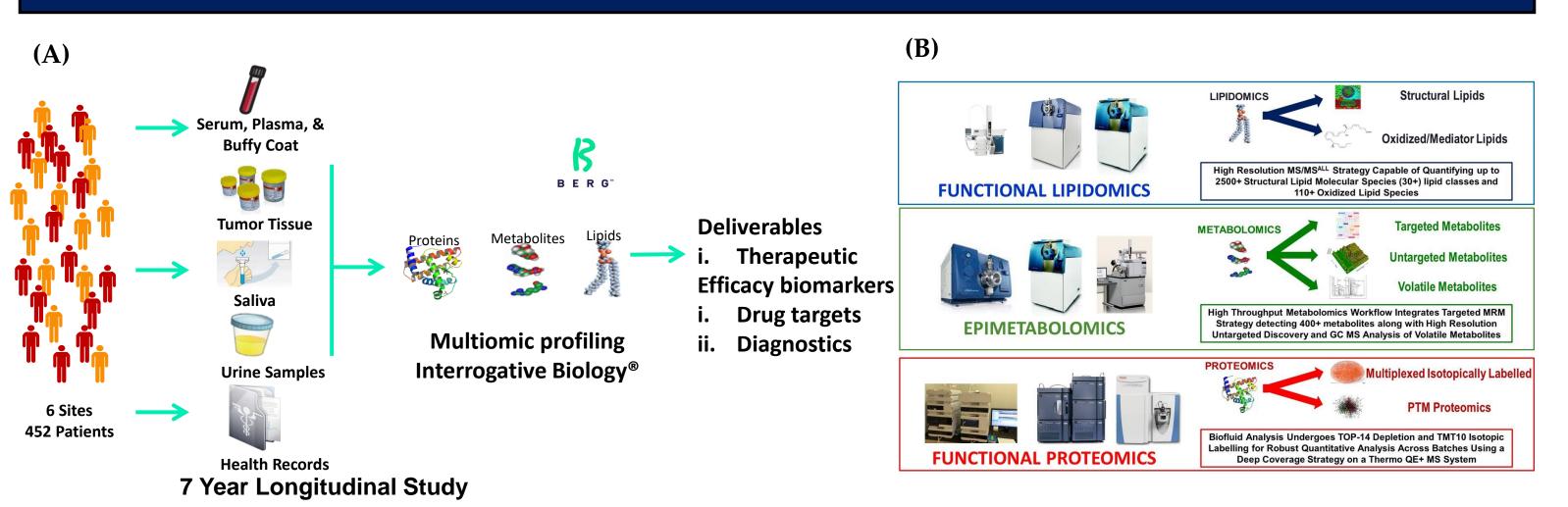
STUDY DESIGN AND MULTI-OMIC PLATFORM

Figure 1. (**A**) Study workflow: longitudinal biospecimen collection with detailed clinical, radiographic, and pathological annotation endpoints to minimize bias; Multiomic network generation using Bayesian Artificial Intelligence to identify novel biomarkers and therapeutic targets. (**B**) Lipidomic, metabolomic, and functional proteomic mass spectrometry platforms employed for bioanalysis.

INFORMATIC WORKFLOW FOR BIOMARKER ANALYSIS

Figure 2. Biological samples are collected from patients and interrogated by multi-omics profiling. Technology-specific pipelines convert raw measurements into processed data, which are integrated with clinical records. This integrated data is analyzed by bAIcis® to develop cause-and-effect networks and to perform statistical analysis. Normalization of biological data is performed by an empirical Bayes method, ComBat (Johnson *et al.* 2007. *Biostatistics*. **8**(1):118-27).
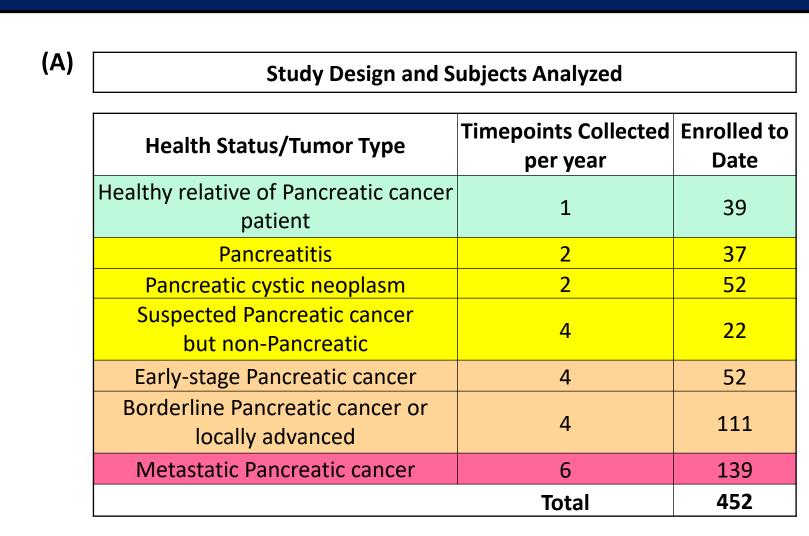
BASELINE CHARACTERISTICS OF FOLFIRINOX-TREATED COHORT

	Non-Responders	Responders
	17	17
AGE - median (min - max)	63 (41 - 85)	66 (49 - 78)
SEX - n(%)		
nMale	12 (%=70.59)	9 (%=52.94)
nFemale	5 (%=29.41)	8 (%=47.06)
BMI - median (min - max)		
BMI_all	25.3 (19.4 - 37.4)	28.2 (20.9 - 33.3)
BMI_male	26.1 (19.4 - 37.4)	28.2 (25.5 - 32.9)
BMI_female	22.8 (22.4 - 36.7)	27.9 (20.9 - 33.3)
Disease Categories - (N)		
Early PAN	2	1
Local PAN	10	13
Metastatic PAN	5	3
Race (N)		
ASIAN	0	0
BLACK	1	1
NATIVE	0	0
OTHERRACE	0	0
PACIFIC	0	0
WHITE	16	16

	Non-Responders	Responders
Tobacco Use - n (%)		
Tobacco use (current)	6 (%=35.29)	1 (%=5.88)
Tobacco use (former)	8 (%=47.06)	10 (%=58.82)
Tobacco use (never)	3 (%=17.65)	6 (%=35.29)
History - n (%) or median (min - max) (n) (IQR)		
Family history of PDAC	1 (%=5.88)	3 (%=17.65)
Diabetic	6 (%=35.29)	3 (%=17.65)
Past Pancreatitis	3	3
Jaundice	4	0
Weight Loss (lbs)	8.5 (1 - 24) (n=12) (IQR=4.375)	7.85 (3 - 11) (n=12) (IQR=3.5)
Pain (N)		
Pain (Mild)	2	4
Pain (Moderate)	1	1
Pain (Severe)	6	4
Lab Values - median (min - max) (N)		
ALB	3.95 (3.1 - 4.7) (n=12)	3.9 (3.2 - 4.5) (n=5)
BILI	0.65 (0.2 - 9.8) (n=14)	1 (0.2 - 4.8) (n=9)
CA199	686 (52 - 9619) (n=13)	345.5 (4 - 12443) (n=14)
CEA	5.8 (2.3 - 29.4) (n=11)	7.3 (1 - 3542) (n=11)
ECOG (N)		
ECOG [0]	7	10
ECOG [1]	10	4
ECOG [2]	0	1
ECOG [3]	0	1
ECOG [4]	0	0

Table 1. Baseline characteristics of responders and non-responders prior to treatment with FOLFIRINOX.
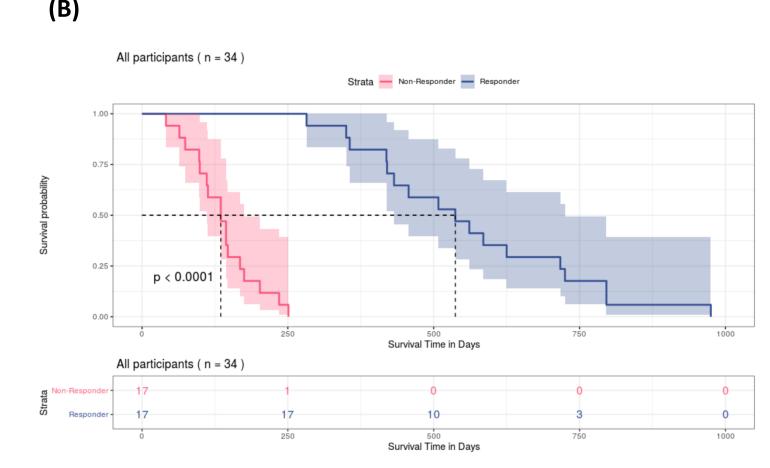
OVERVIEW OF BIOMARKER COHORT AND CLINICAL OUTCOME

(A)

Study Design and Subjects Analyzed

Health Status/Tumor Type	Timepoints Collected per year	Enrolled to Date
Healthy relative of Pancreatic cancer patient	1	39
Pancreatitis	2	37
Pancreatic cystic neoplasm	2	52
Suspected Pancreatic cancer but non-Pancreatic	4	22
Early-stage Pancreatic cancer	4	52
Borderline Pancreatic cancer or locally advanced	4	111
Metastatic Pancreatic cancer	6	139
Total		452

Figure 3. (**A**) Clinical study design for enrollment of clinical groups in Project Survival®, a prospective pancreatic cancer biomarker study. (**B**) Survival outcome of FOLFIRINOX treated patients.
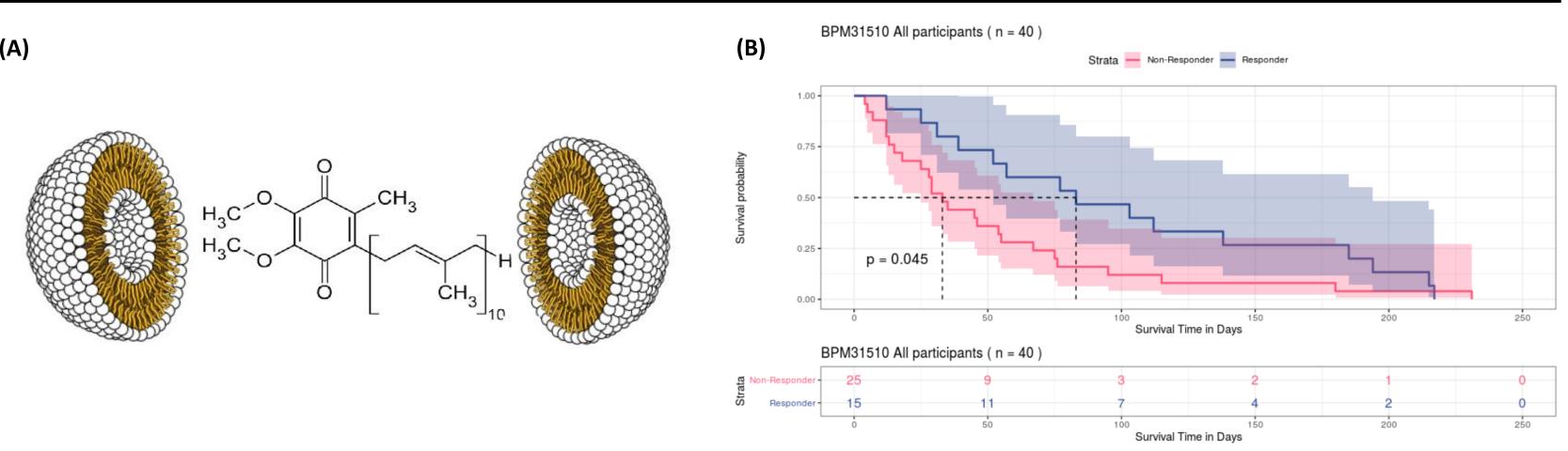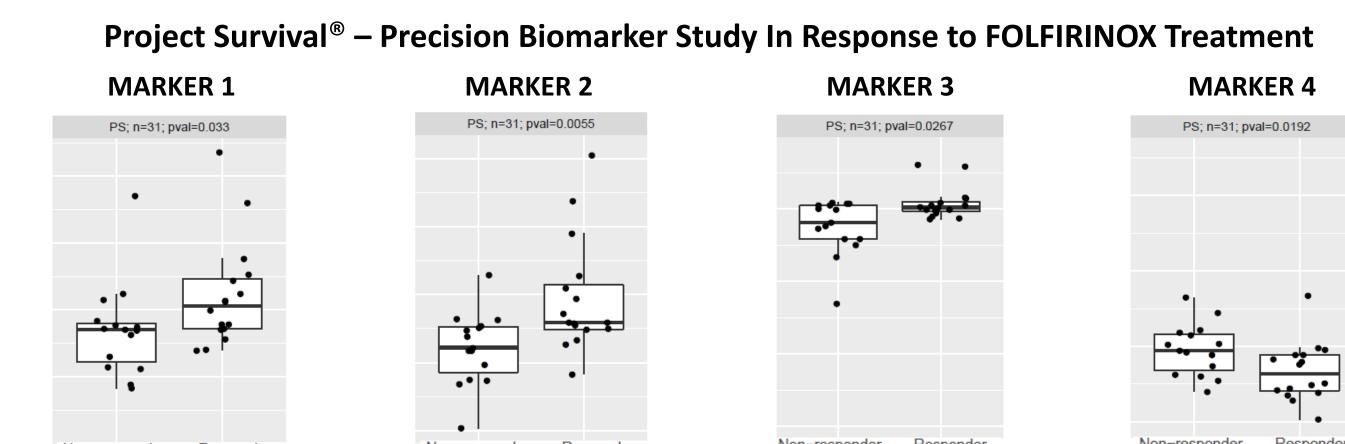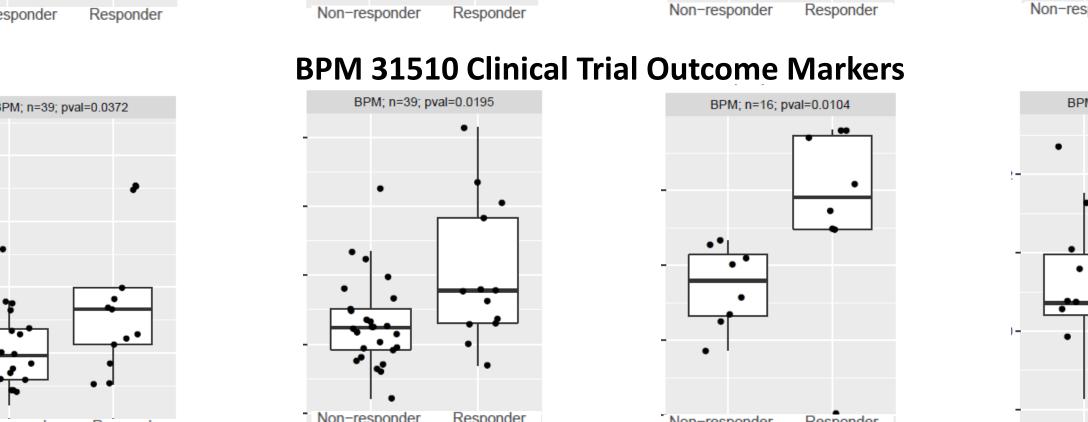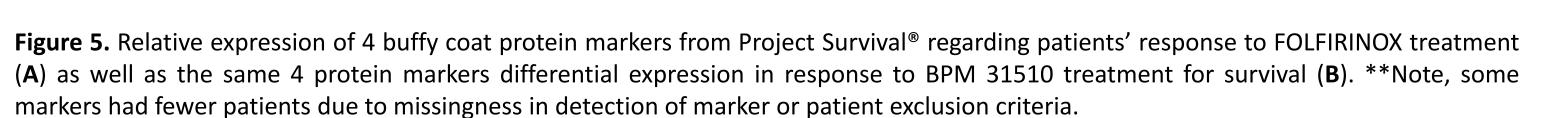
BPM 31510 TREATMENT CLINICAL OUTCOME

Figure 4. BPM 31510 is CoQ10-lipid conjugate, in which, oxidized CoQ10 has been incorporated into a mixture of lipids as a nanoparticle, enabling delivery of supraphysiological concentrations of CoQ10 to the mitochondria (**A**). Pancreatic adenocarcinoma patients treated with BPM 31510 were separated into non-responders and responders based on clinical criteria (**B**).

IDENTIFICATION OF DIAGNOSTIC PROTEIN MARKERS IN BUFFY COAT

Figure 5. Relative expression of 4 buffy coat protein markers from Project Survival® regarding patients' response to FOLFIRINOX treatment (**A**) as well as the same 4 protein markers differential expression in response to BPM 31510 treatment for survival (**B**). **Note, some markers had fewer patients due to missingness in detection of marker or patient exclusion criteria.

DIAGNOSTIC OUTPUT OF BUFFY COAT PROTEIN MARKERS

(A)

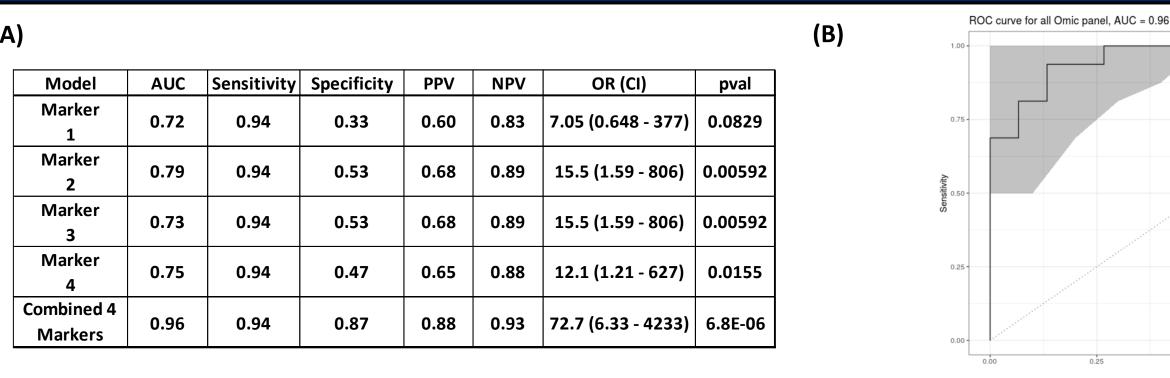| Model	AUC	Sensitivity	Specificity	PPV	NPV	OR (CI)	pval
Marker 1	0.72	0.94	0.33	0.60	0.83	7.05 (0.648 - 377)	0.0829
Marker 2	0.79	0.94	0.53	0.68	0.89	15.5 (1.59 - 806)	0.00592
Marker 3	0.73	0.94	0.53	0.68	0.89	15.5 (1.59 - 806)	0.00592
Marker 4	0.75	0.94	0.47	0.65	0.88	12.1 (1.21 - 627)	0.0155
Combined 4 Markers	0.96	0.94	0.87	0.88	0.93	72.7 (6.33 - 4233)	6.8E-06

Figure 6. (**A**) Diagnostic output of individual and combined buffy coat protein markers. (**B**) ROC curve assessment for combined markers.

CONCLUSIONS

Utilizing 2 independent studies, a prospective pancreatic cancer biomarker study (Project Survival®) as well as a Phase II clinical trial, we were able to identify protein biomarkers in buffy coat that stratified response to FOLFIRINOX, and for BPM 31510 refractory to FOLFIRINOX. This classifier demonstrated promising diagnostic utility that will be investigated further as potential predictive markers of clinical outcomes in pancreatic adenocarcinoma.

ACKNOWLEDGEMENTS