Plasma-based detection of pancreatic cancer: A multiomics approach

Teng-Kuei Hsu[1], Tzu-Yu Liu[1], Billie Gould[1], Christine Decapite[2], Amer Zureikat[3], Alessandro Paniccia[3], Eric Ariazi[1], Marvin Bertin[1], Richard Bourgon[1], Kaitlyn Coil[1], Hayley Donnella[1], Adam Drake[1], Julie M. Granka[1], Preet Kaur[1], Maggie C. Louie[1], Shivani Mahajan[1], Amit Pasupathy[1], Ofer Shapira[1], Peter Ulz[1], Chun Yang[1], C. Jimmy Lin[1] and Randall Brand[2]

1. Freenome Inc., South San Francisco
2. Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA
3. Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA

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Background & Objectives

Background

- Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers, with an overall five-year survival rate of 11%[1]
- Potential curative resection is possible if the tumor is detected at an early stage, with a five-year survival rate of 42% [1]
- The only current FDA-cleared biomarker for PDAC is the carbohydrate antigen 19-9 (CA19-9), which is intended for monitoring response to therapy but not for early detection
- CA19-9 blood tests have varying sensitivity to detect PDAC and are prone to false positives in the presence of other underlying pancreatic conditions and to false negatives in subpopulations unable to express CA19-9

Objective

- The goal of our proof-of-concept study was to determine if a multiomics approach using methylation profiling of cell-free DNA and CA19-9 would be better than CA19-9 alone in detecting PDAC

1. Cancer Stat Facts: Pancreatic Cancer. Available at: https://seer.cancer.gov/statfacts/html/pancreas.html. Accessed July 9, 2021

Study design and analysis pipeline

UPMC Study

Study Cohort

PDAC n=39

51% male

Mean age: 74.9 yrs

Stage II (n=9)
Stage III (n=11)
Stage IV (n=19)

Negatives n=36

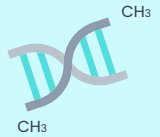
33% male

Mean age: 74.3 yrs

Healthy (n=17)
Benign pancreatic abnormalities (n=19)

Methods

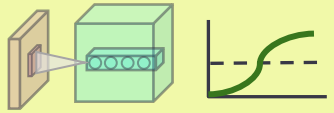
cfDNA Methylation



Targeted methylation profiling

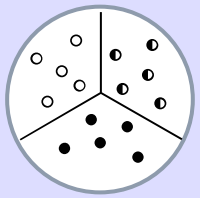
CA19-9 Protein

Quantitation of plasma CA19-9 abundance



Machine Learning Model

Combines methylation and CA19-9 signals to build a joint multiomics prediction of PDAC status



Classifier Generation

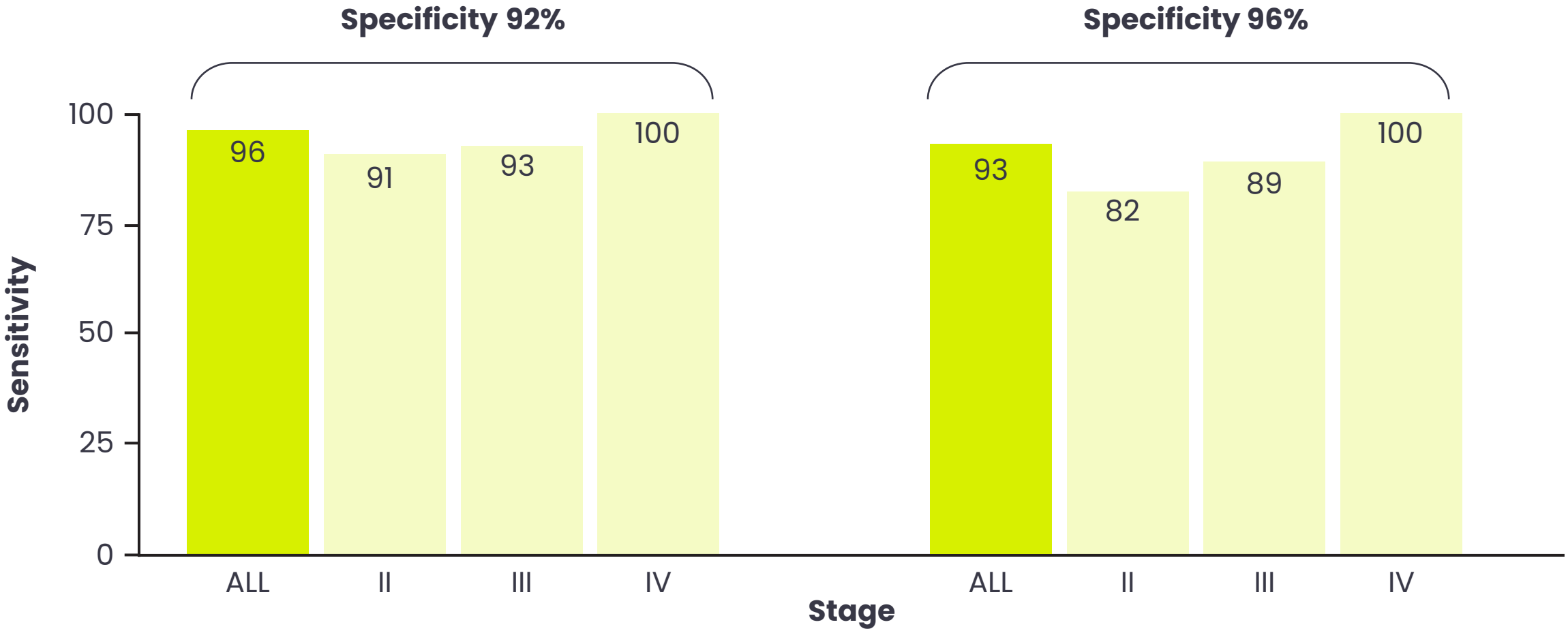
Train and evaluate models with three fold cross-validation (five resamplings). Each sample was assessed in cross-validation test sets by a model that had never seen that sample in training

Samples used in model training and performance assessment

Sample source	n	Assay(s)	Class	Data use
UPMC healthy controls	17	CA19-9, cfDNA methylation	Negative	Model training and performance assessment
UPMC benign pancreatic abnormalities	19	CA19-9, cfDNA methylation	Negative	Model training and performance assessment
UPMC PDAC participants	39	CA19-9, cfDNA methylation	Positive	Model training and performance assessment
Other healthy controls	95	cfDNA methylation	Negative	Supplemental model training
PDAC tumor tissue samples	29	cfDNA methylation	Positive	Supplemental model training

Performance estimates are based on plasma samples from healthy controls and participants with benign pancreatic abnormalities (the "negative" group), and PDAC participants (the "positive" group), from the UPMC cohort. Two additional sample cohorts were used to supplement machine learning model training for cfDNA methylation alone.

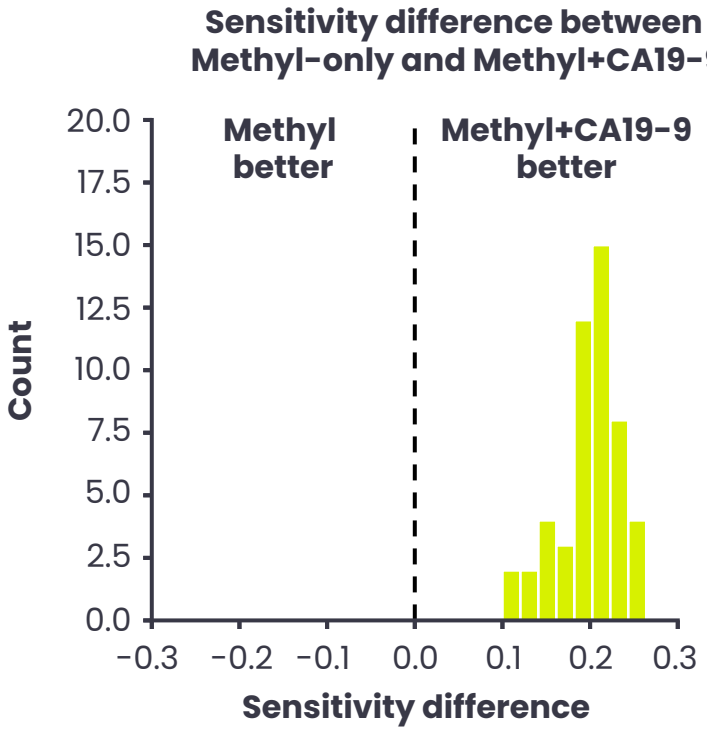
The multiomics approach achieved high sensitivity and specificity for PDAC

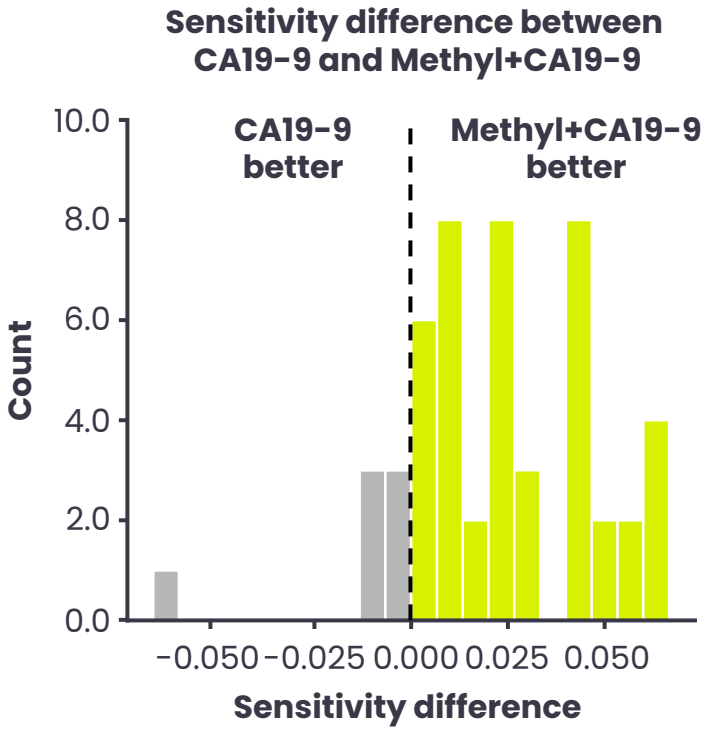


We first considered decision thresholds that yielded 92% specificity. We also evaluated model performance at a higher specificity level (96%). To achieve this with small discrete sample numbers we used a combination of the 92% and 100% specificity levels in our iterated three-fold cross-validation.

The multiomics approach showed significantly greater sensitivity than methylation-only or CA19-9-only models

	92% Specificity	96% Specificity
Sensitivity of **Methyl+CA19-9**	96%	93%
Sensitivity of **Methyl**	87%	74%
Sensitivity of **CA19-9**	88%	87%

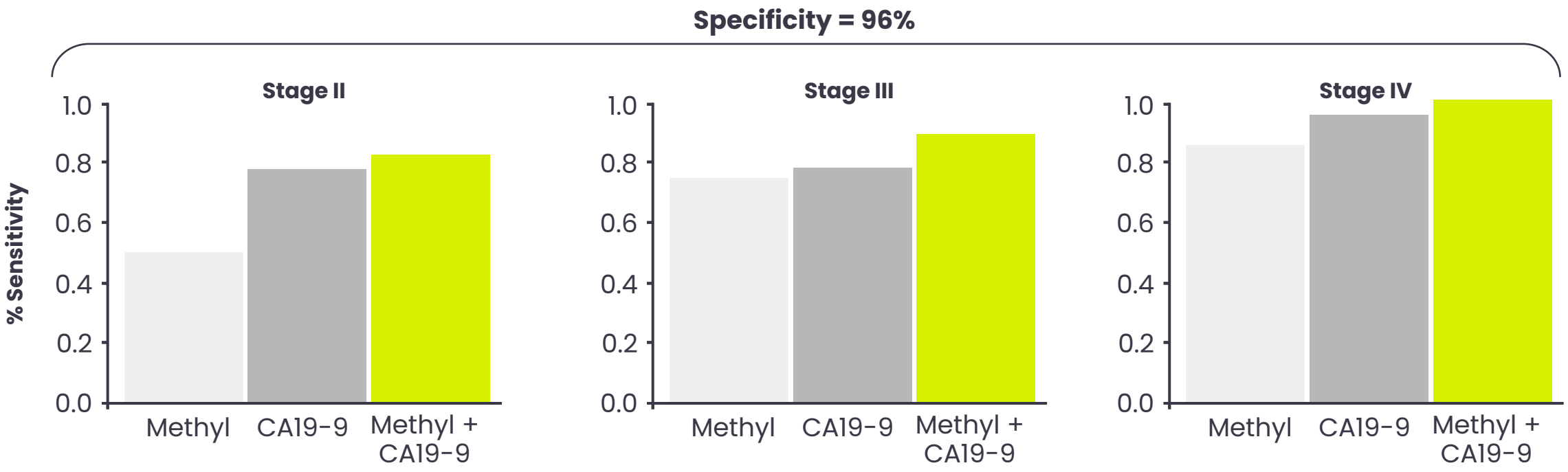




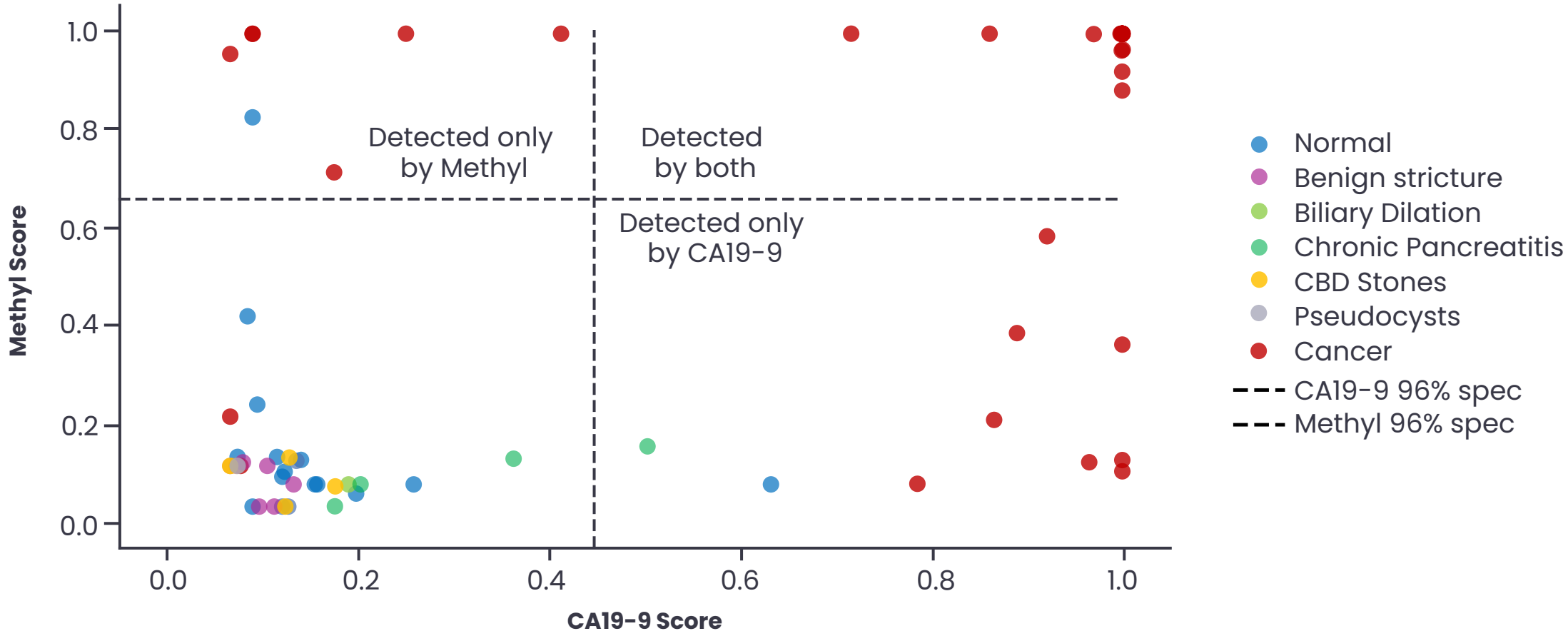
- Model sensitivity was improved when signals from CA19-9 and cfDNA methylation were combined
- After repeating our training and performance estimation process 50 times with different cross-validation sampling, we found the sensitivity at 96% specificity of Methyl+CA19-9 model was significantly greater than that of both the Methyl and CA19-9 models (Wilcoxon signed-rank test $p=8x10^{-10}$ and $3x10^{-7}$, respectively)
- The Methyl+CA19-9 model had greater sensitivity than the Methyl model in all 50 runs and greater sensitivity than the CA19-9 model in 42 out of 50 runs

Sensitivity of the multiomics model was greater than that of methylation-only or CA19-9-only models across all stages

Stage	n	Sensitivity of Methyl+CA19-9	Sensitivity of Methyl	Sensitivity of CA19-9	Specificity
II	9	82%	50%	78%	96%
III	11	89%	75%	78%	96%
IV	19	100%	86%	96%	96%

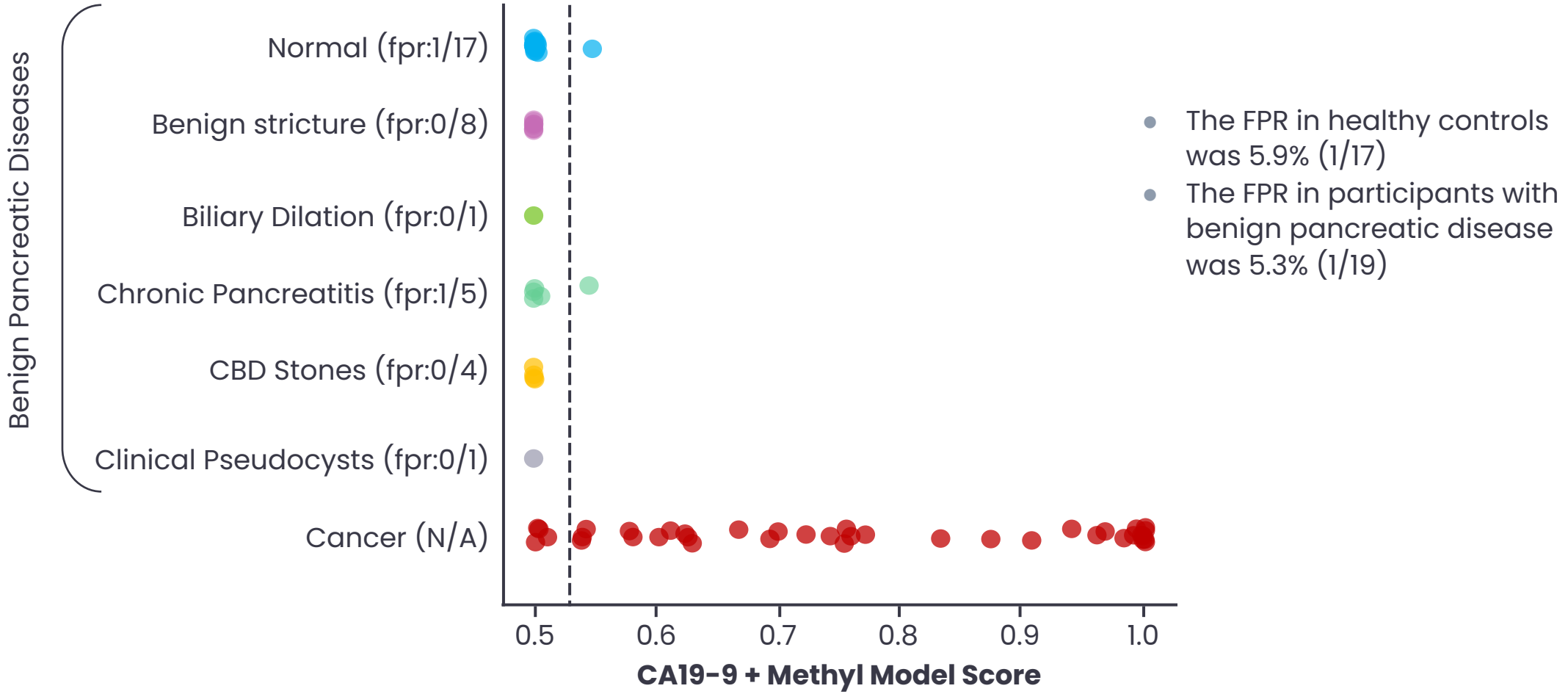


The multiomics model showed greater sensitivity because single models detect different cancer samples



Per-fold sample scores from the CA19-9-only and methylation-only models. Points are colored by sample type. Positive model calls occur above or to the right of the average 96% specificity cutoff dashed lines shown.

False positive rate (FPR) in healthy controls and in participants with benign pancreatic disease were equivalent



Average per-fold Methyl+CA19-9 multiomics model scores colored by sample type. Samples above the average 96% specificity cutoff (dashed line) were called as positive for pancreatic cancer.

Conclusions and Acknowledgements

Conclusions:

- These proof-of-concept data demonstrate the promise of using a multiomics approach to develop a more sensitive and specific test for the early detection of pancreatic cancer, which could be further developed for use in screening populations.
- In this initial multiomics model for detecting pancreatic cancer, benign pancreatic conditions do not cause an elevation in the rate of false positive calls in comparison to healthy individuals.
- This discovery study is limited by small sample size, and further work is needed to verify that results are generalizable to a larger population.
- Additional studies are underway, focusing on early-stage (stage I/II) disease and larger cohorts, to validate these results.

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