GI09
06/27/2020
11:53am

Surinder Batra, Ph.D., University of Nebraska Medical Center

GI09
06/26/2020
6:00am

Validation of Mucin Based Biomarkers for Early Diagnosis of Pancreatic Neoplasms

Surinder K Batra

A National Cancer Institute Designated Cancer Center

FRED & PAMELA BUFFETT CANCER CENTER

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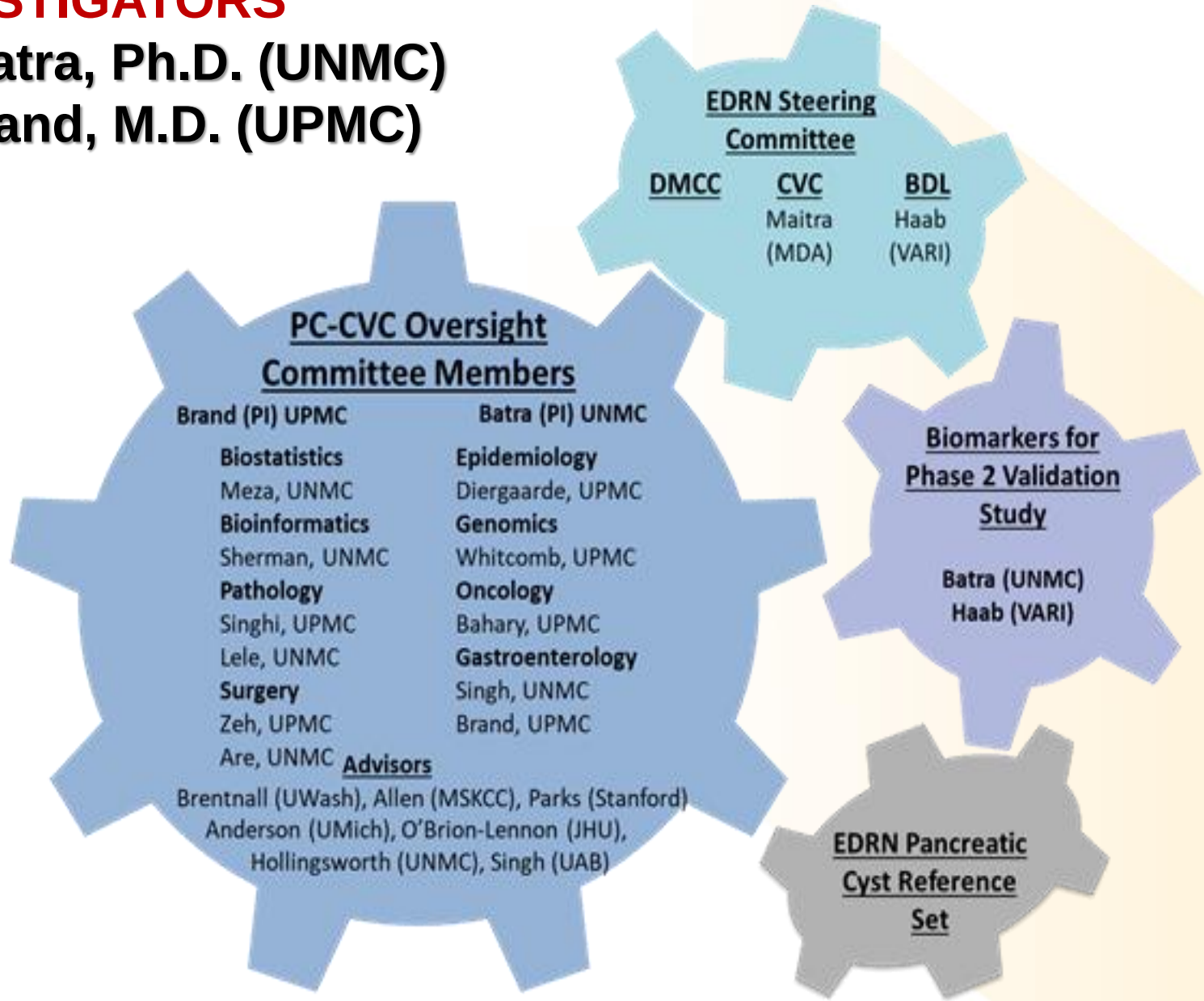
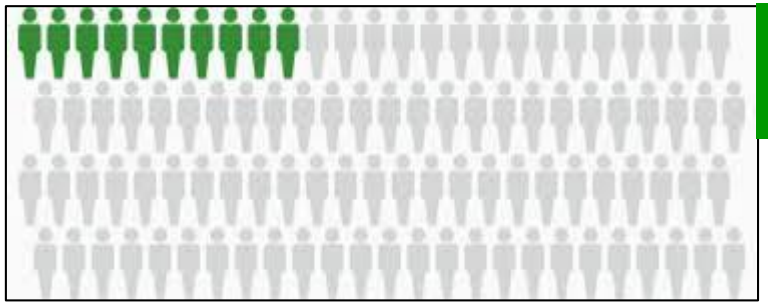


Pancreatic Cancer: An enigma



Percent Surviving 5 Years

10%

New Cases- 2020	Deaths-2020
57,600	47,050

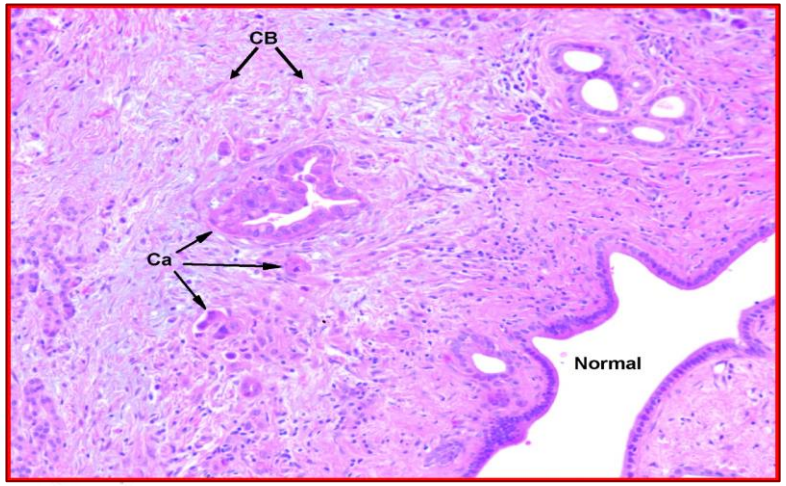
Pancreatic cancer is the only malignancy where survival rate has not been increased over the last **40** years

By 2030 Pancreatic cancer will be the **2nd** leading cause of cancer related deaths in US!!

90% of patients die after diagnosis of pancreatic cancer

Challenges to Patient Care

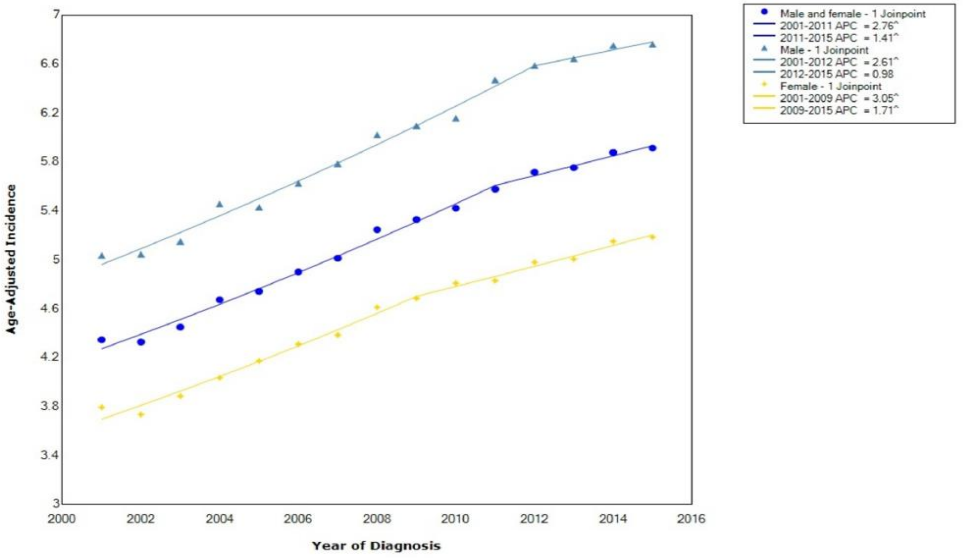
- **Chemotherapy and Radiotherapy Resistant**
- **Highly Heterogeneous**
- **Lack of Robust models**
- **LACK of early diagnostic and prognostic marker(s)**



Mahadevan and Von Hoff, CCR, 2007

Pancreatic Cancer: INCIDENCE ON RISE

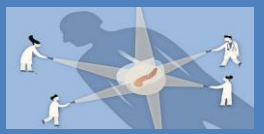
- **In the U.S., its incidence has crept upward by about 0.5 percent annually for more than a decade.**



Patel et al, Cureus, 2018

IMPROVED DETECTION MODALITIES

Refined ways of testing biopsied tissue and higher-resolution imaging



SMOKING

1 IN 5 PC CASE

AGING

(More than three quarters of new patients are between 55 and 84 years old)

PANCREATIC CANCER IS ON THE RISE

DIABETES

Late onset DM has emeged as most potential risk factor for PC

OBESITY

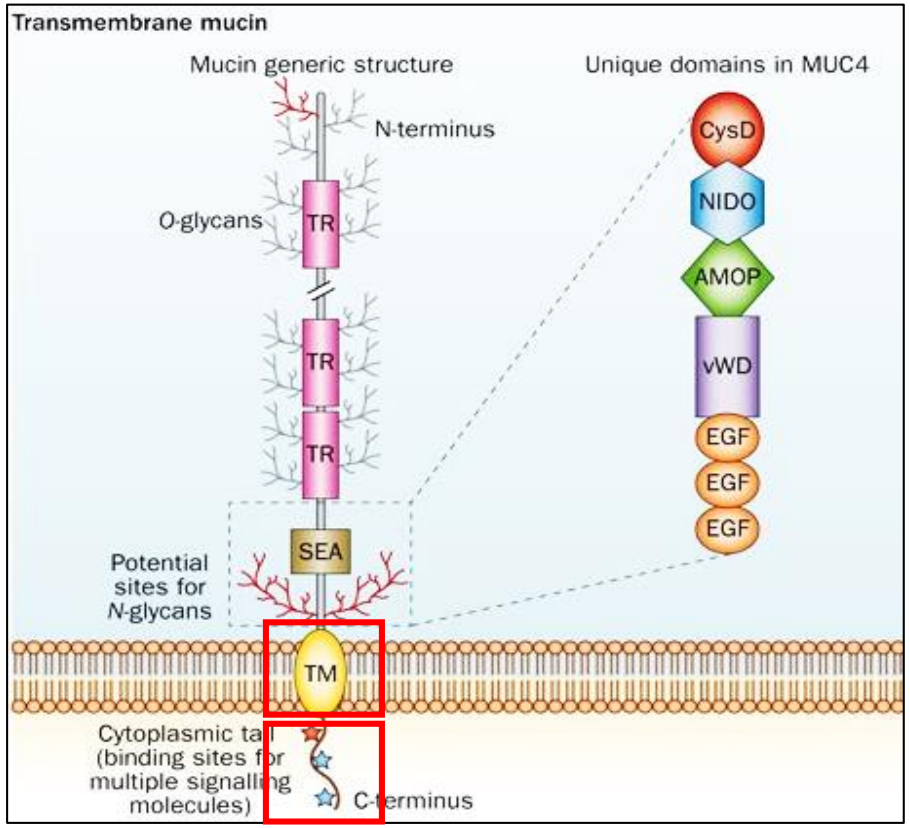
DIABESTY

TIME BOMB

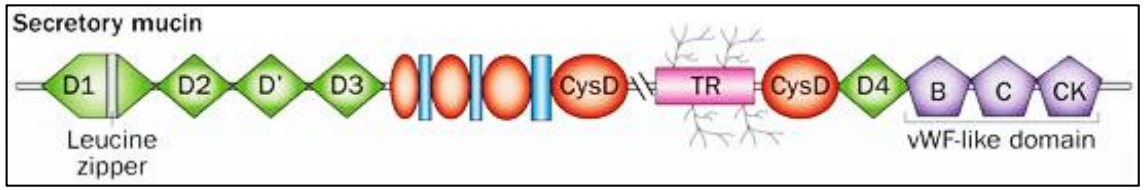
CANCER CENTER

Mucins: Transmembrane and Secreted Members

21 members, broadly classified into:



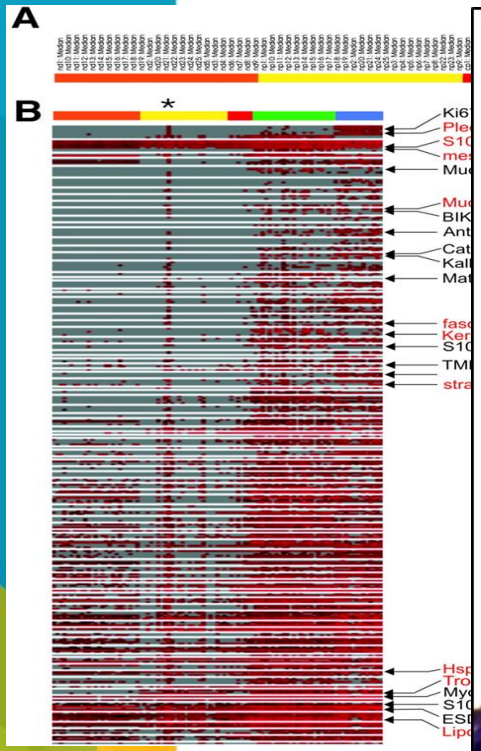
MUC1
MUC3A/B
MUC4
MUC12
MUC13
MUC16
MUC17
MUC20
MUC21

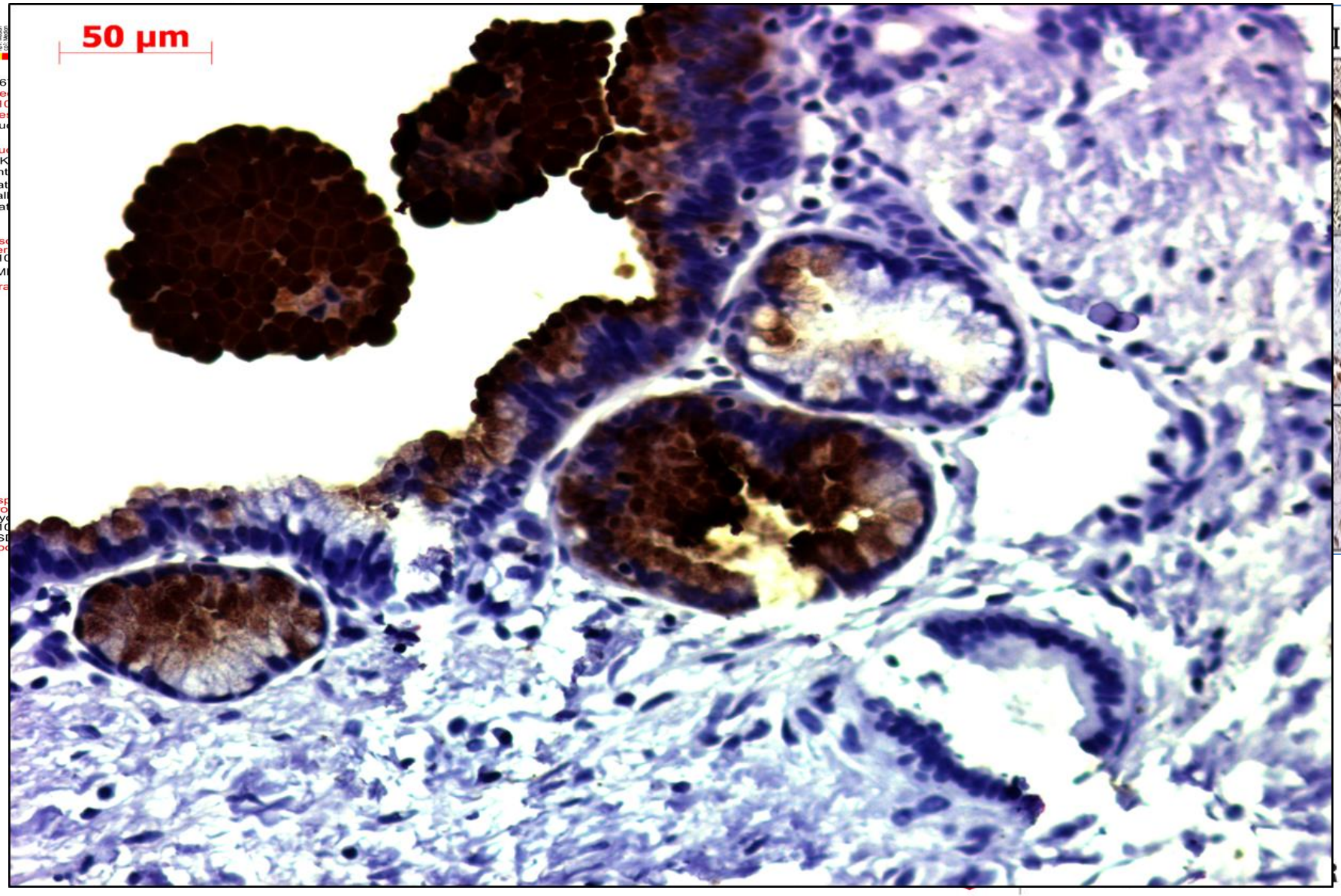


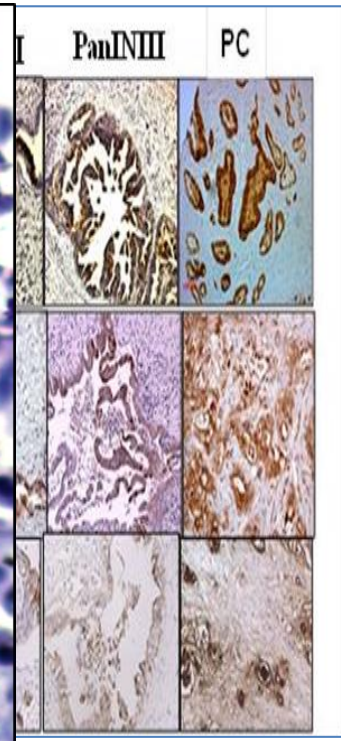
MUC2
MUC5B
MUC5AC
MUC6

Kaur et al., Nat Rev Gastro Hepatol. 2013

EXPRESSION PROFILE OF MUCINS IN PANCREATIC PATHOLOGIES







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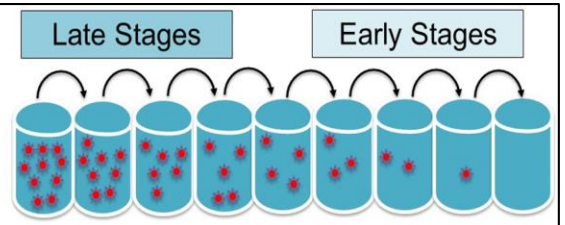
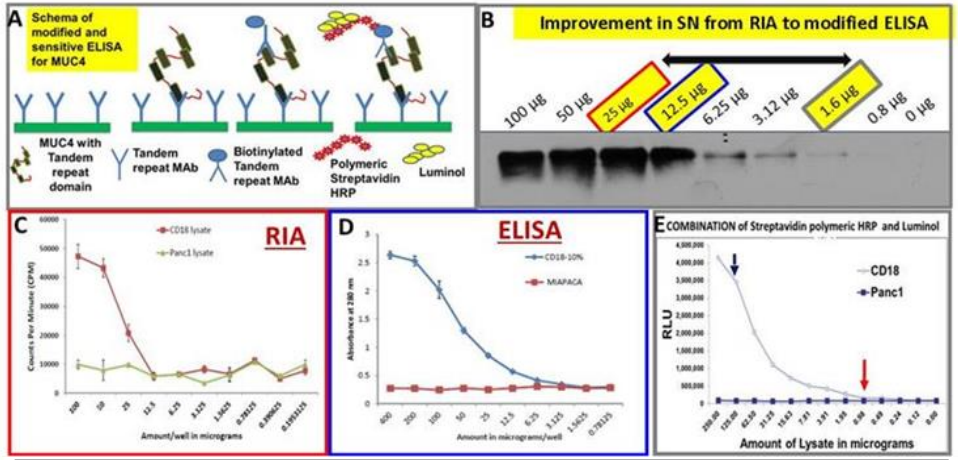
ULTRASENSITIVE TECHNOLOGIES

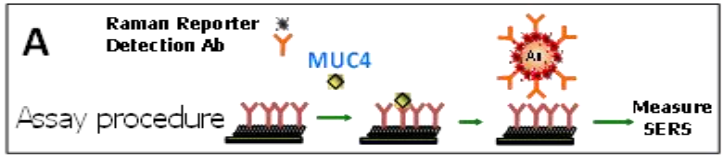
- *Sensitivity*
- *Specificity*
- *Reproducibility*
- *Appropriate Sample Set*

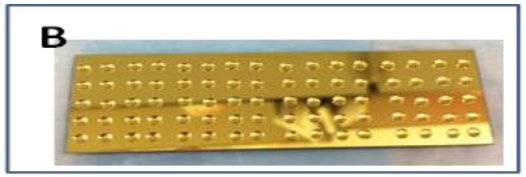


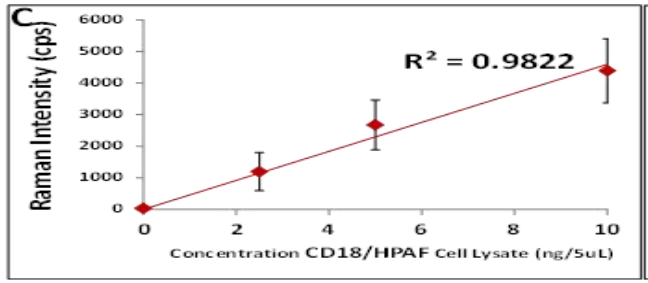
Early Stage Disease-Low Levels of Biomarkers

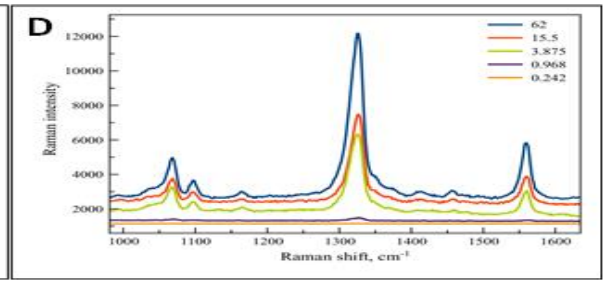
Ultrasensitive Technology

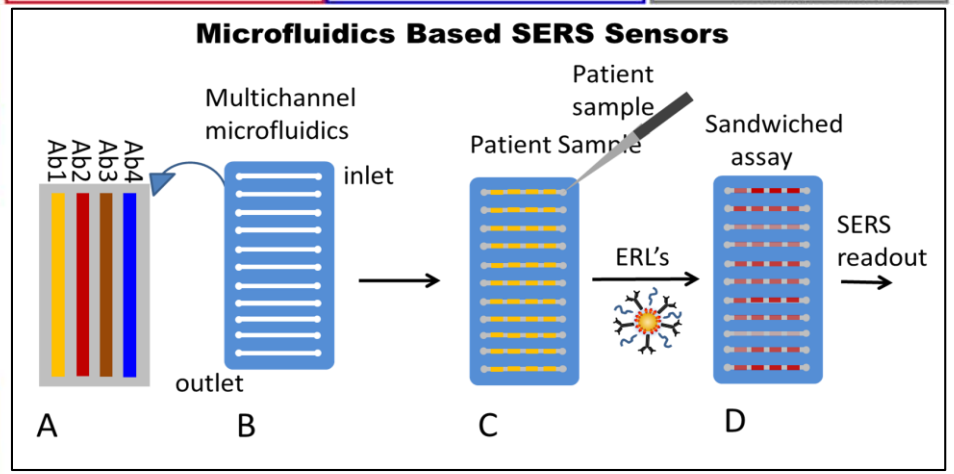










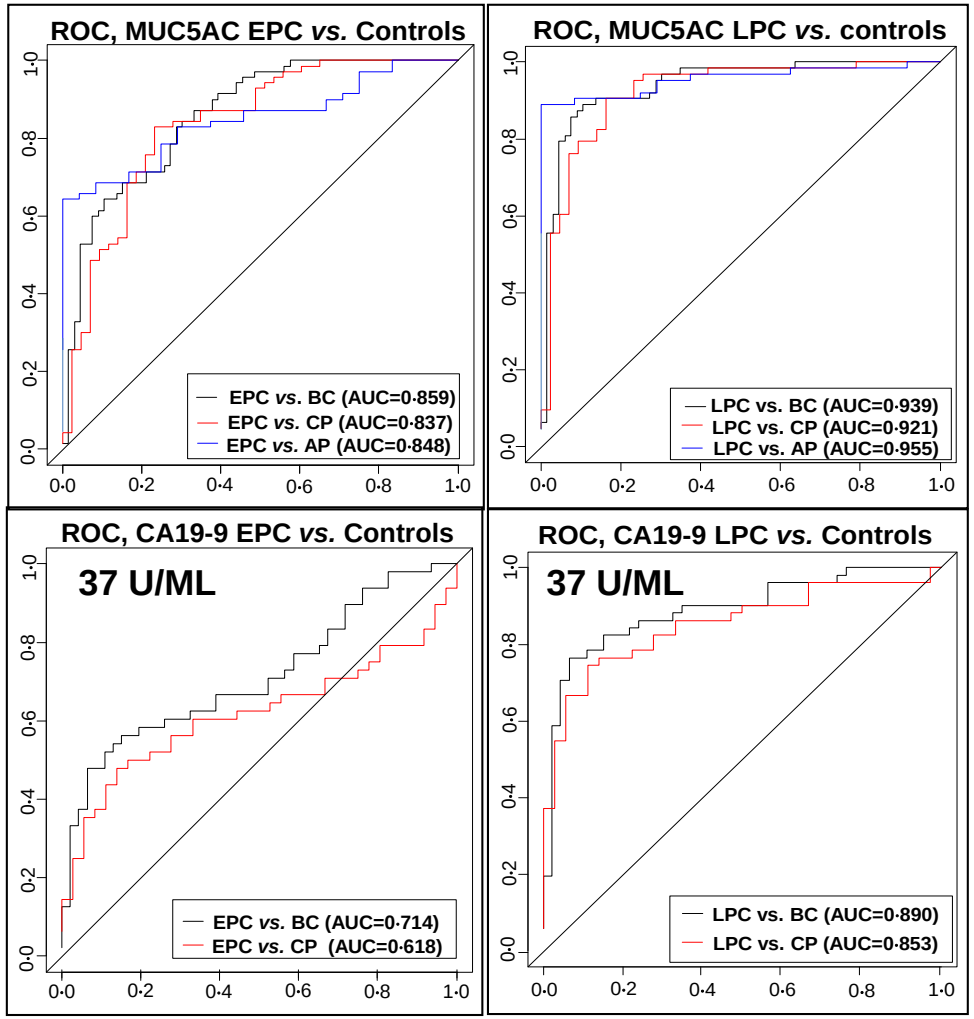


LIMIT OF DETECTION

ELISA	12.5 µg
Radioimmunoassay (RIA)	25 µg
Ultrasensitive ELISA (uELISA)	3.25 µg
SERS	0.6ng

CENTER

DIAGNOSTIC EFFICACY OF MUC5AC and CA19.9



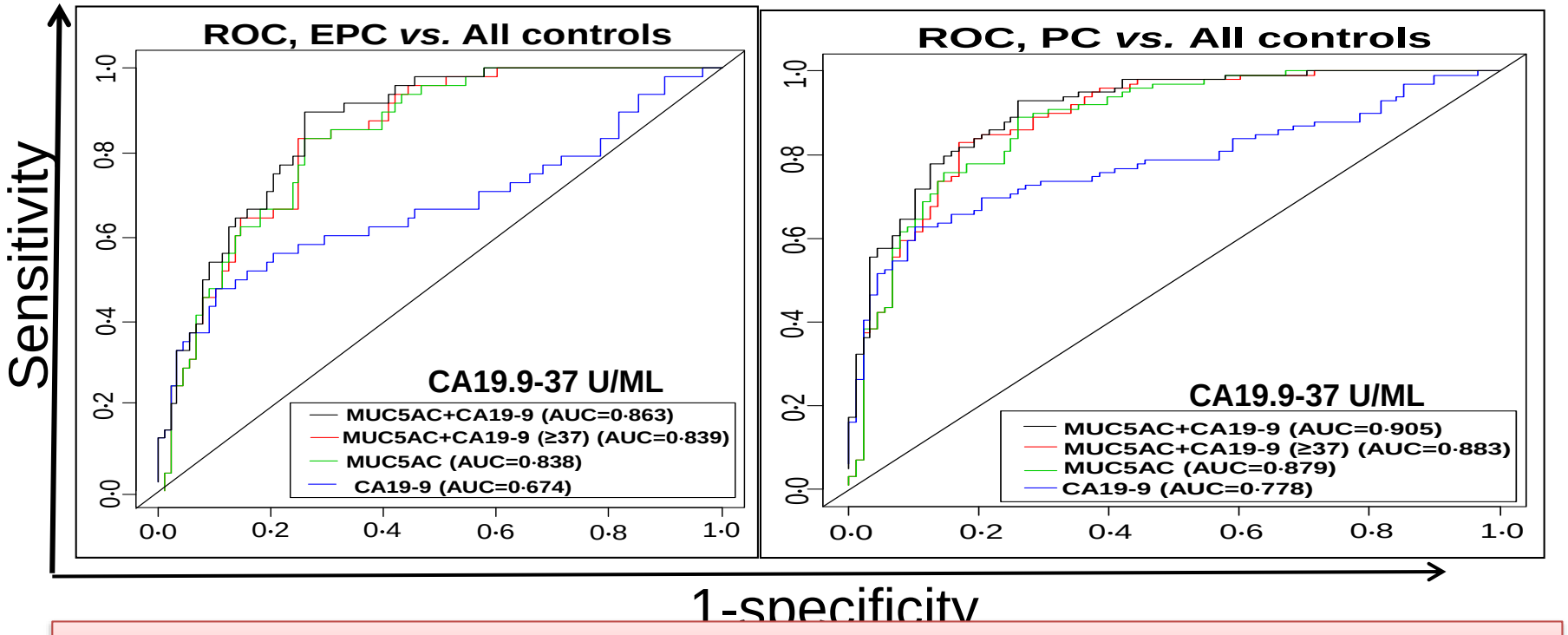
MUC5AC				
Comparison	AUC	Estimated Threshold	Specificity	Sensitivity
EPC vs. BC	0.86	≥22.40	0.87	0.67
EPC vs. CP	0.84	≥22.40	0.77	0.83

CA19-9				
Comparison	AUC	Estimated Threshold	Specificity	Sensitivity
EPC vs. BC	0.71	≥115.32	0.89	0.48
EPC vs. CP	0.62	≥115.32	0.86	0.48

Kaur S… Brand RE, Batra SK. **Am J Gastroenterol.** 2017

MUC5AC is potential diagnostic markers for Early stage PC cases

MUC5AC IN COMBINATION WITH CA19.9



THE COMBO(MUC5AC AND CA19.9) IMPROVES THE SENSITIVITY AND SPECIFICITY FOR DIFFERENTIATING EPC FROM CONTROLS

	Comparison	AUC	SE	Threshold	SP	SN
EPC vs. BC	MUC5AC	0.84	0.04	≥20·40	0·7	0·83
	CA19-9	0.57	0.05	>=37	0·48	0·67
	Ln(MUC5AC) + CA19-9	0.85	0.04	P(EPC) ≥0·553	0·72	0·85
EPC vs.CP	MUC5AC	0.82	0.05	>=20.404	0.76	0.84
	CA19-9	0.50	0.05	>=37	0.32	0.7
	Ln(MUC5AC) + CA19-9	0.84	0.043	P(EPC)>= 0.472	0.78	0.86

N=199 where both MUC5AC and CA19.9 values were available; CA19.9

Kaur S… Brand RE, Batra SK. **Am J Gastroenterol.** 2017

DIAGNOSTIC PERFORMANCE OF MUC5AC IN VALIDATION SETS

	Estimated Sensitivity	Estimated Specificity	Total cases	AUC	PPV	NPV	AC
Validation Set I- MAYO CLINIC N=94 MUC5AC CUT-OFF ≥20.4NG/ML							
PC vs. BC	75% (39/52)	73% (16/22)	74	0.74	87%	55%	74%
PC vs. CP	75% (39/52)	79% (15/19)	71	0.77	91%	54%	76%
EPC vs. BC	**68%** (17/25)	**73%** (16/22)	47	**0.70**	74%	67%	70%
EPC vs. CP	**68%** (17/25)	**79%** (15/19)	44	**0.74**	81%	65%	73%
LPC vs. BC	81% (22/27)	73% (16/22)	49	0.77	79%	76%	78%
LPC vs. CP	81% (22/27)	79% (15/19)	46	0.80	85%	75%	80%
Blinded Validation Set II UPMC-N=341 MUC5AC CUT-OFF ≥20.4NG/ML							
PC vs. BC	68% (107/157)	83% (94/113)	270	0.76	84%	65%	74%
PC vs. CP	68% (107/157)	72% (36/50)	207	0.70	88%	42%	69%
EPC vs. BC	**65%** (53/82)	**83%** (94/113)	195	**0.74**	74%	76%	75%
EPC vs. CP	**65%** (53/82)	**72%** (36/50)	132	**0.68**	79%	55%	67%
LPC vs. BC	73% (53/73)	83% (94/113)	186	0.78	74%	82%	79%
LPC vs. CP	73% (53/73)	72% (36/50)	123	0.72	79%	64%	72%

MUC5AC IS POTENTIAL DIAGNOSTIC MARKER FOR IDENTIFYING EARLY STAGE PANCREATIC CANCER (STAGE 2B)

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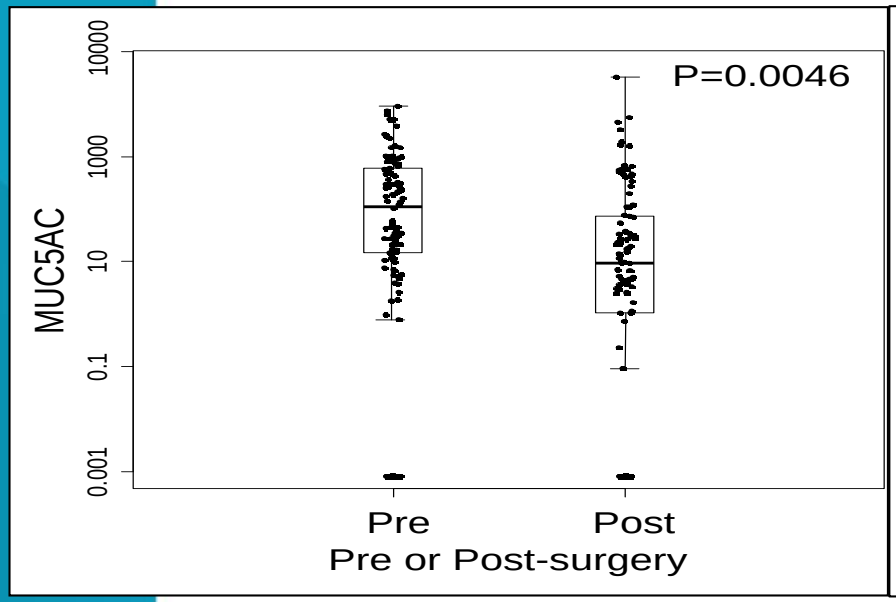
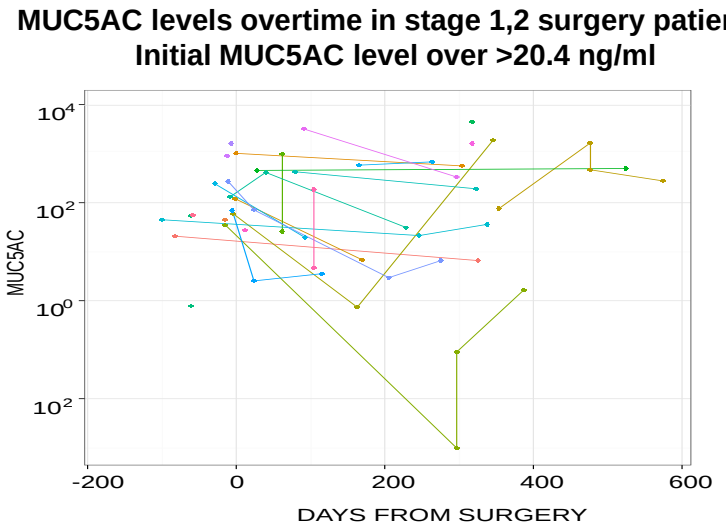
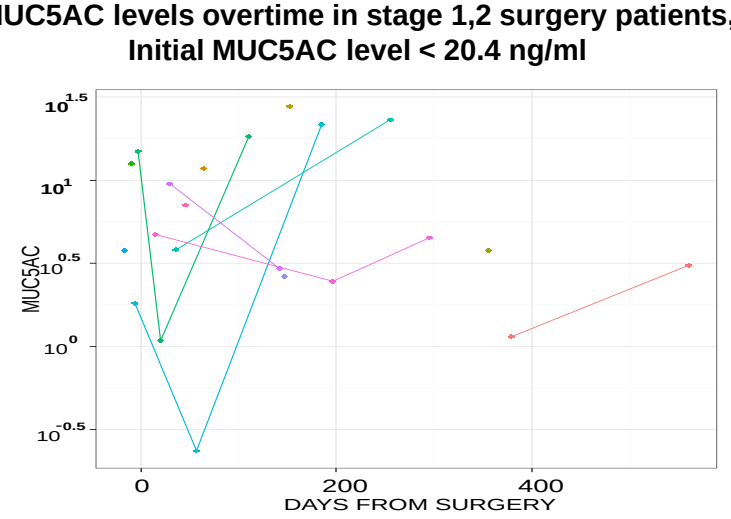
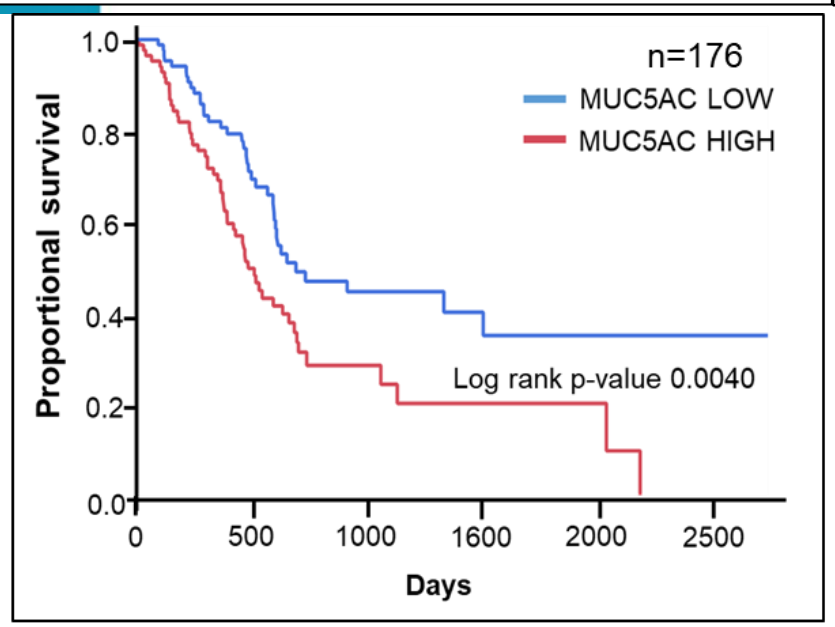
MUC5AC, A MARKER OF POOR PROGNOSIS









Adapted from TCGA analysis

- Overexpression of MUC5AC is associated with **poor prognosis** of PC patients.
- IN ADDITIONAL TO DIAGNOSTIC AND PROGNOSTIC SIGNIFICANE, **DOES MUC5AC HAS ANY FUNCTIONAL IMPLICATIONS?**

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Distinct features of MUC5AC

➢ ***De-novo*** expressed **secreted** mucin**:**

- Top-most differentially expressed mucin.
- Unique domain combination.
- Serum abundance correlate with disease progression.

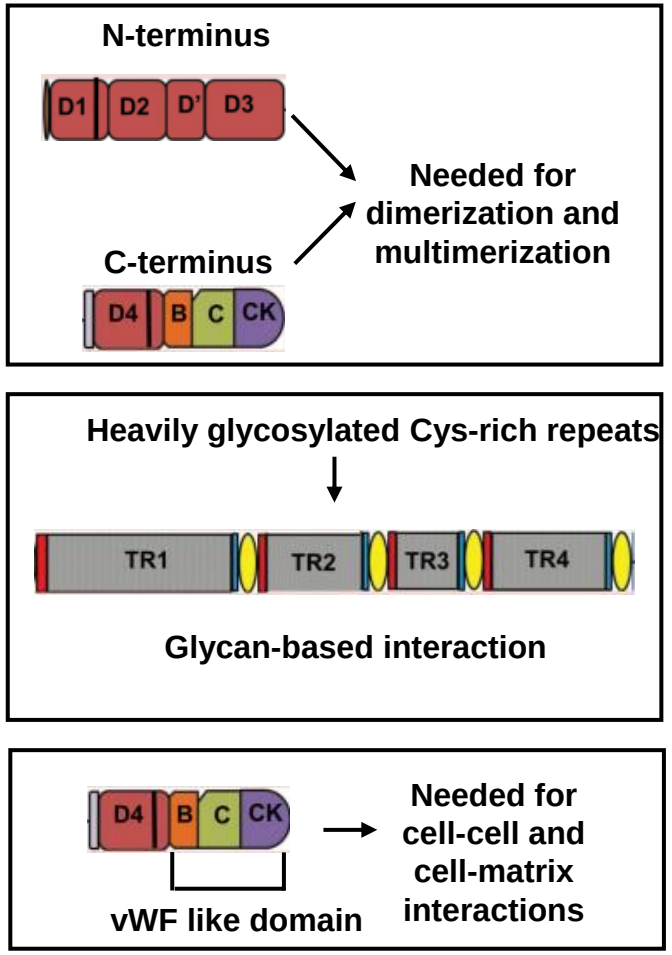
➢ **Secreted** MUC5AC may be more than a biomarker.

- Mucinous neoplasms have been connected to **patients' subtypes** across various studies.
- Association to **drug resistance**.

Molecular subtypes of pancreatic cancer

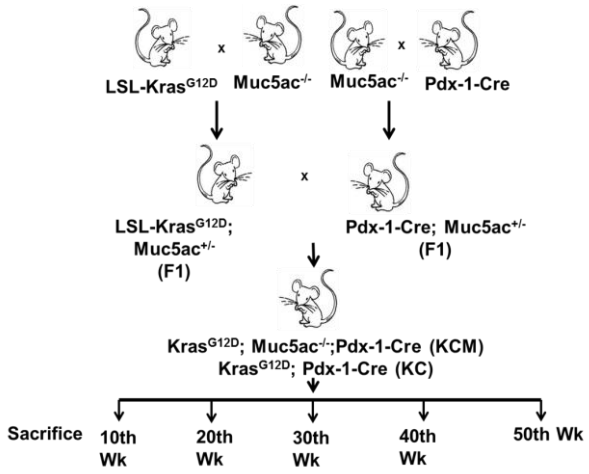
Eric A. Collisson, Peter Bailey, David K. Chang & Andrew V. Biankin

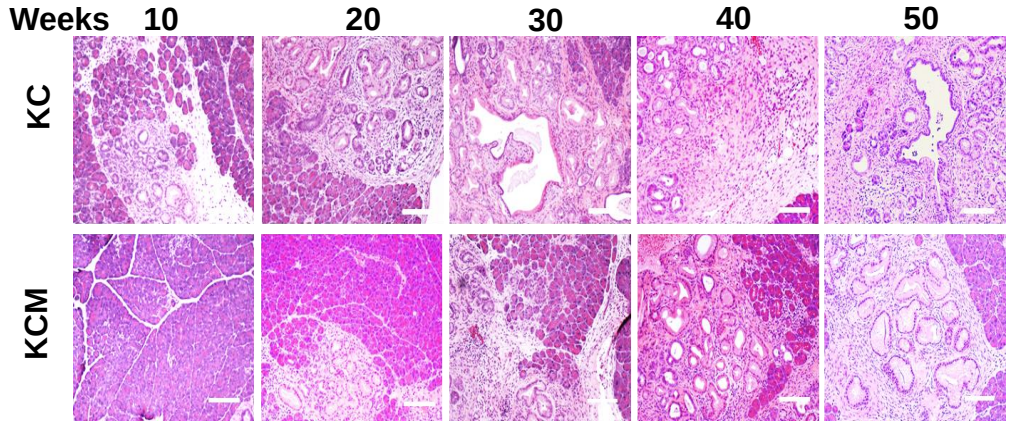
Nature Reviews Gastroenterology & Hepatology **16**, 207–220(2019) | Cite this article



Krishn and Ganguly et al., Carcinogenesis. 2018

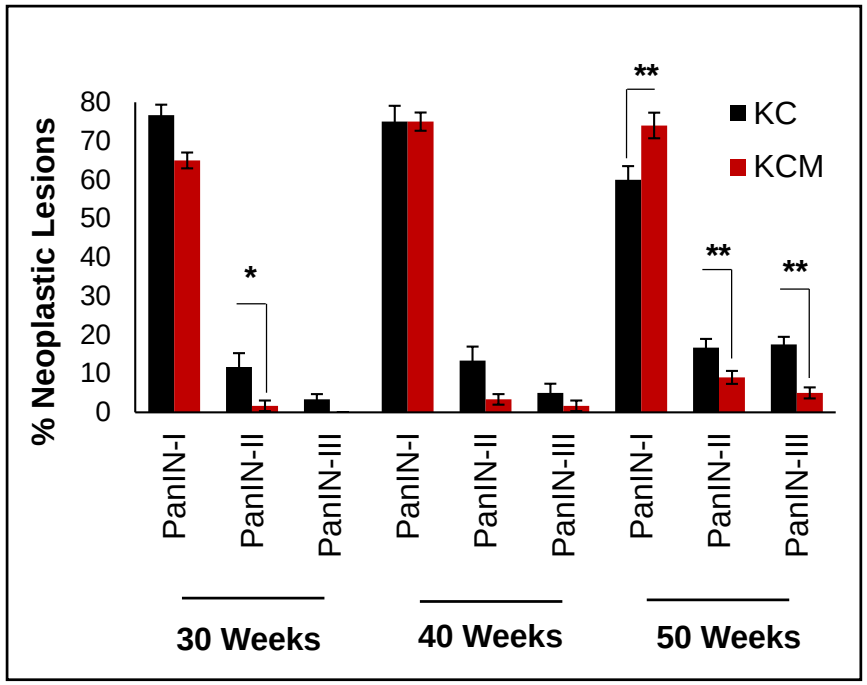
Functional Contribution of Muc5ac in PC-GEMM

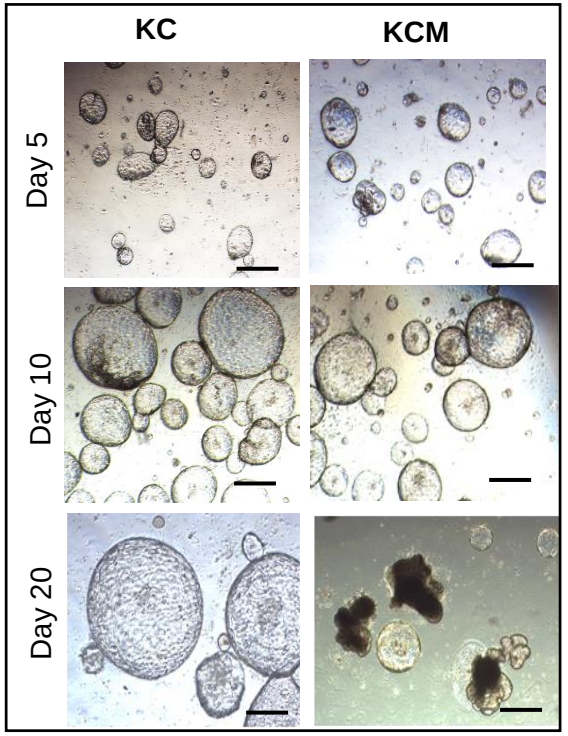


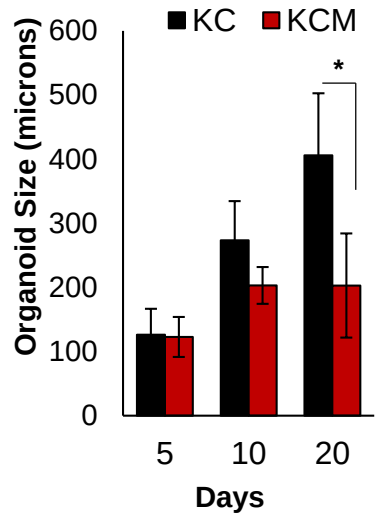


KC: KrasG12D, Pdx-cre

KCM: KrasG12D, Pdx-cre, Muc5ac-/-





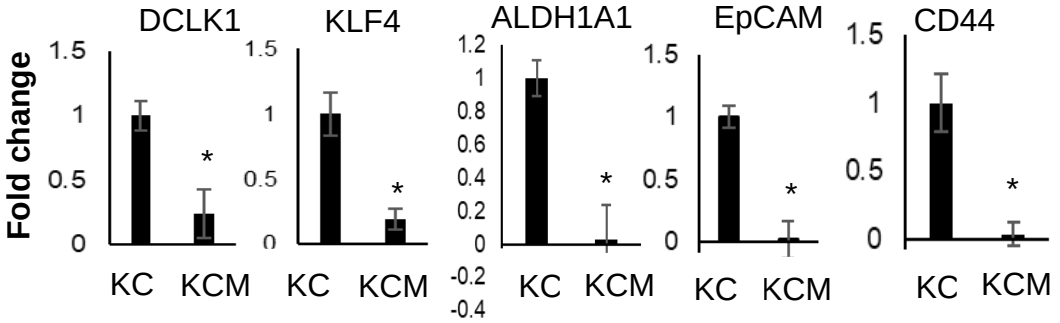


Genetic deletion of Muc5ac delays neoplastic onset and PanIN progression.

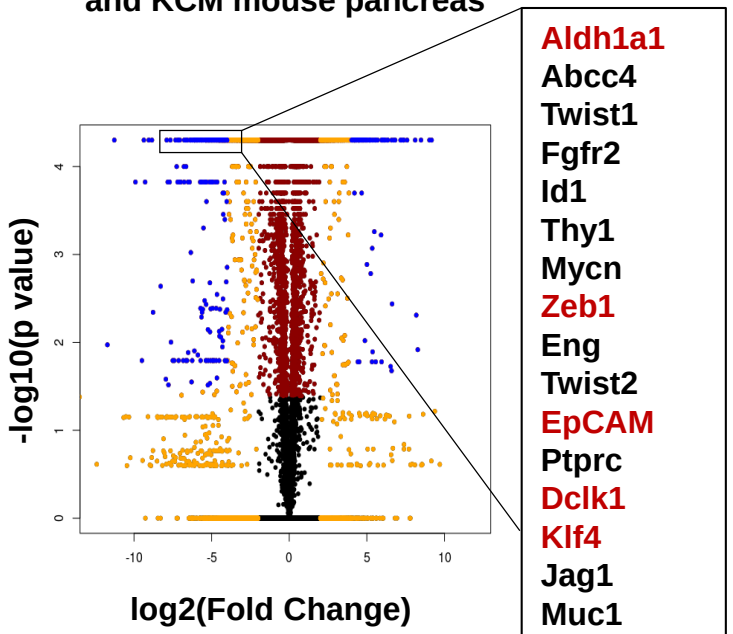
Transcriptomic analysis from KC and KCM pancreatic tumors

KC: KrasG12D, Pdx-cre

KCM: KrasG12D, Pdx-cre, Muc5ac-/-

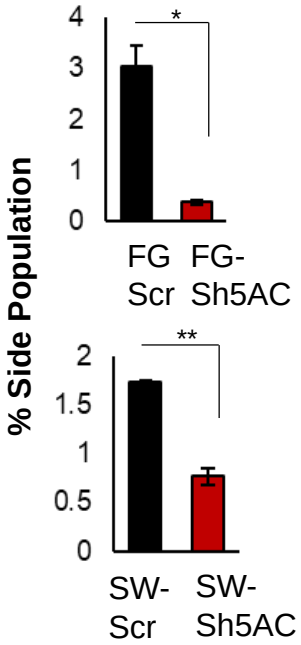


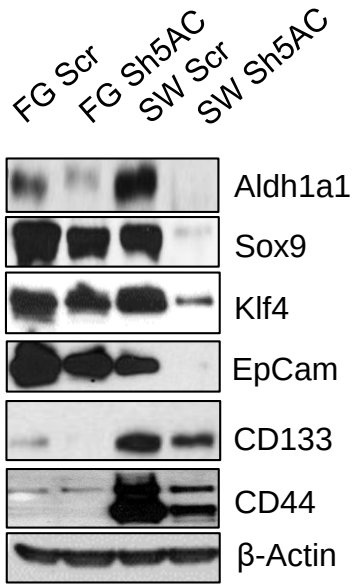
RNA seq. from 50 weeks KC and KCM mouse pancreas



Aldh1a1
Abcc4
Twist1
Fgfr2
Id1
Thy1
Mycn
Zeb1
Eng
Twist2
EpCAM
Ptprc
Dclk1
Klf4
Jag1
Muc1
CD44
Plat

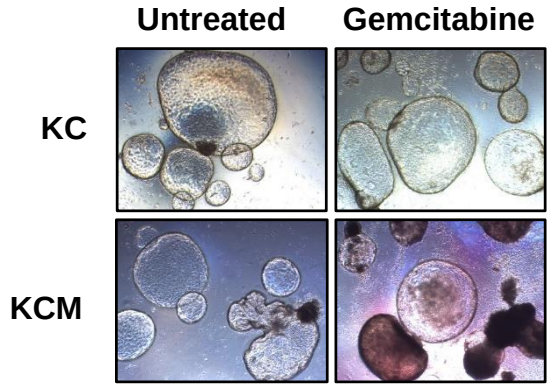
Human PC cell lines

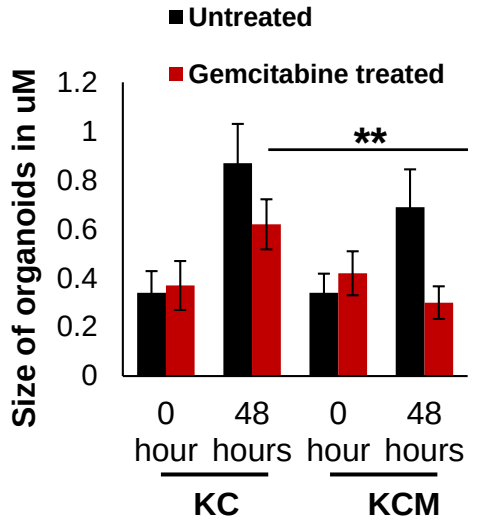


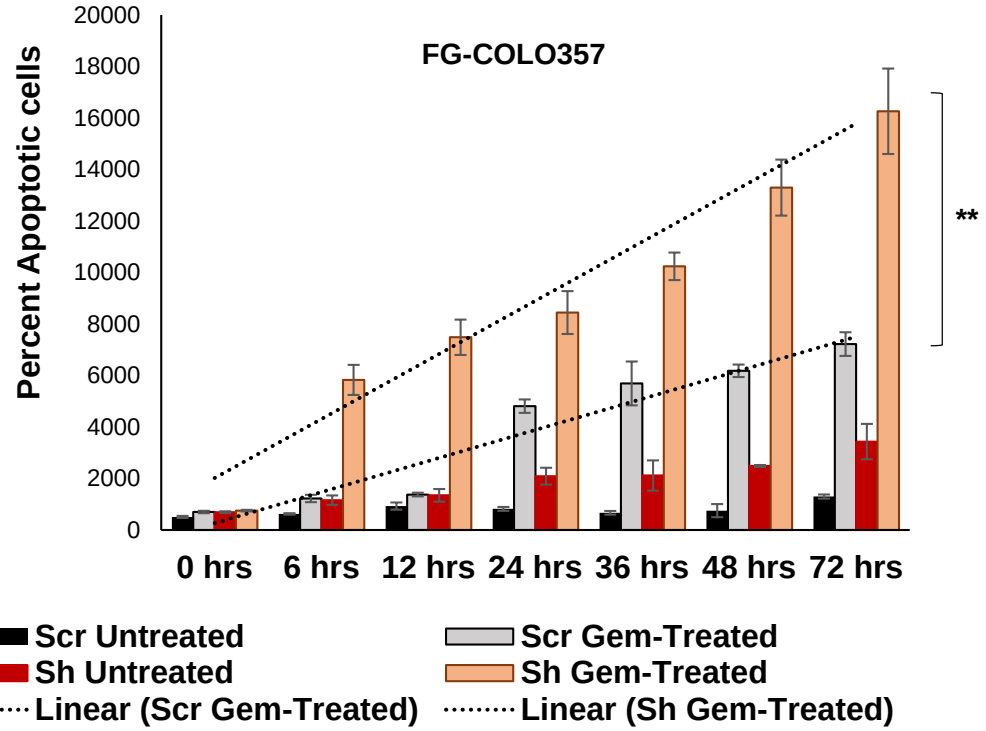


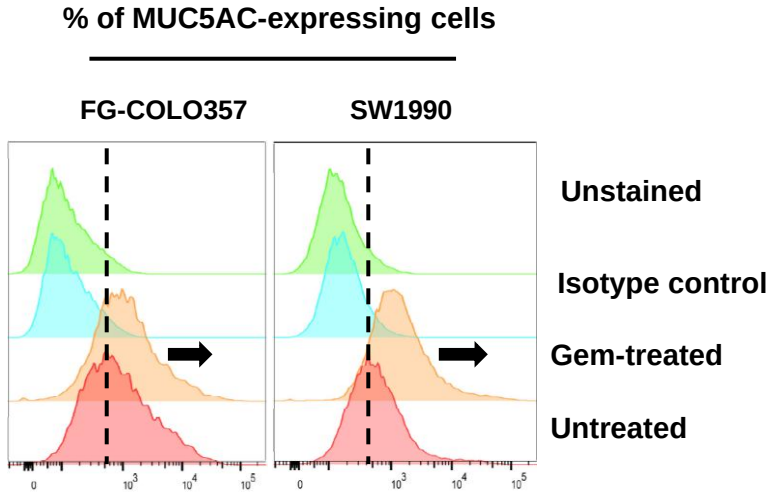
Abrogation of MUC5AC resulted in significant disruption of **cancer stem cells (CSCs)** in PC-GEMM and human PC cell lines

Does Muc5ac functionally contribute to CSC maintenance?



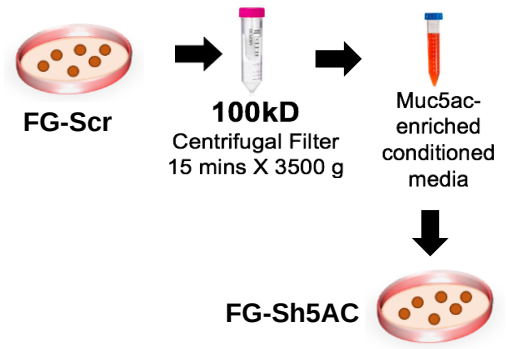


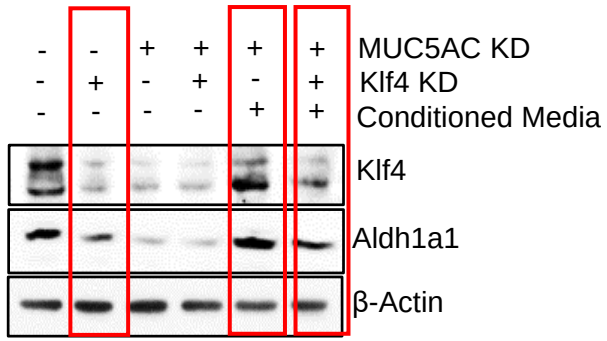


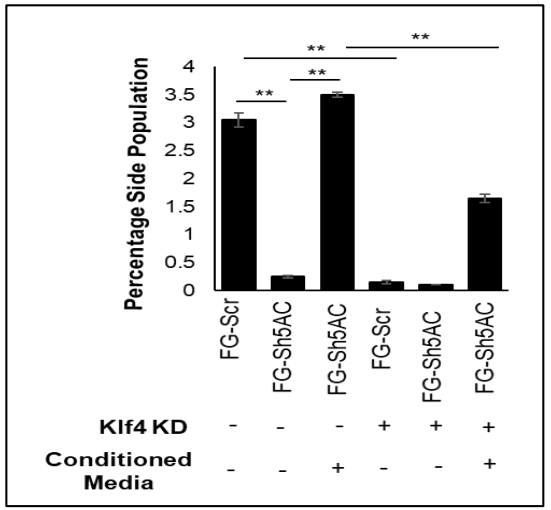


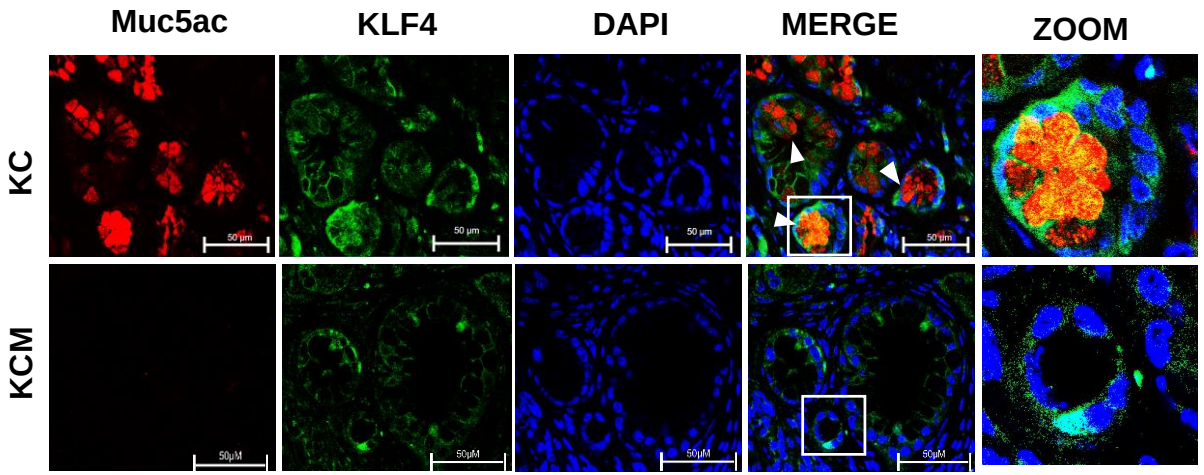
Depletion of Muc5ac sensitizes mouse organoids and human PC cells to gemcitabine.

Secreted MUC5AC enriches CSCs via upregulation of KLF4

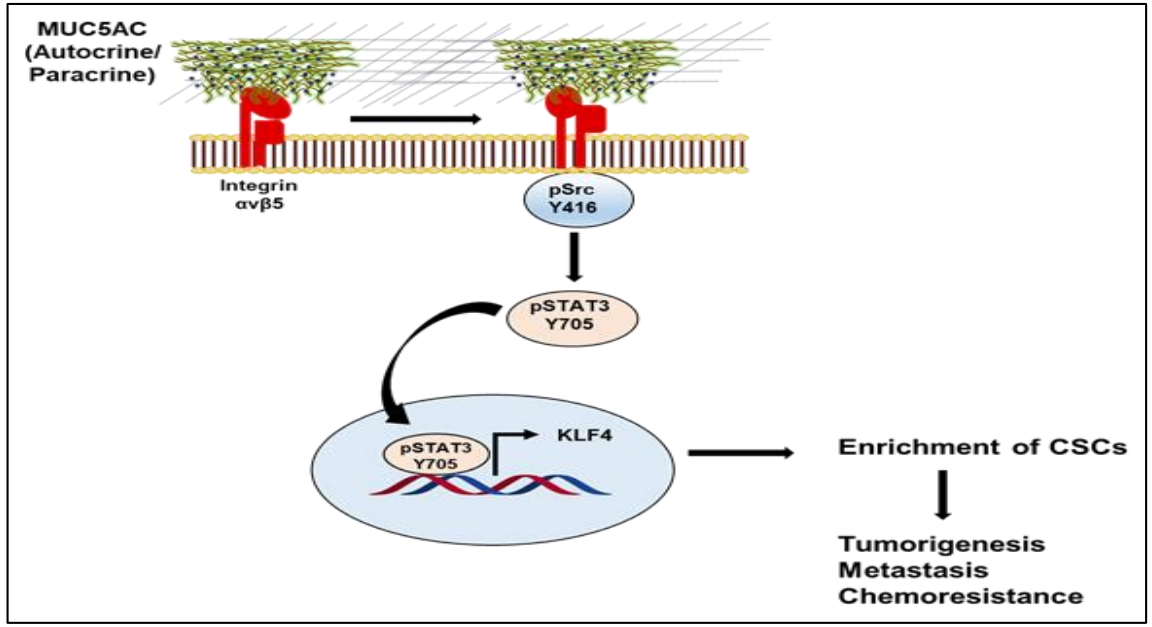




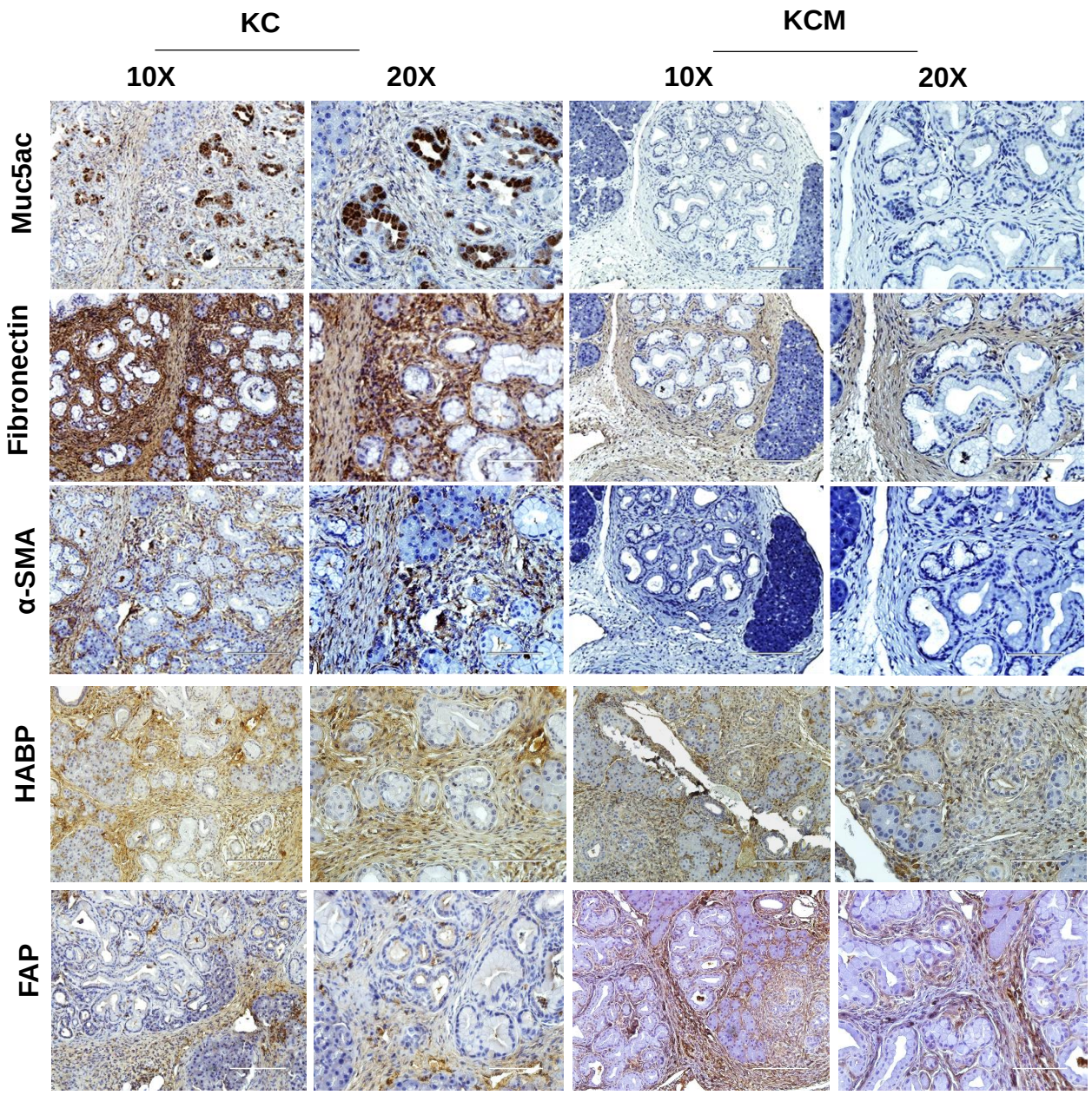




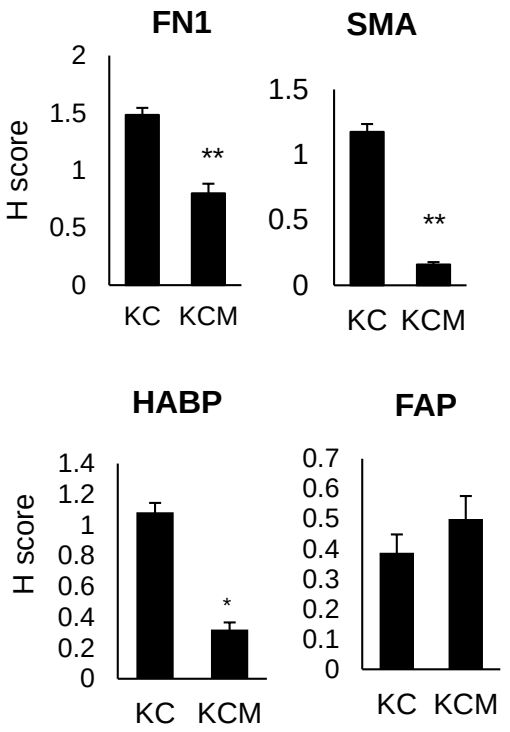
MUC5AC/KLF4 axis contributes towards CSC maintenance... Both in autocrine and paracrine



On STROMAL FRONT: Muc5ac-KO mouse showed modulation of PC stroma



Quantification of IHC (n=7)



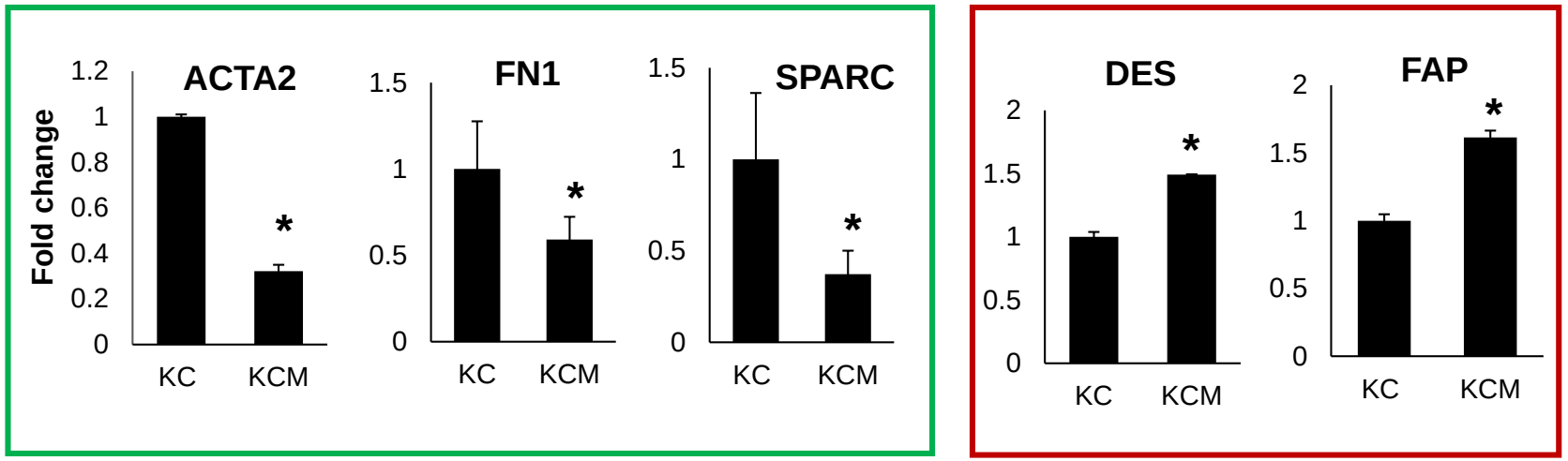
Muc5ac-KO mouse showed modulation of PC stroma

*p<0.05

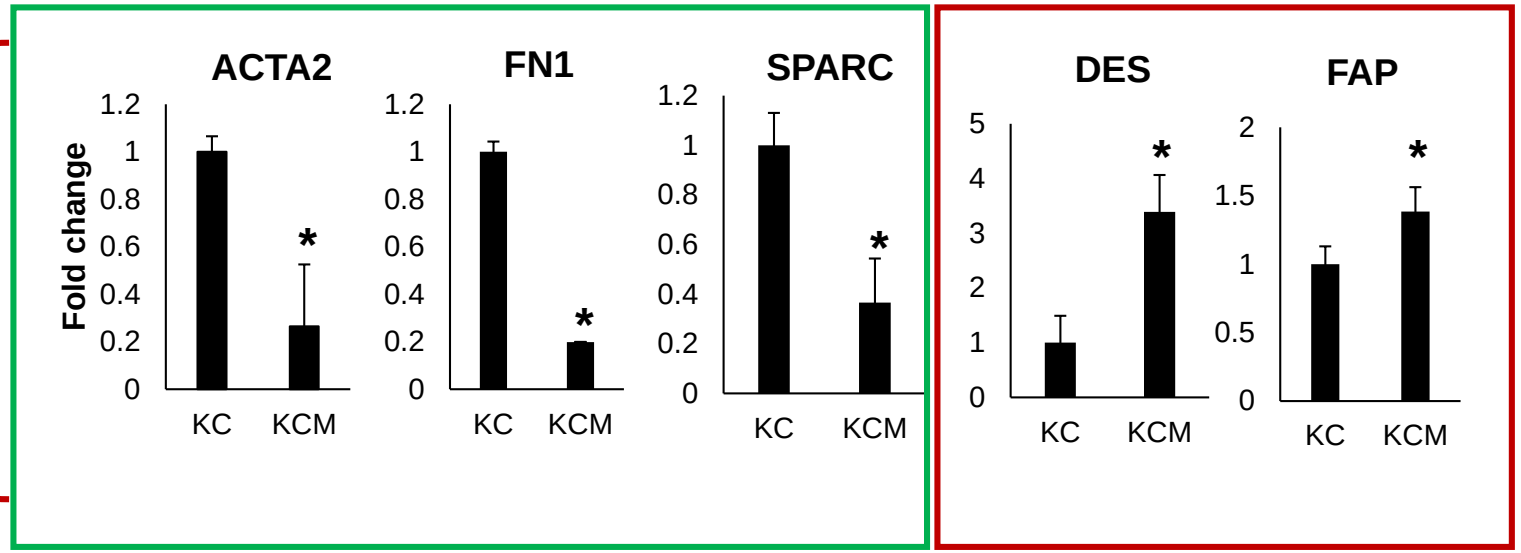
Downregulated

Upregulated

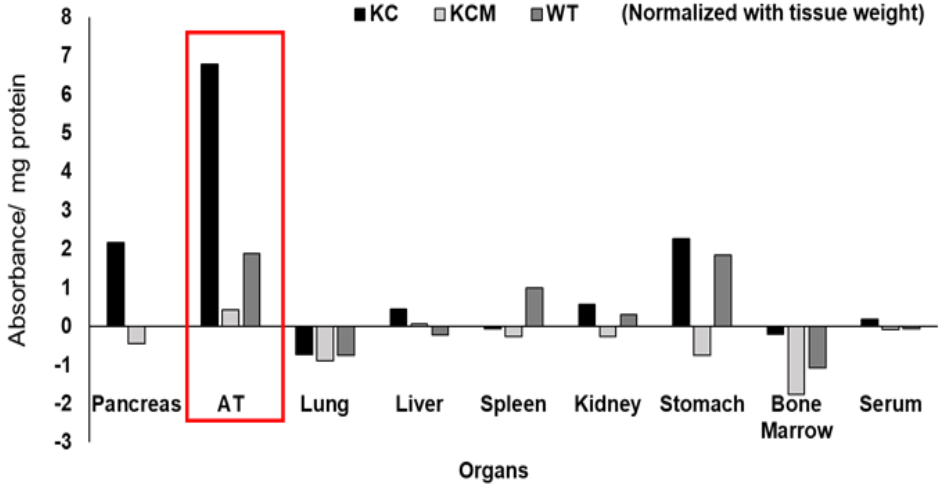
Tumor (50 weeks)

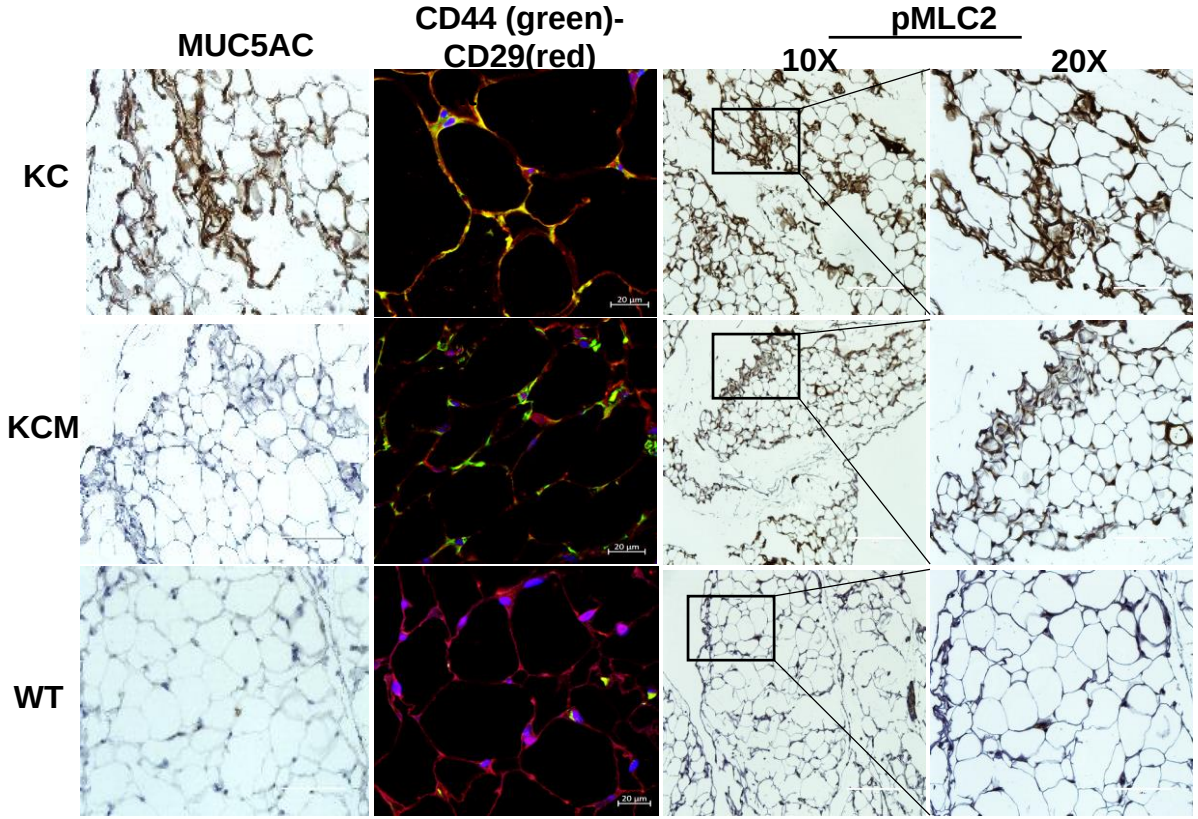


CAFs Isolated from Tumor



On Systemic Front: MUC5AC-mediated clustering of CD44 and CD29 may lead to cytoskeletal reorganization of ADMSCs





CD44-CD29 co-localization

↓

Rho/Rac activation

↓

Cytoskeletal reorganization

↓

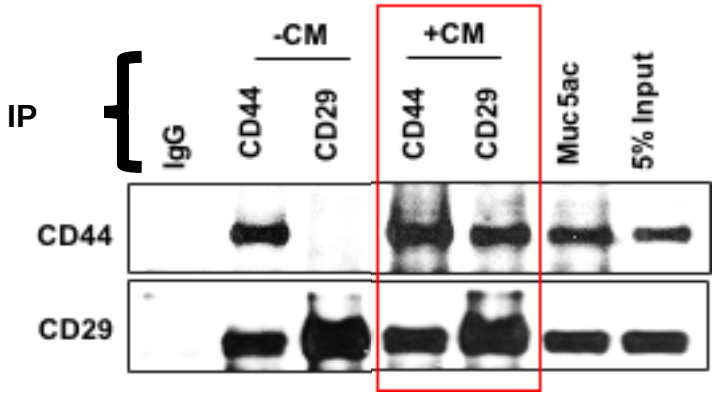
Phosphorylation of myosin light chain (pMLC2)

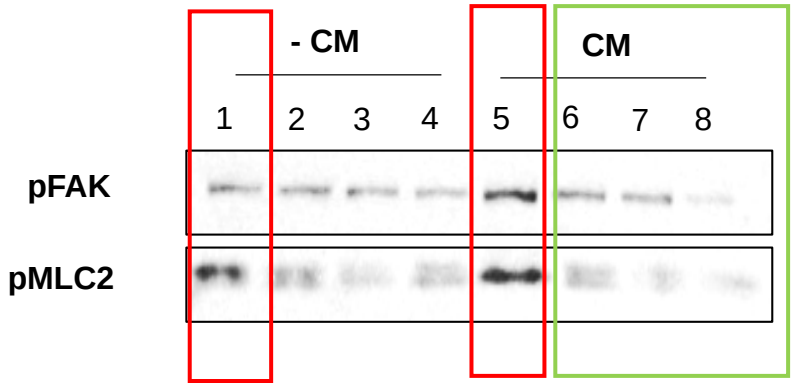
↓

Migration of ADMSCs

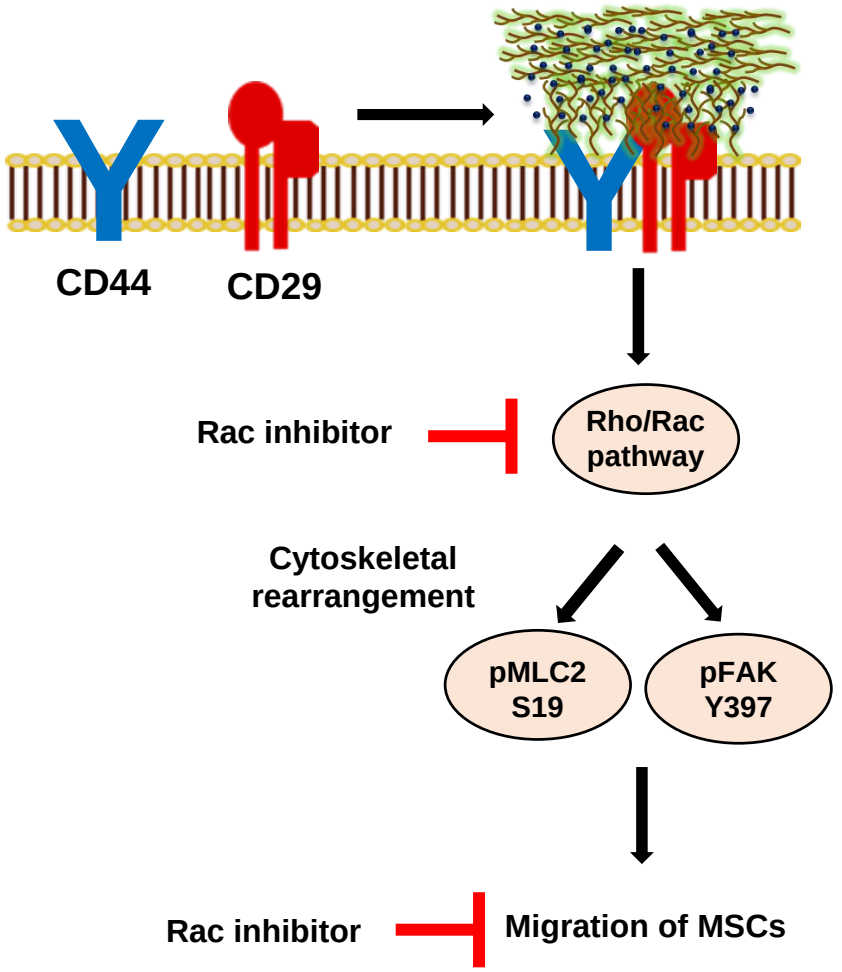
- Differential expression of MUC5AC is observed in adipose tissue
- CD44 and CD29 are **colocalized on the MSCs** of KC- adipose tissue....

MUC5AC-mediated clustering of CD44 and CD29 lead to cytoskeletal reorganization of ADMSCs





1, 5 : No Rac1 inhibitor
2, 6: 0.1 uM Rac1 inhibitor
3, 7: 1uM Rac1 inhibitor
4, 8: 10uM Rac1 inhibitor



INTERIM-SUMMARY

MUC5AC: DIAGNOSTIC and FUNCTIONAL IMPLICATIONS

- Elevated levels of MUC5AC are present in early stages of pancreatic cancer patients.

- The combination of MUC5AC and CA19.9 improve differentiation of:

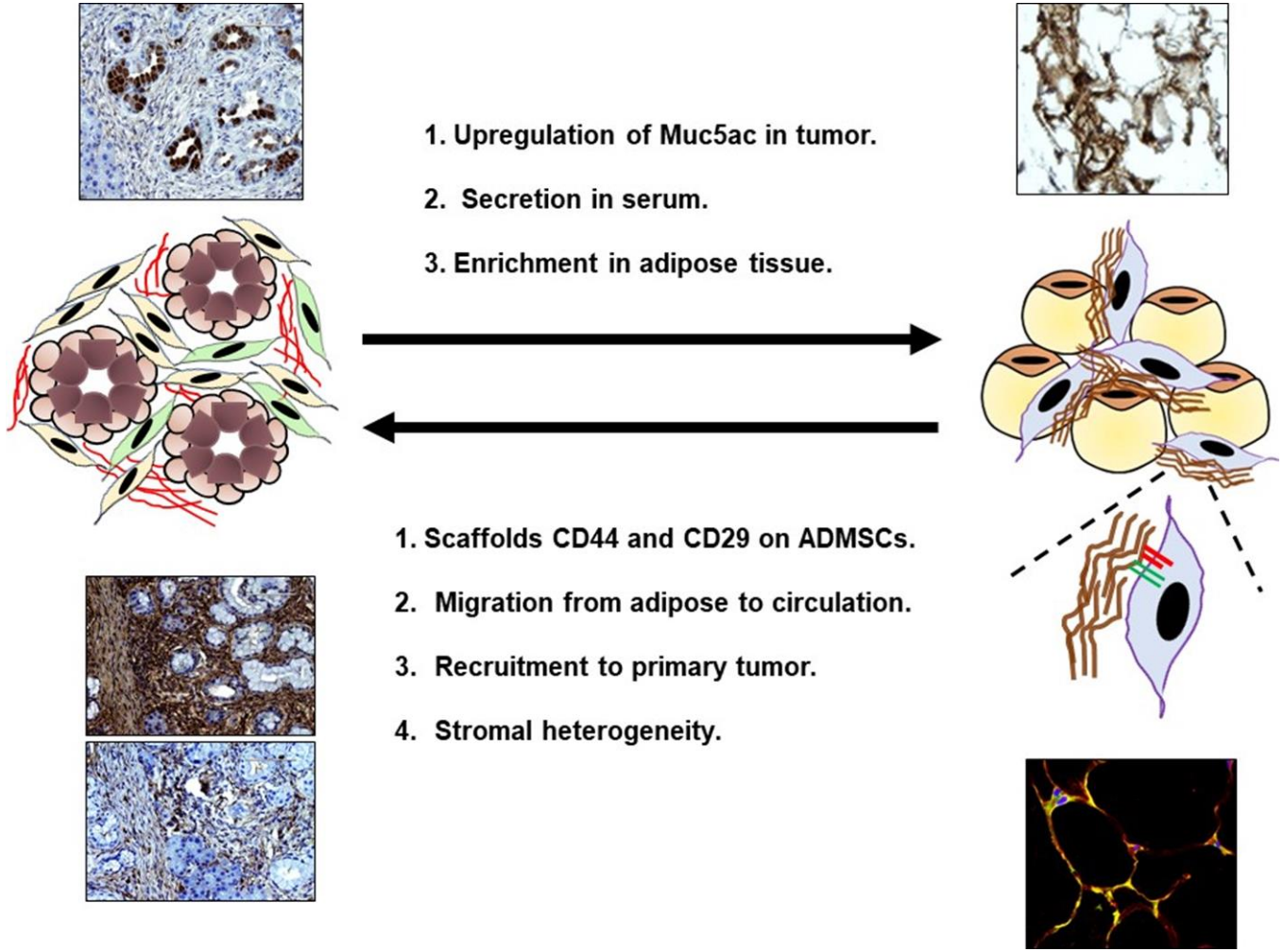
  A. EPC cases from benign controls
  B. EPC and chronic pancreatitis cases in comparison to CA19.9 alone.

- Functionally, MUC5AC facilitates enrichment of cancer stem cells

- Modulates tumor stroma

- Scaffolds CD44 and CD29 on ADMSCs

- Migration from adipose to circulation



Major Hurdles to Development Pancreatic Cancer Biomarkers

➢ *Lack of appropriate Sample Set*

✓ REFERENCE SET FROM EDRN
✓ BAKE-OFF SETS FROM CVC PROGRAM

REFERENCE SET

❖**242 blinded samples collected by NCI under EDRN program**

DIAGNOSIS	SAMPLES	AGE AND DIABETES ADJUSTED
HEALTHY CONTROLS	58	53
CHRONIC PANCREATITIS	59	57
ACUTE BILIARY OBSTRUCTIONS	30	30
STAGE IA/IB/IIA	52	51
STAGE IIB	41	37
TOTAL CASES	240	228
TOTAL PANCREATIC CANCER	93	88

❖Patients till stage 2B were present in the reference set

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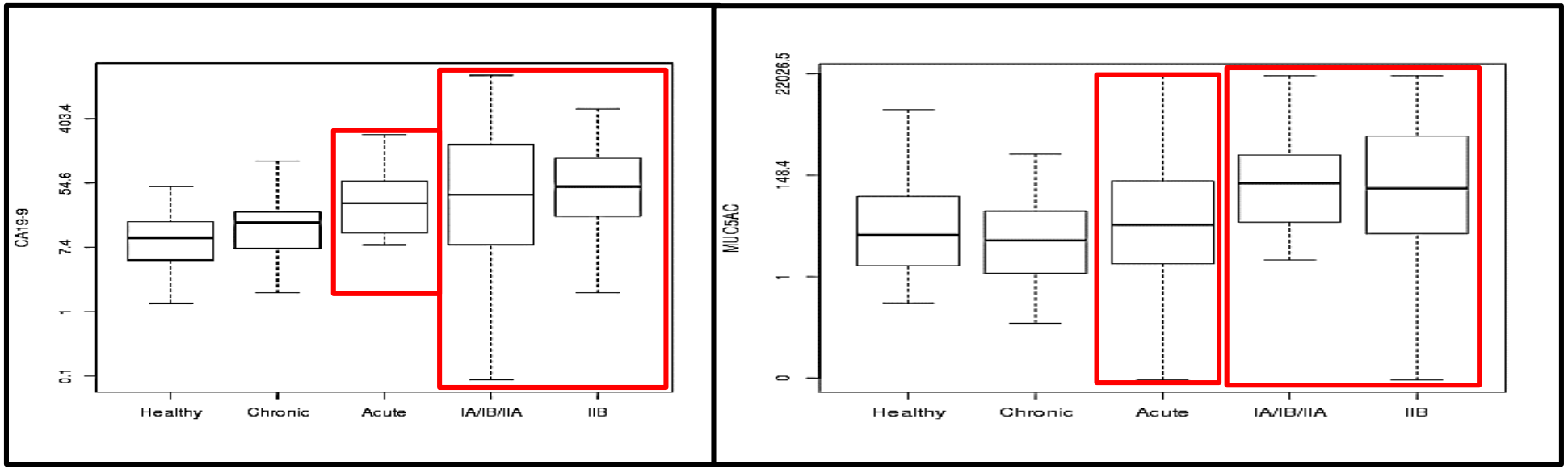
REFERENCE SET: CA19.9 AND MUC5AC LEVELS



- **Elevated levels of both MUC5AC and CA19.9 were observed in PC early stages in comparison to various control groups**
- **Elevated levels of CA19.9 were observed in acute biliary obstruction cases while MUC5AC showed varying trend in this group**

Dr. Huang FHRC

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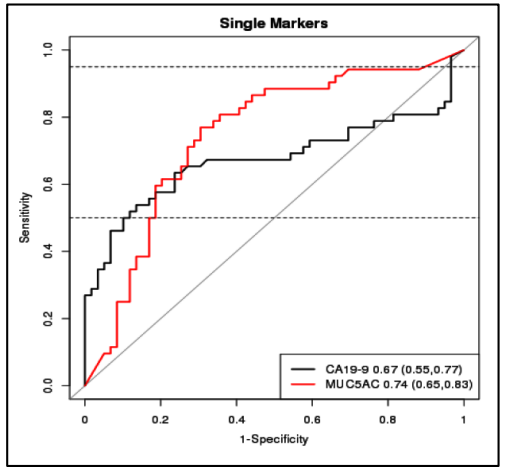
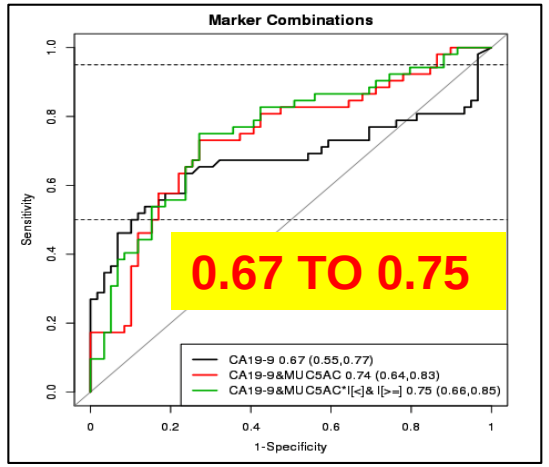
MULTICENTRIC VALIDATION OF MUC5AC IN REFERENCE SET FROM EDRN

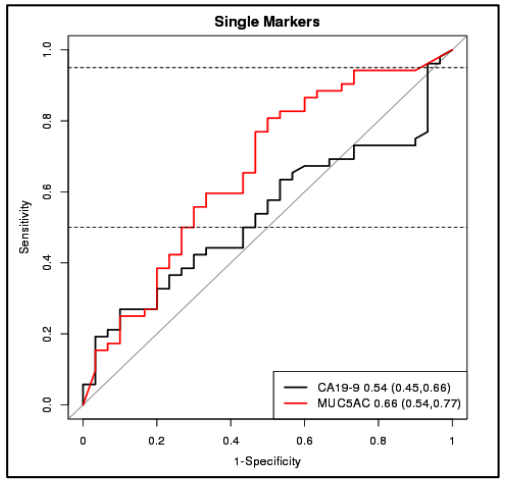
STAGE IA/IB/IIA VS CHRONIC PANCREATITIS

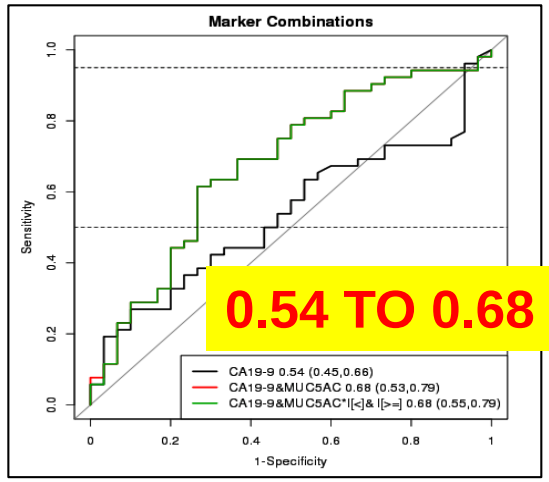




0.67 TO 0.75

STAGE IA/IB/IIA *VS* ACUTE BILIARY OBSTRUCTIONS





0.54 TO 0.68

MUC5AC IMPROVES THE DIAGNOSTIC PERFORMANCE OF CA19.9

BAKE-OFF SAMPLE SET-1

- 183 specimens of pancreatic cancer cases and controls collected from three different sites
  - University of Pittsburgh
  - MD Anderson
  - Mayo Clinic

- **Direct comparison of the potential biomarkers in comparative study**
  - Individual cancer types
  - Benign types

Individual cancer type
1- **A**denocarcinoma,
2 -**M**ucinous tumors,
3- **E**ndocrine tumors,
4- **C**holangiocarcinoma,
5- **A**mpullary

Individual benign type
1-**H**ealthy Control,
2-**B**enign Biliary Obstruction,
3-**C**hronic diabetic,
4-**C**hronic Pancreatitis

- **Investigators were blinded to sample set**

DATA ANALYSES
Dr. Ying Huang, Ph.D. FHRC

- ROC curves was generated for individual marker (panel); the marker's performance is compared to CA19-9 wrt.
  - AUC
  - Specificity at 90% sensitivity
  - Sensitivity at 90% specificity

- Marker Performance Was Evaluated Across Individual Cancer Types as well as Individual Control Groups

- **MARKER PERFORMANCE IN COMBINAITON WITH CA19.9**

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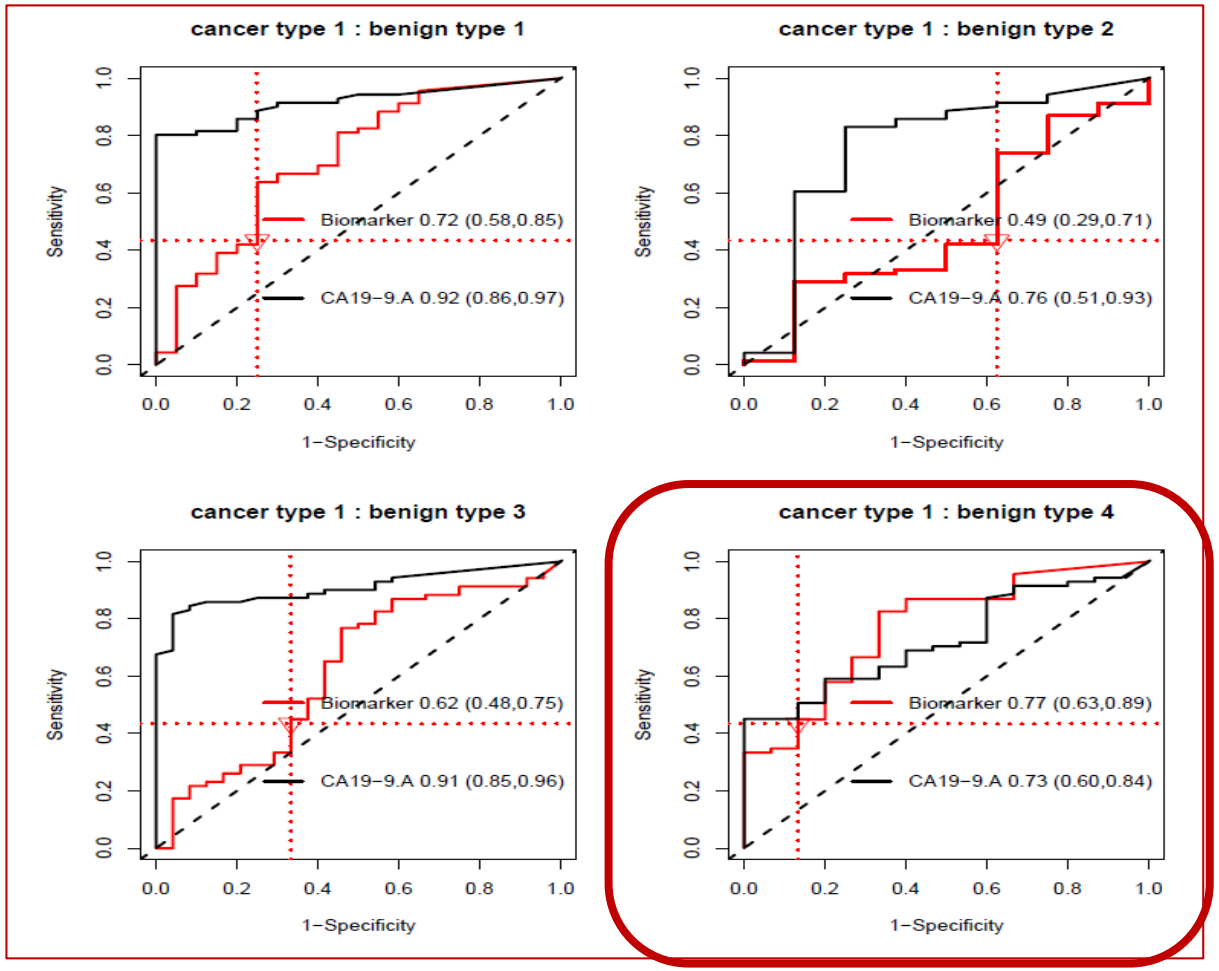
DIAGNOSTIC PERFORMANCE OF MUC5AC IN DIFFERENTIATING ADENOCARCINOMA FROM INDIVIDUAL CONTROL TYPE



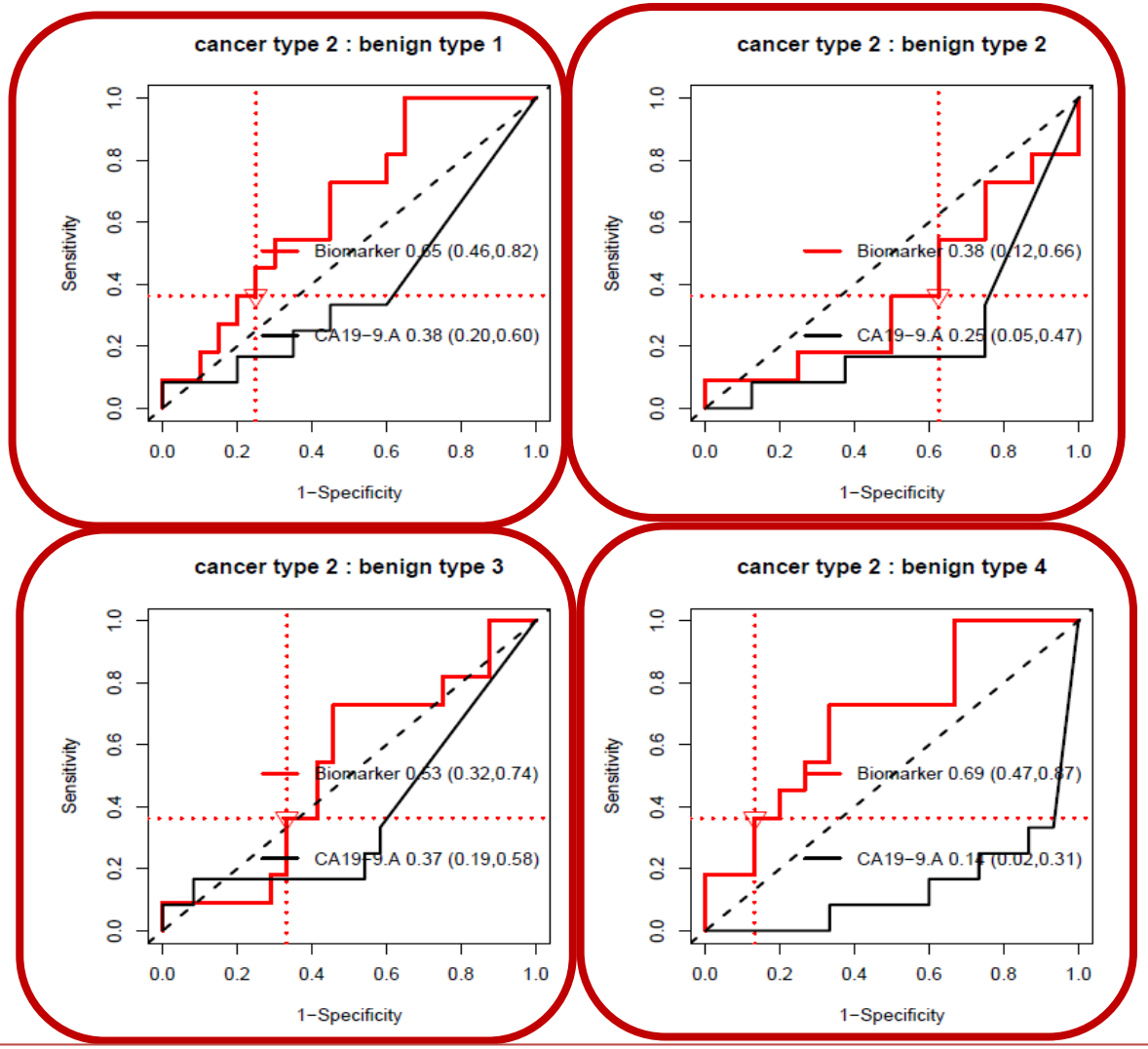
Individual cancer type
1- **A**denocarcinoma,
2 -**M**ucinous tumors,
3- **E**ndocrine tumors,
4- **C**holangiocarcinoma,
5- **A**mpullary

Individual benign type
1-**H**ealthy Control,
2-**B**enign Biliary Obstruction,
3-**C**hronic diabetic,
4-**C**hronic Pancreatitis

Cancer type:Benign type		AUC		SensSpec90		SpecSens90	
1:4	Biomarker	0.77	(0.63,0.89)	0.35	(0.24,0.71)	0.33	(0.00,0.73)
1:4	CA19-9.A	0.73	(0.60,0.84)	0.45	(0.35,0.66)	0.33	(0.00,0.60)
1:4	Difference	0.05	(-0.12,0.21)	-0.10	(-0.31,0.22)	0.00	(-0.33,0.53)

MUC5AC PERFORMED BETTER THAN CA19.9 IN DIFFERENTATING CHRONIC PANCREATITIS CASES FROM CA19.9 THAT WAS USED AT ITS OPTIMAL CUT-OFF

DIAGNOSTIC PERFORMANCE OF MUC5AC IN DIFFERENTIATING MUCINOUS TUMORS FROM INDIVIDUAL CONTROL TYPE



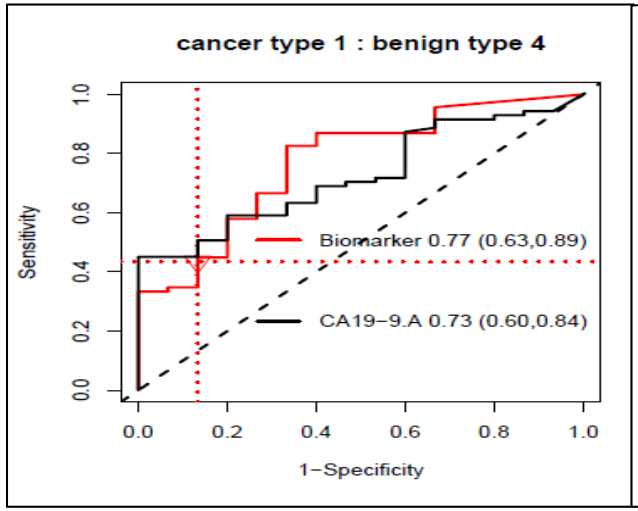
CA19.9 USED AT ITS OPTIMAL PERFORMANCE

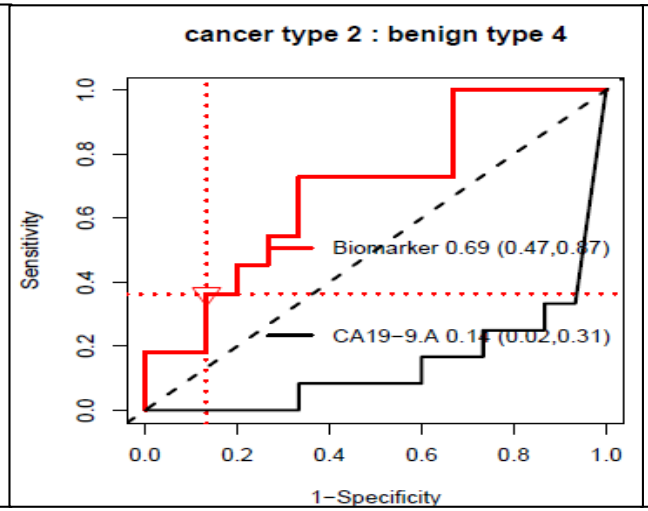
Individual cancer type
1- **A**denocarcinoma,
2 -**M**ucinous tumors,
3- **E**ndocrine tumors,
4- **C**holangiocarcinoma,
5- **A**mpullary

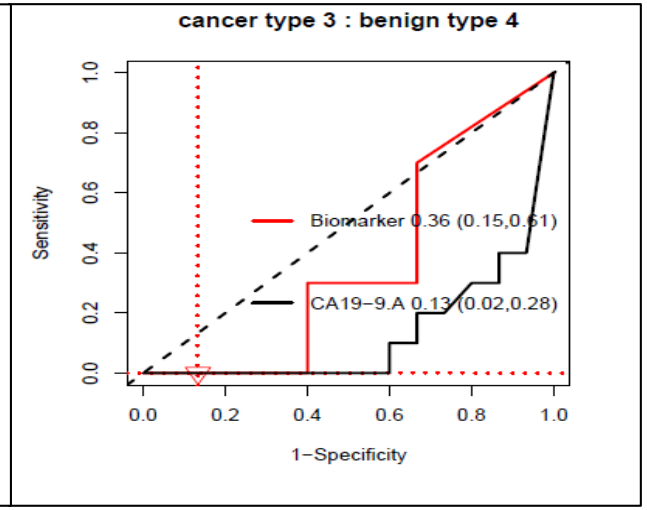
Individual benign type
1-**H**ealthy Control,
2-**B**enign Biliary Obstruction,
3-**C**hronic diabetic,
4-**C**hronic Pancreatitis

MUC5AC PERFORMED BETTER THAN CA19.9 IN DIFFERENTATING ALL TYPES OF BENIGN CASES FROM MUCINOUS TUMORS

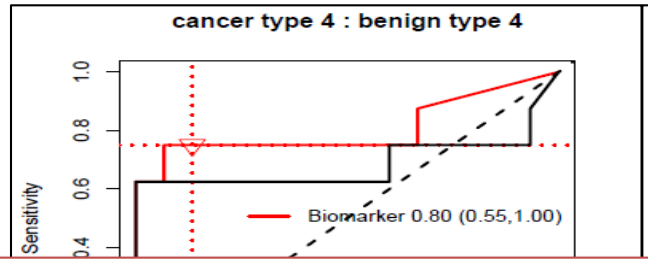
INDIVIDUAL CANCER TYPES VS. CHRONIC PANCREATITIS

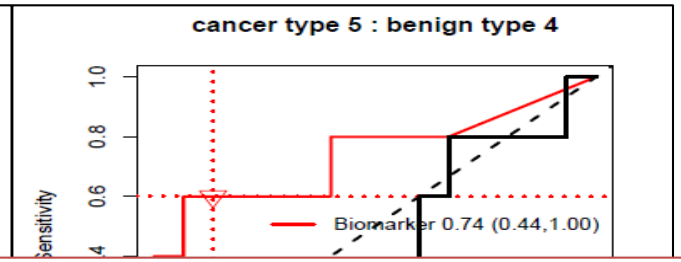






1:4	Biomarker	0.77	(0.63,0.89)	0.35	(0.24,0.71)	0.33	(0.00,0.73)	2:4	Biomarker	0.69	(0.47,0.87)	0.18	(0.00,0.70)	0.33	(0.13,0.80)	3:4	Biomarker	0.36	(0.15,0.61)	0.00	(0.00,0.00)	0.00	(0.00,0.47)
1:4	CA19-9.A	0.73	(0.60,0.84)	0.45	(0.35,0.66)	0.33	(0.00,0.60)	2:4	CA19-9.A	0.14	(0.02,0.31)	0.00	(0.00,0.00)	0.00	(0.00,0.00)	3:4	CA19-9.A	0.13	(0.02,0.28)	0.00	(0.00,0.00)	0.00	(0.00,0.00)
1:4	Difference	0.05	(-0.12,0.21)	-0.10	(-0.31,0.22)	0.00	(-0.33,0.53)	2:4	Difference	0.55	(0.27,0.79)	0.18	(0.00,0.70)	0.33	(0.13,0.80)	3:4	Difference	0.23	(-0.03,0.51)	0.00	(0.00,0.00)	0.00	(0.00,0.47)





Individual cancer type
1- **A**denocarcinoma,
2 -**M**ucinous tumors,
3- **E**ndocrine tumors,
4- **C**holangiocarcinoma,
5- **A**mpullary

4:4	CA19-9.A	0.69	(0.39,0.95)	0.62	(0.25,0.88)	0.00	(0.00,0.60)	5:4	CA19-9.A	0.44	(0.13,0.75)	0.20	(0.00,0.60)	0.07	(0.00,0.53)			
4:4	Difference	0.12	(-0.02,0.30)	0.12	(-0.25,0.38)	0.00	(0.00,0.60)	5:4	Difference	0.30	(-0.05,0.72)	0.40	(0.00,0.80)	-0.07	(-0.47,0.93)			

MUC5AC PERFORMED BETTER THAN CA19.9 IN DIFFERENTATING CHRONIC PANCREATITIS CASES IN ALL TUMOR TYPES

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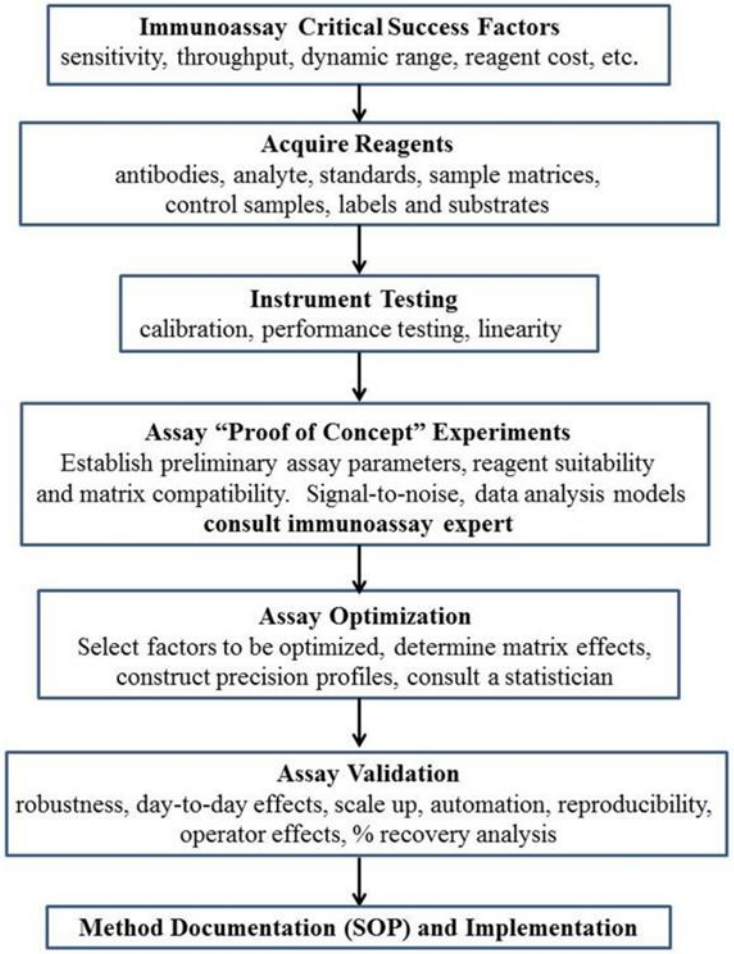
CHALLENGES WITH CLINICAL ASSAY ON MUCINS

- Mucins are high molecular weight complex glycoproteins
- Reductions in levels of MUC4 and MUC5AC is observed over time IN STANDARDS PREPARED FROM CELL LYSATE
- For development of clinical grade, we focused our efforts to develop MUCIN-RICH STANDARD.
- Evaluated its performance to detect MUC4, MUC5AC, AND CA19.9.
- Evaluated its stability, sensitivity specificity, robustness, and reproducibility.



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DEVELOPMENT OF CLINICAL GRADE STANDARD CONTAINING MUCIN-RICH FRACTIONS

Criteria

(3) Biomarkers (CA19.9, MUC5AC, MUC4)

Approach

- ELISA data
  - Antibody pairs pre-determined
- Blood Interfering Component
  - Bilirubin
  - Hemoglobin
  - Intralipid
  - Biotin

How are you going to get enough starting material?

Had to have quick growth

CD-18, Capan-2, A549, CFPAC-1

- Cell Line selection
  - No human material
  - No recombinant protein
  - No synthetic peptide
- Location
  - MUC4 primarily membrane bound
  - CA19.9 and MUC5AC in cell culture supernatant and membrane associated
- Total Lysate
  - Isolation/Prep Steps/Method Development
    - GIT Ultracentrifugation
      - ✗ Scale-up concerns
    - Porcine Mucin Method (gastric scrapings)
      - Solid-Liquid-Liquid Extraction (SLLE)
        - 1:15 Hexane: Water Ratio

↓

Isolate Aqueous Phase and Concentrate:
(Excludes organic, hydrophobic lipids, and membranous material)

↓

Size Exclusion Chromatography (SEC)
Superose 6 Increase Column

Large Proteins
(MUC4, MUC5AC, CA19.9) + Other Large Proteins

→

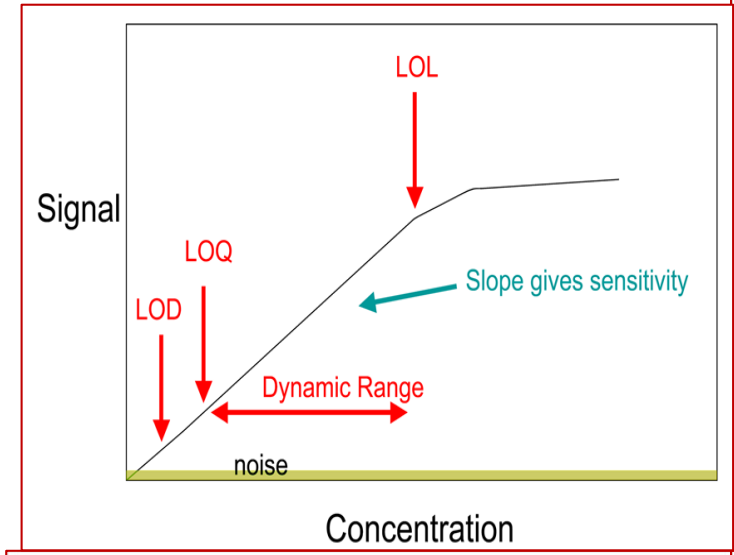
Large Proteins
(MUC4, MUC5AC, CA19.9)
+
Other Large Proteins

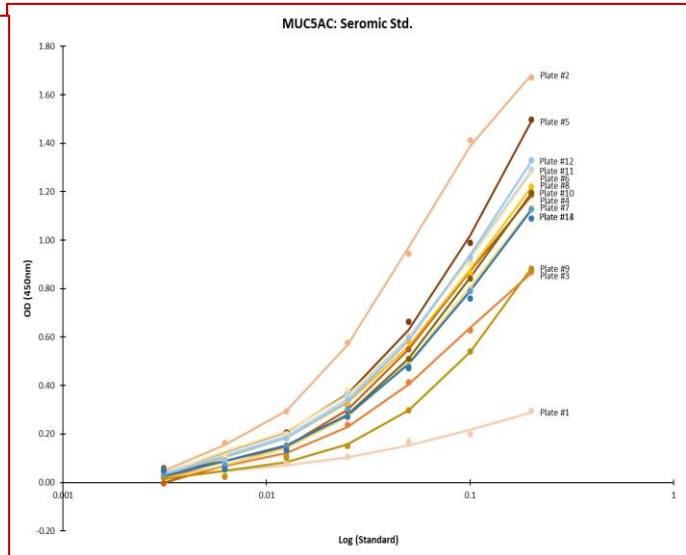
"SDT" Seromic Standard
10 mM Phosphate Buffer, 170 mM NaCl, pH 7.0

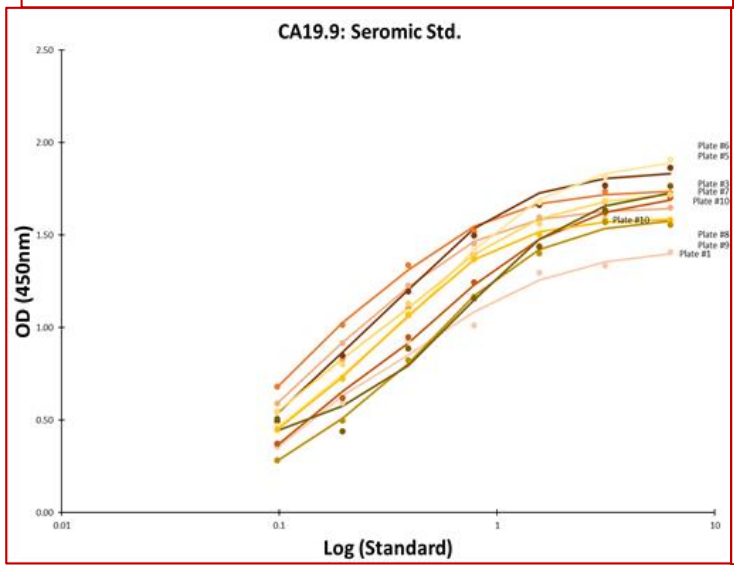
- Lyophilized
- Store at -80°C
- Store in 0.1% BSA
- Preserve in Human Serum Matrix

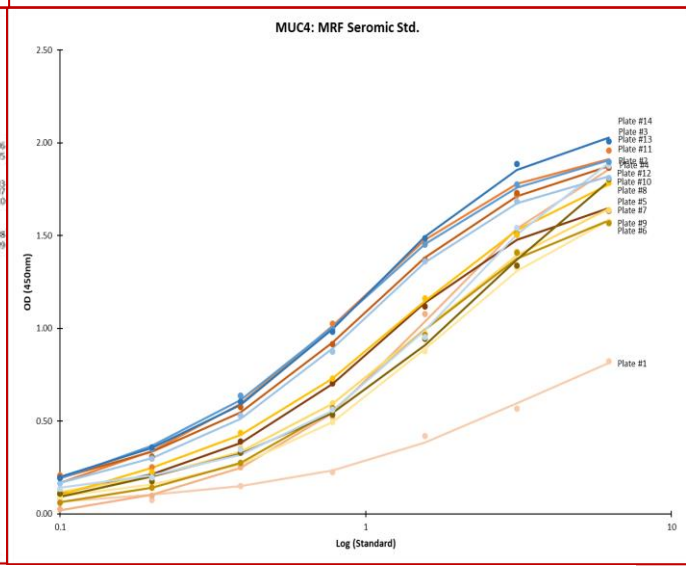
Sample Testing

Stability Testing





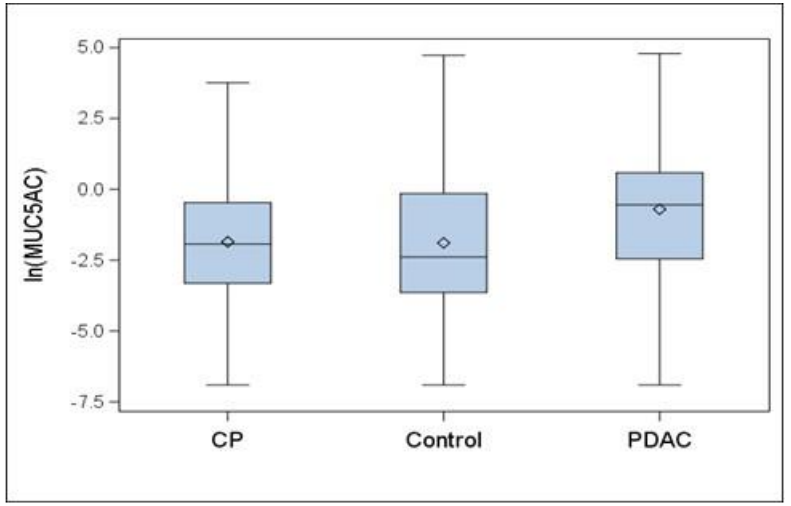


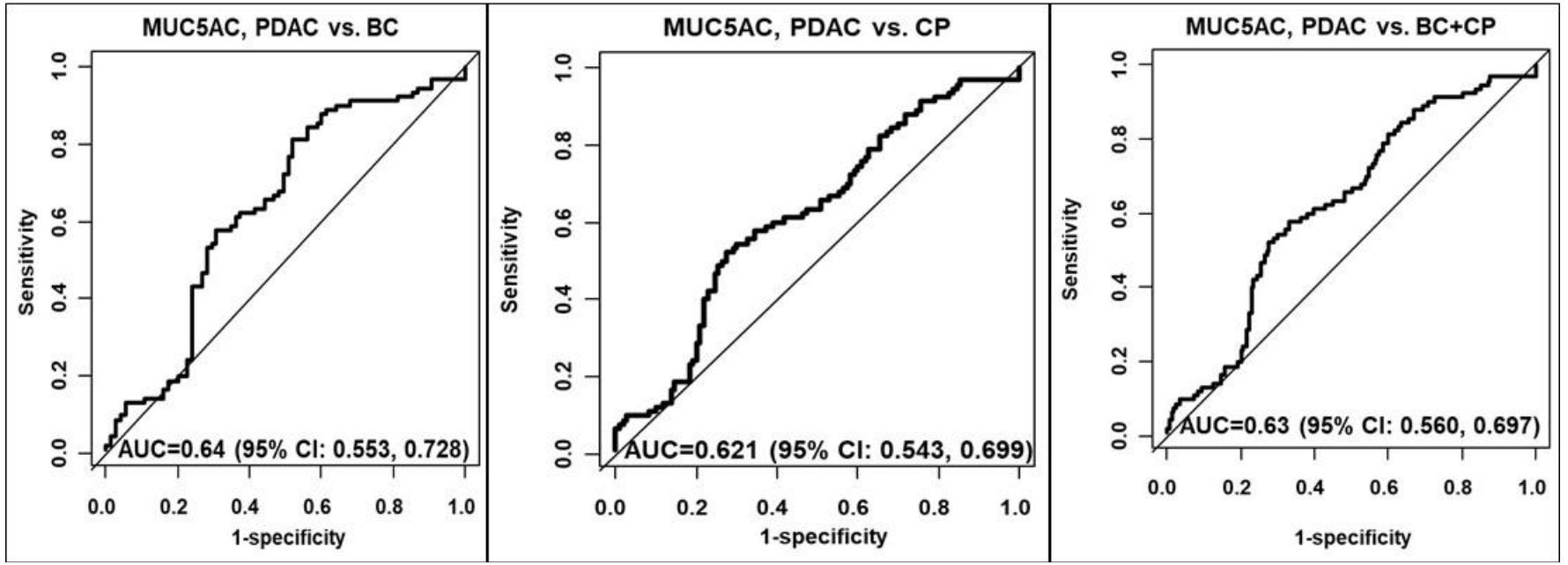


NEWLY PREPARED MUCIN-RICH STANDARD WAS USED FOR FURTHER STUDIES

MUC5AC PERFORMANCE IN VALIDATION SET FROM CVC PROGRAM

- N= 250 CASES
- ACQUIRED IN BLINDED MANNER FROM UPMC



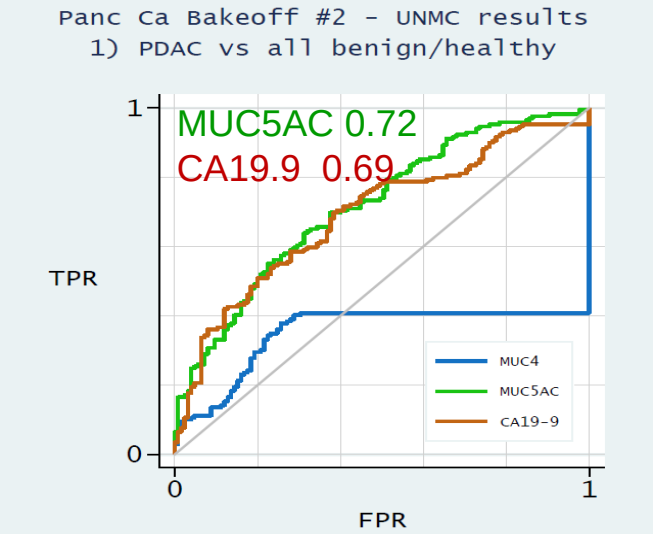


MUC5AC SHOWED AUC IN RANGE OF 0.62- 0.64 FOR DIFFERENTIATING VARIOUS CONTROL GROUPS FROM PC CASES

BAKE-OFF SAMPLE SET-2

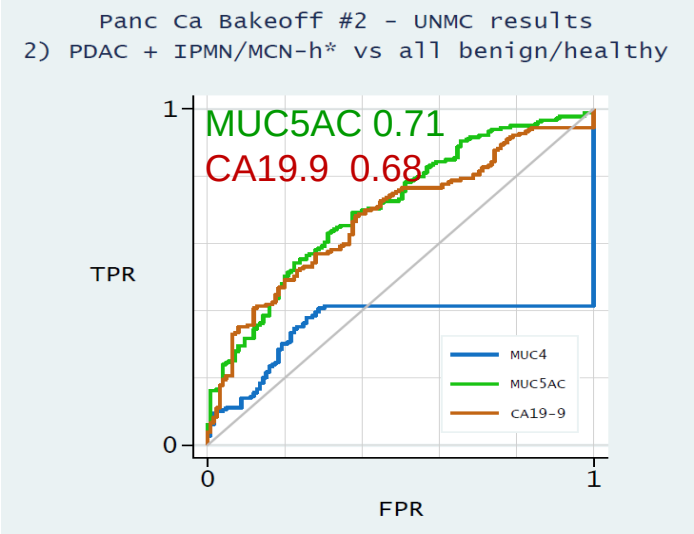
- Pancreatic conditions included pancreatic ductal adenocarcinoma (PDAC), other pancreatic cancers, neoplasms, benign pancreatic conditions, and some healthy controls.

PDAC vs. all benign/healthy



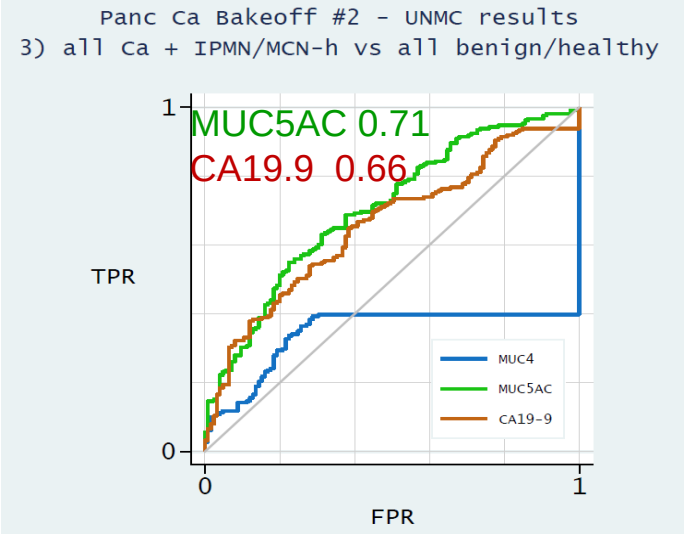
marker	AUC	sens(spec=.90)	spec(sens=.90)
CA19-9	0.69 (0.63,0.75)	0.36 (0.20,0.49)	0.23 (0.15,0.31)
MUC4	0.35 (0.29,0.42)	0.14 (0.08,0.24)	0.00 (0.00,0.00)
difference*	-0.34 (-0.42,-0.25)	-0.22 (-0.35,-0.07)	-0.23 (-0.31,-0.15)
p (difference)	<< .001	.0012	<< .001
MUC5AC	0.72 (0.66,0.77)	0.33 (0.22,0.45)	0.34 (0.23,0.43)
difference*	0.03 (-0.04,0.09)	-0.03 (-0.17,0.12)	0.11 (-0.02,0.22)
p (difference)	.45	.68	.066

PDAC + IPMN/MCN-H vs. all benign/healthy



marker	AUC	sens(spec=.90)	spec(sens=.90)
CA19-9	0.68 (0.62,0.74)	0.35 (0.20,0.46)	0.22 (0.13,0.31)
MUC4	0.36 (0.29,0.43)	0.14 (0.08,0.25)	0.00 (0.00,0.00)
difference*	-0.32 (-0.40,-0.24)	-0.21 (-0.33,-0.05)	-0.22 (-0.31,-0.13)
p (difference)	<< .001	.0015	<< .001
MUC5AC	0.71 (0.65,0.76)	0.32 (0.22,0.43)	0.34 (0.22,0.43)
difference*	0.03 (-0.03,0.09)	-0.03 (-0.16,0.11)	0.12 (-0.02,0.23)
p (difference)	.35	.62	.068

All cancers + IPMN/MCN-H vs. all benign/healthy

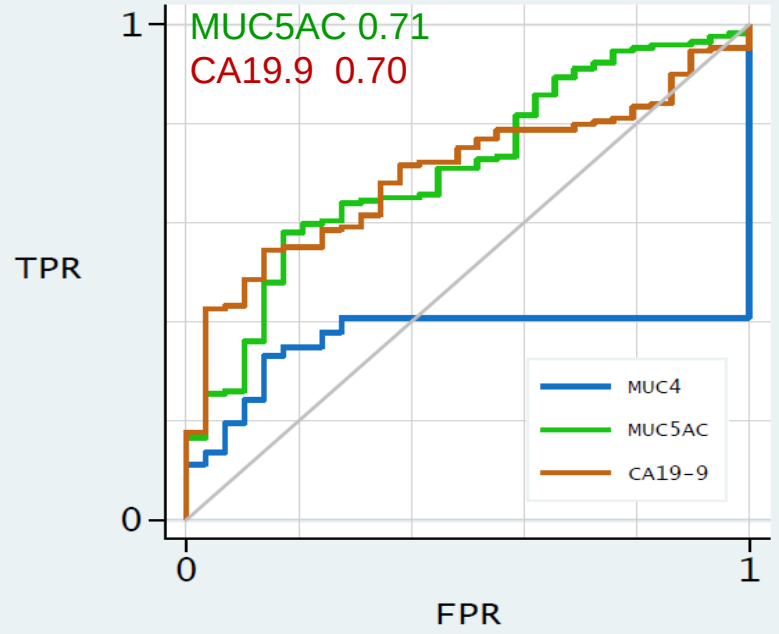


marker	AUC	sens(spec=.90)	spec(sens=.90)
CA19-9	0.66 (0.60,0.72)	0.32 (0.19,0.43)	0.22 (0.12,0.30)
MUC4	0.34 (0.28,0.41)	0.14 (0.09,0.24)	0.00 (0.00,0.00)
difference*	-0.31 (-0.39,-0.23)	-0.18 (-0.29,-0.05)	-0.22 (-0.30,-0.12)
p (difference)	<< .001	.0024	<< .001
MUC5AC	0.71 (0.65,0.76)	0.30 (0.20,0.43)	0.33 (0.23,0.42)
difference*	0.05 (-0.02,0.11)	-0.02 (-0.15,0.11)	0.10 (-0.01,0.23)
p (difference)	.12	.77	.083

AUC of >0.7 was observed for MUC5AC for differentiating various control groups from PDAC cases

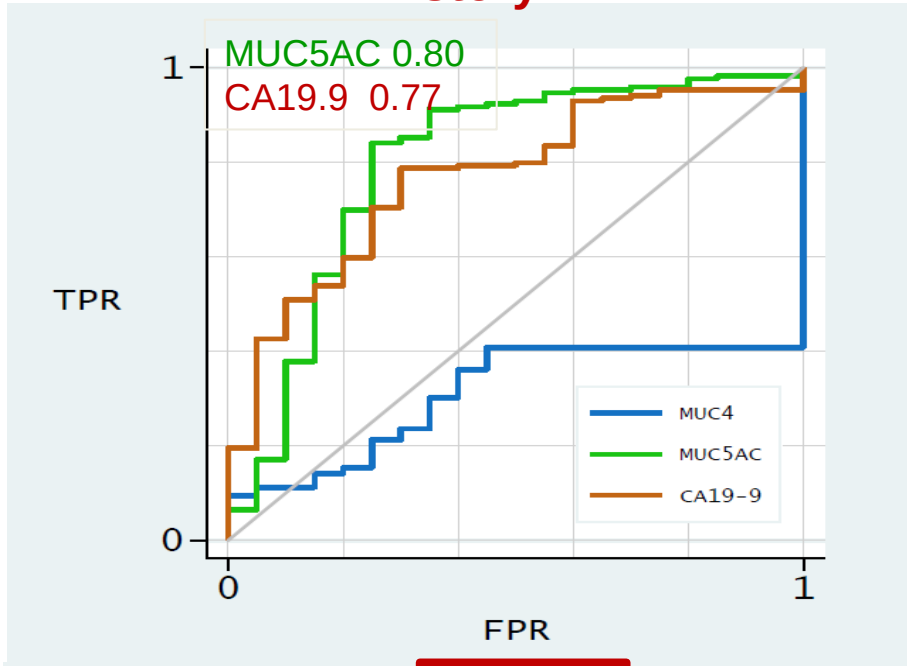
CONTROLS WITH AND WITHOUT FAMILY HISTORY OF PC VERSUS PDAC CASES

PDAC vs. HC control without family history



marker	AUC	sens(spec=.90)	spec(sens=.90)
CA19-9	0.70 (0.62,0.78)	0.43 (0.17,0.60)	0.10 (0.00,0.24)
MUC4	0.37 (0.29,0.44)	0.20 (0.09,0.39)	0.00 (0.00,0.00)
difference*	-0.33 (-0.43,-0.23)	-0.24 (-0.43,-0.01)	-0.10 (-0.24,0.00)
p (difference)	<< .001	.031	.098
MUC5AC	0.71 (0.61,0.80)	0.26 (0.15,0.62)	0.31 (0.14,0.48)
difference*	0.01 (-0.09,0.11)	-0.17 (-0.33,0.17)	0.21 (-0.03,0.41)
p (difference)	.86	.18	.061

PDAC vs. HC control with family history

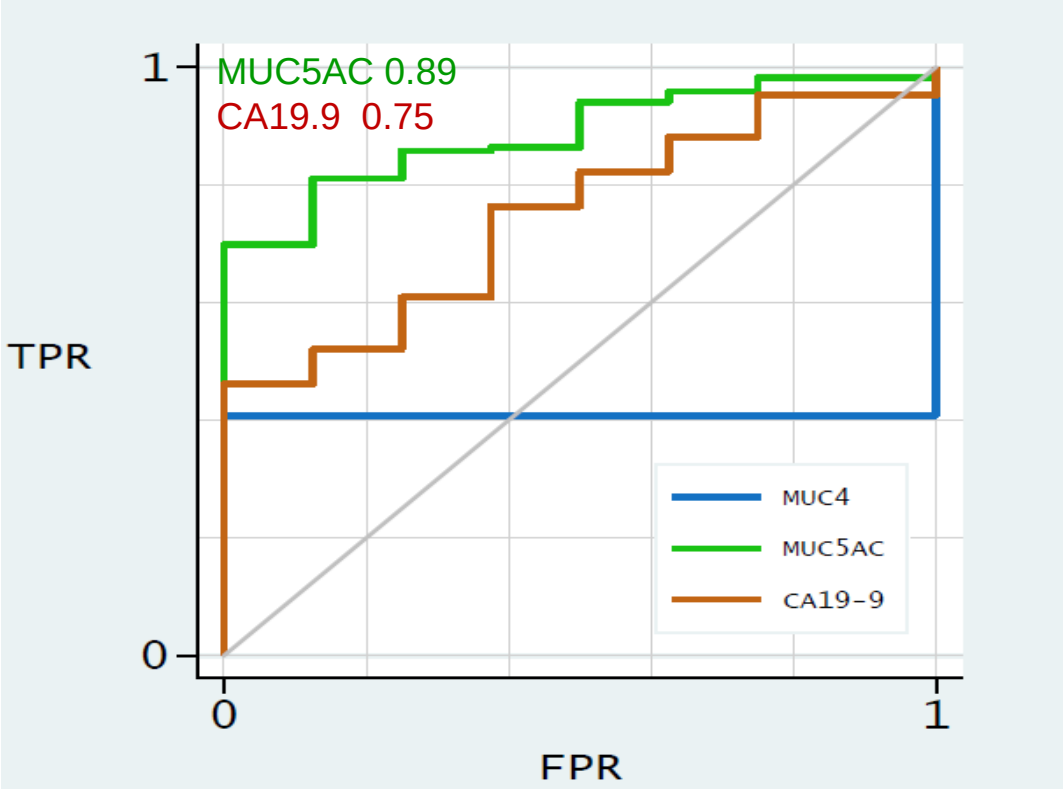


marker	AUC	sens(spec=.90)	spec(sens=.90)
CA19-9	0.77 (0.66,0.86)	0.43 (0.16,0.66)	0.40 (0.20,0.60)
MUC4	0.31 (0.23,0.39)	0.11 (0.07,0.21)	0.00 (0.00,0.00)
difference*	-0.46 (-0.56,-0.36)	-0.31 (-0.53,-0.06)	-0.40 (-0.60,-0.20)
p (difference)	<< .001	.022	< .001
MUC5AC	0.80 (0.67,0.91)	0.17 (0.04,0.79)	0.65 (0.35,0.85)
difference*	0.03 (-0.05,0.11)	-0.25 (-0.37,0.19)	0.25 (-0.05,0.50)
p (difference)	.44	.085	.077

AUC OF 0.8 WAS OBSERVED FOR MUC5AC FOR DIFFERENTIATING HEALTHY CONTROL WITH FAIMLY HISTORY OF PDAC IN COMPARISON TO AUC OF 0.71 FOR CONTROLS WITHOUS FAIMLY HISTORY

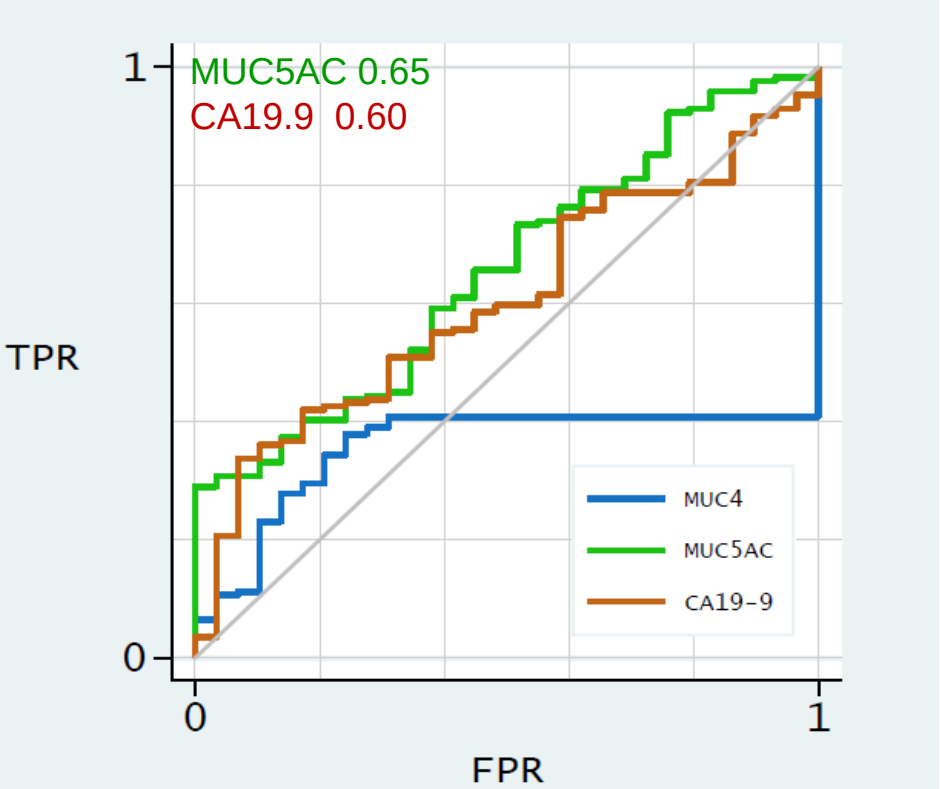
Other control groups: Benign Biliary obstruction

PDAC vs. Benign Biliary Obstruction



marker	AUC	sens(spec=.90)	spec(sens=.90)
CA19-9	0.75 (0.60,0.87)	0.46 (0.40,0.70)	0.25 (0.00,0.63)
MUC4	0.41 (0.34,0.49)	0.41 (0.34,0.49)	0.00 (0.00,0.00)
difference*	-0.34 (-0.49,-0.18)	-0.05 (-0.30,0.04)	-0.25 (-0.63,0.00)
p (difference)	<< .001	.51	.14
MUC5AC	0.89 (0.80,0.95)	0.70 (0.64,0.88)	0.50 (0.13,0.88)
difference*	0.14 (0.04,0.26)	0.24 (0.10,0.43)	0.25 (0.00,0.63)
p (difference)	.016	.0065	.13

PDAC vs. Chronic pancreatitis



marker	AUC	sens(spec=.90)	spec(sens=.90)
CA19-9	0.60 (0.50,0.70)	0.34 (0.03,0.46)	0.10 (0.00,0.24)
MUC4	0.36 (0.28,0.43)	0.11 (0.05,0.35)	0.00 (0.00,0.00)
difference*	-0.25 (-0.36,-0.13)	-0.22 (-0.34,0.21)	-0.10 (-0.24,0.00)
p (difference)	<< .001	.12	.1
MUC5AC	0.65 (0.56,0.75)	0.31 (0.25,0.44)	0.24 (0.10,0.41)
difference*	0.05 (-0.06,0.17)	-0.03 (-0.14,0.34)	0.14 (-0.07,0.34)
p (difference)	.39	.81	.17

MUC5AC PERFORMED BETTER THAN CA19.9 IN DIFFERENTATING CHRONIC PANCREATITIS AND BENIGN BILIARY OBSTRUCTION

INTERIM-SUMMARY

- OBSERVED AUC FOR MUC5AC WERE BETTER THAN CA19.9 IN DIFFERENTATING **PDAC FROM CHRONIC PANCREATITIS**

- DIFFERENCES WERE OBSERVED AT OPTIMAL VALUE OF CA19.9

- **MUC5AC PERFORMANCE BETTER or COMPARABLE TO CA19.9 IN DIFFERENTIATING VARIOUS CONTROL GROUPS FROM PDAC IN VARIOUS SETS INCLUDING TRAINING, VALIDATION, REFERENCE SET FROM EDRN, BAKE-OFF SET 1, BAKE-OFF SET 2.**

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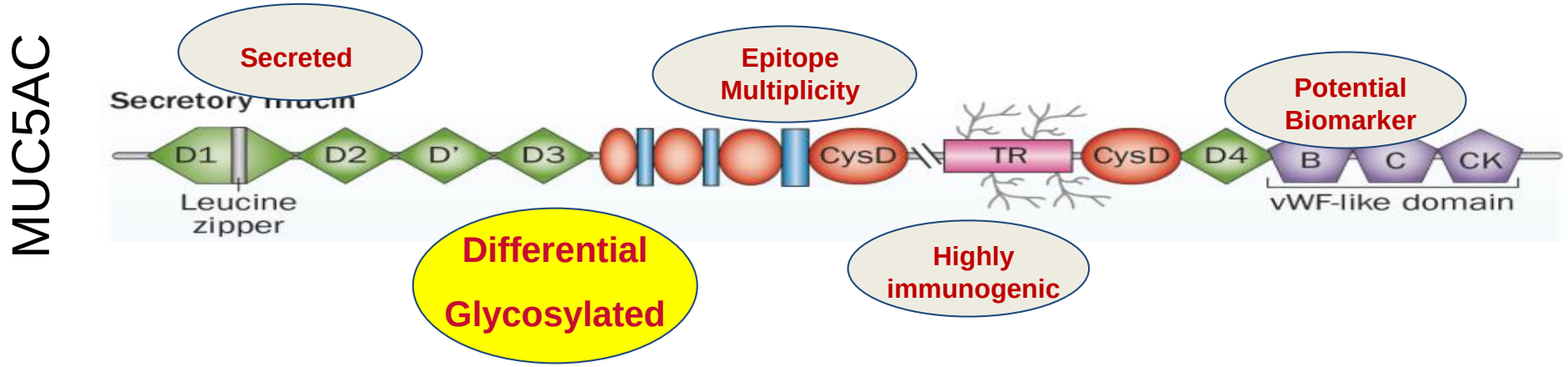
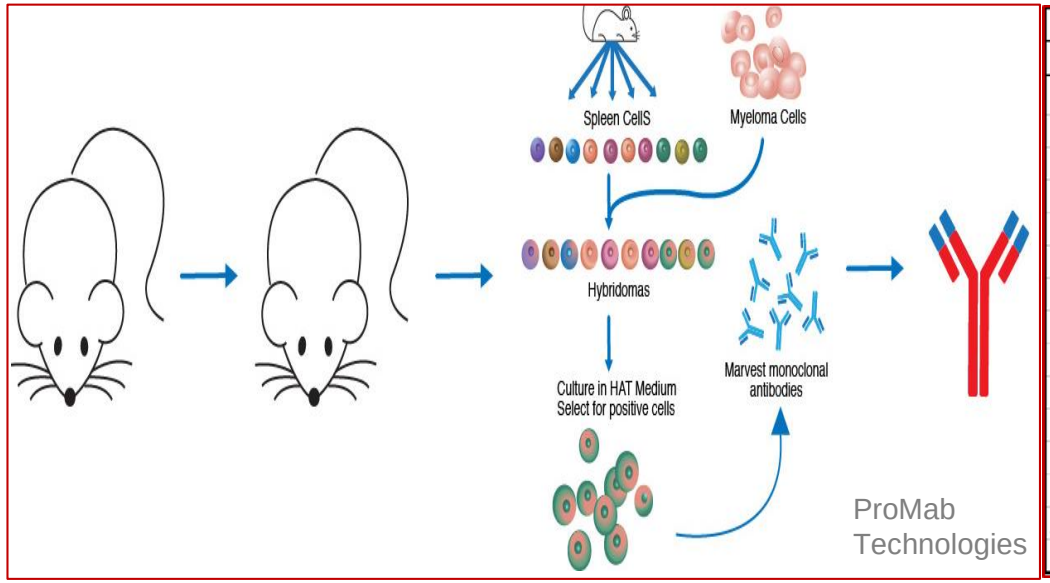
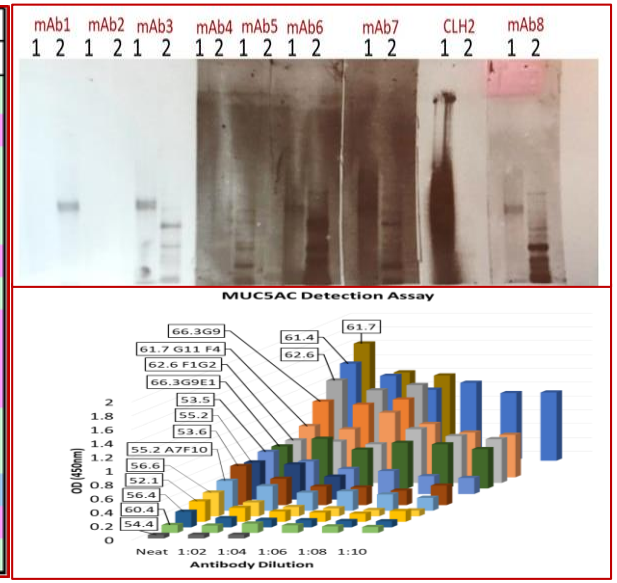
GENERATION AND CHARACTERIZATION OF NOVEL MONOCLONAL ANTIBODIES AGAINST MUC5AC





Hybrid		Clone	ELISA	
Name	Number	Number	Peptide	Protein
AA_1 P6 2/1	51	51.1	0.683	1.077
AA1P6 1/2	52	52.1	0.056	0.297
AA1P6 3/6	53	53.1	0.095	0.757
AA1 4/9	54	54.4	2.94	OVERFLW
AA2 P6 2/6	55	55.2	0.52	3.8
AA1P6 1/1	56	56.4	1.05	0.27
AS 2/17	57	57.1	0.11	0.844
AA1 2/24	58	58.1	1.24	0.22
AS 3/10	59	59.1	OVERFLW	0.19
AS 4/7	60	60.4	1.75	0.27
AA1 2/11	61	61.7	0.404	OVERFLW
AA1 2/23	62	62.6	0.19	2.12
PP3/8	63	63.1	0.92	0.435
AA1 P6 4/1-1	64	64.1	0.075	0.29
AS P6 1/3	66	66.3	3.881	2.235

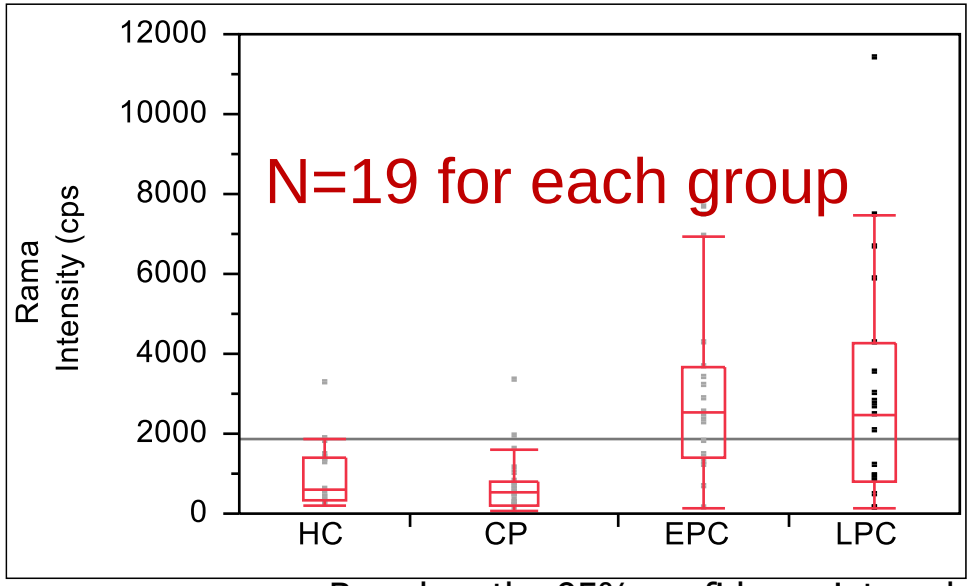


ONGOING: Evaluation and comparison of MUC5AC performance across malignancies using newly developed antibodies

Detection of MUC4 in PC Patient Serum

- Healthy Controls (HC)
- Chronic Pancreatitis (CP)
- Early Stage PC (EPC)
- Late Stage PC (LPC)

Comparison	AUC	Threshold	Specificity	Sensitivity
PC vs. HC	0.798	>= 2079	0.947	0.605
EPC vs. HC	0.842	>= 2242	0.947	0.632
LPC vs. HC	0.754	>= 2079	0.947	0.579
PC vs. CP	0.843	>=1198	0.870	0.763
EPC vs. CP	0.883	>=1224	0.870	0.895
LPC vs. CP	0.803	>=831	0.783	0.789



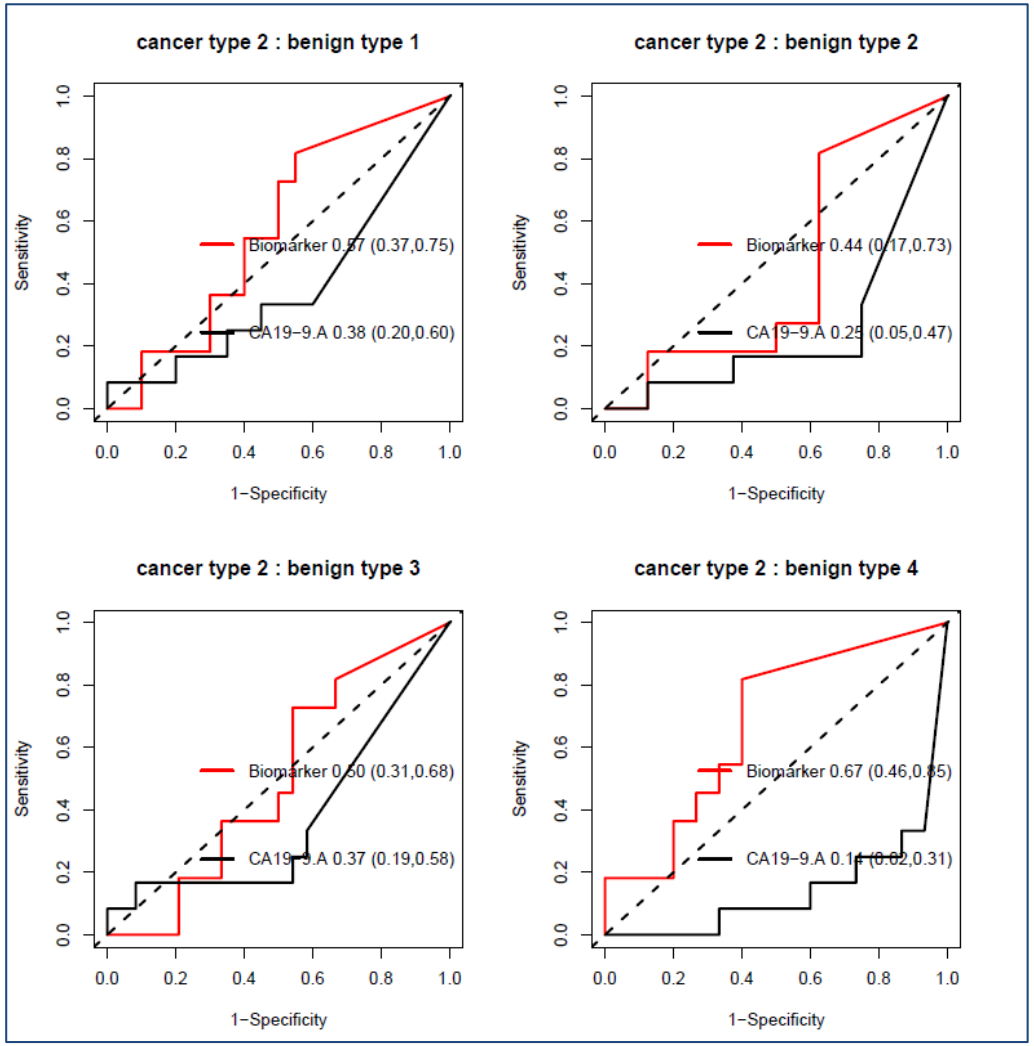
- Based on the 95% confidence interval Raman Intensity discriminates:

- PC vs. HC
- EPC vs. HC (p=0.0053)
- LPC vs. HC (p=0.0050)

- PC vs. CP
- EPC vs. CP (p=0.0011)
- LPC vs. CP (p=0.0011)

MUC4 is 61% sensitive and 95% specific in differentiating PC from HC.
MUC4 was 76% sensitive and 95% specific in differentiating PC from CP.

DIAGNOSTIC PERFORMANCE OF MUC4 IN DIFFERENTIATING MUCINOUS TUMORS FROM INDIVIDUAL CONTROL TYPE

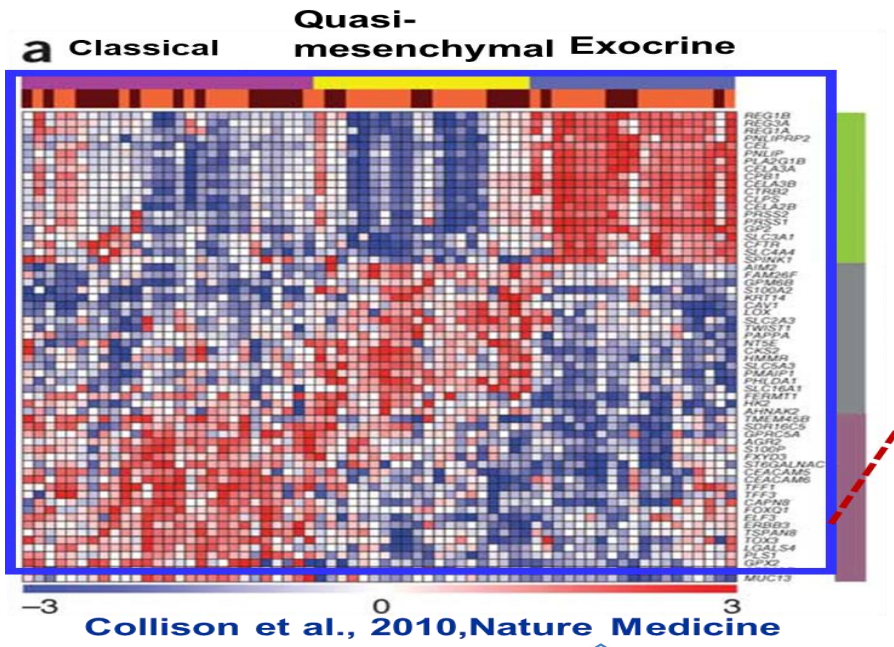


Cancer type:Benign type		AUC		SensSpec90		SpecSens90	
2:1	Biomarker	0.57	(0.37,0.75)	0.18	(0.00,0.40)	0.00	(0.00,0.65)
2:1	CA19-9.A	0.38	(0.20,0.60)	0.08	(0.00,0.33)	0.00	(0.00,0.00)
2:1	Difference	0.19	(-0.11,0.44)	0.10	(-0.25,0.36)	0.00	(0.00,0.65)
2:2	Biomarker	0.44	(0.17,0.73)	0.00	(0.00,0.36)	0.00	(0.00,0.62)
2:2	CA19-9.A	0.25	(0.05,0.47)	0.00	(0.00,0.25)	0.00	(0.00,0.00)
2:2	Difference	0.19	(-0.14,0.51)	0.00	(-0.17,0.33)	0.00	(0.00,0.62)
2:3	Biomarker	0.50	(0.31,0.68)	0.00	(0.00,0.27)	0.00	(0.00,0.56)
2:3	CA19-9.A	0.37	(0.19,0.58)	0.17	(0.00,0.42)	0.00	(0.00,0.00)
2:3	Difference	0.12	(-0.17,0.41)	-0.17	(-0.42,0.11)	0.00	(0.00,0.56)
2:4	Biomarker	0.67	(0.46,0.85)	0.18	(0.00,0.58)	0.00	(0.00,0.80)
2:4	CA19-9.A	0.14	(0.02,0.31)	0.00	(0.00,0.00)	0.00	(0.00,0.00)
2:4	Difference	0.53	(0.26,0.78)	0.18	(0.00,0.58)	0.00	(0.00,0.80)

Table 5: AUC, sensitivity corresponding to specificity = 90% (SensSpec90), specificity corresponding to sensitivity = 90% (SpecSens90) of biomarker of interest, CA19-9, and their difference for separating cancer type 2 from benign type $1, \ldots, 4$; reported confidence intervals were obtained by bootstrapping with 1000 resamples.

MUC4 PERFORMED BETTER THAN CA19.9 IN DIFFERENTATING MUCINOUS TUMORS FROM CA19.9

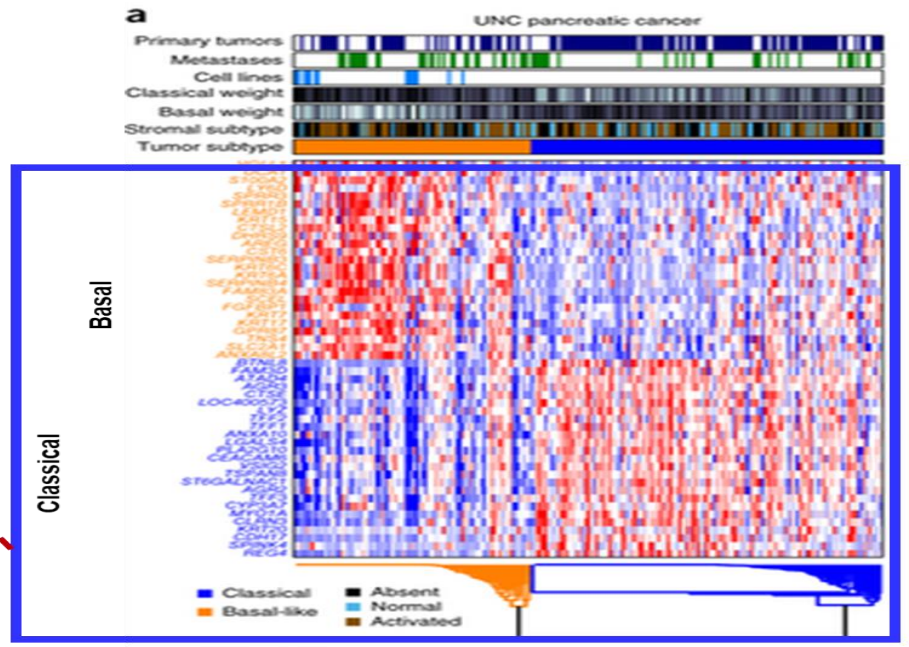
High throughput Sequencing Studies in PDAC identified various distinct SUBTYPES



Collison et al., 2010,Nature Medicine

Meta-analyses studies

TFF1
TFF1
TFF2
TFF3
S100P
CECAM5



Moffitt et al., 2015, Nature Genetics

?

Can we develop additive biomarker panel?

?

Combining Mucins (MUC4, MUC5AC, MUC16), and most differentially expressed markers of early stage PC [MIC-1, CECAM5 and TFFs (TFF1, TFF2 and TFF3)]

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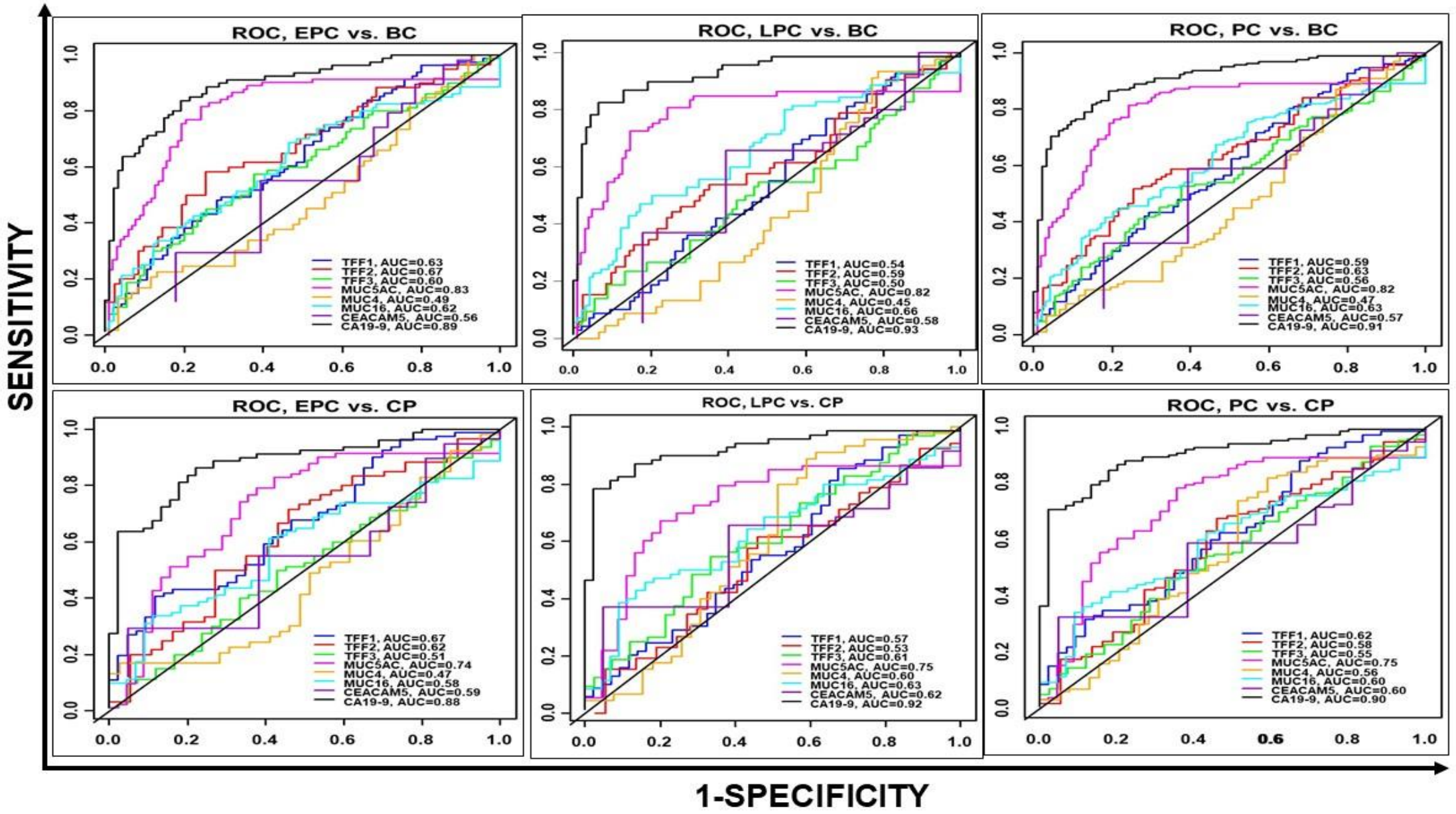
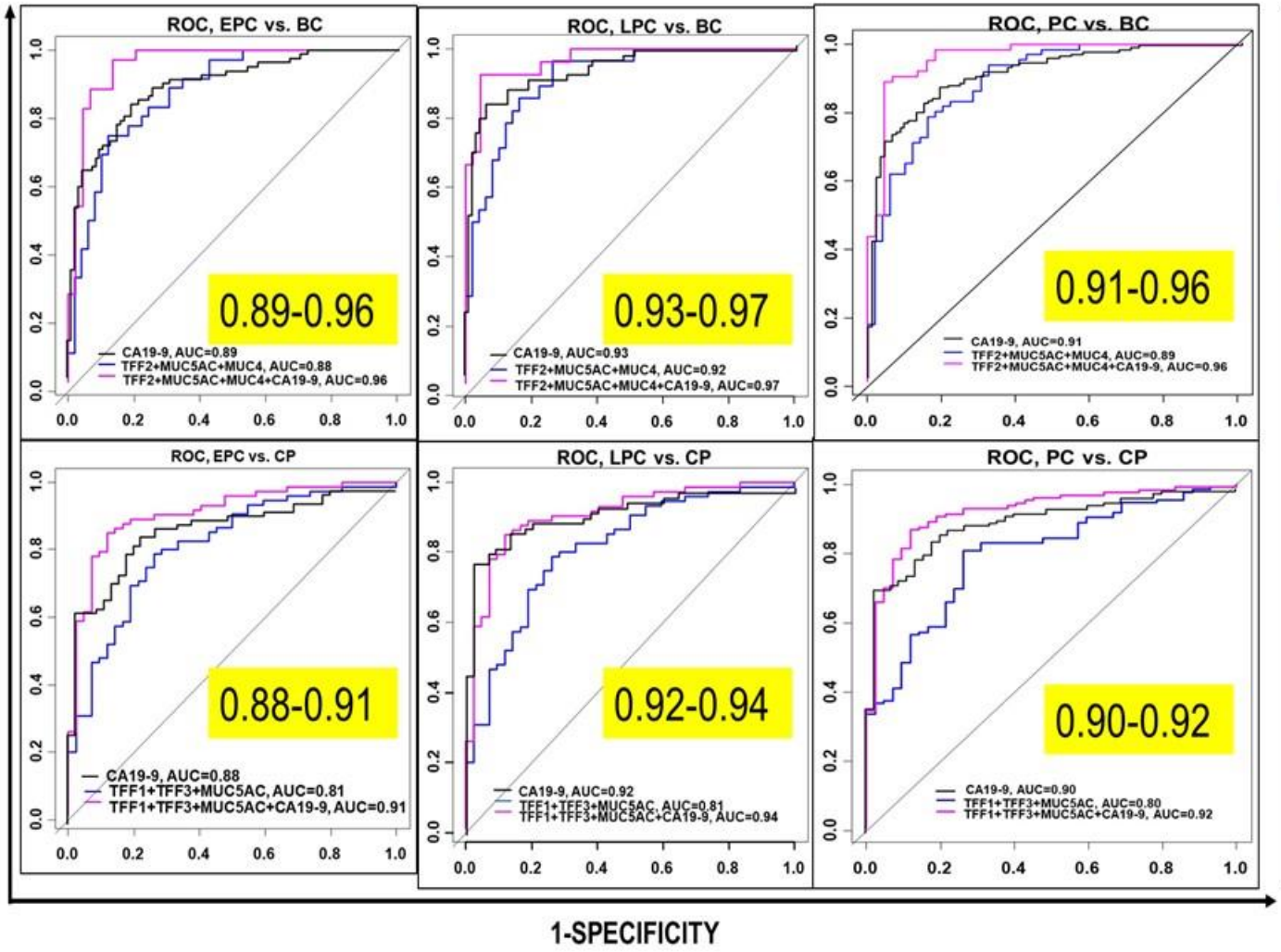
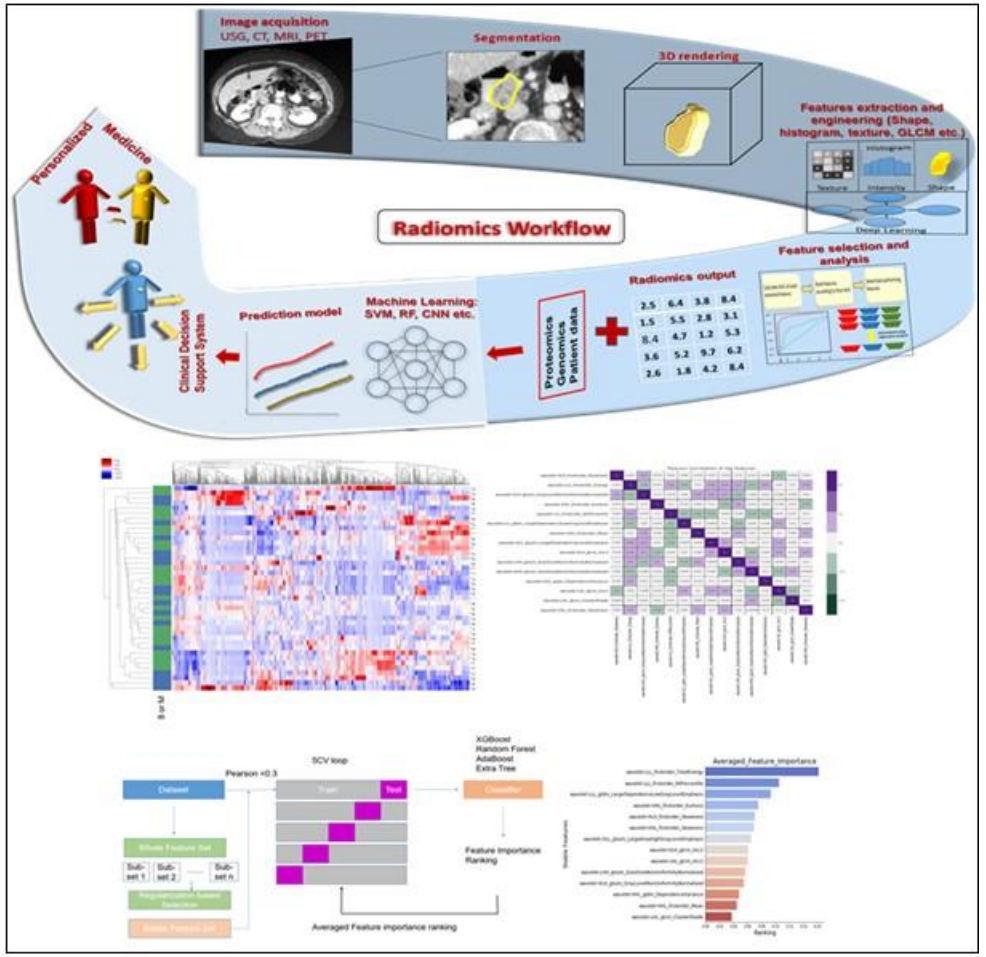
PERFORMANCE OF INDIVIDUAL MARKER(S)



DEVELOPMENT OF COMBINATORIAL BIOMARKER PANEL



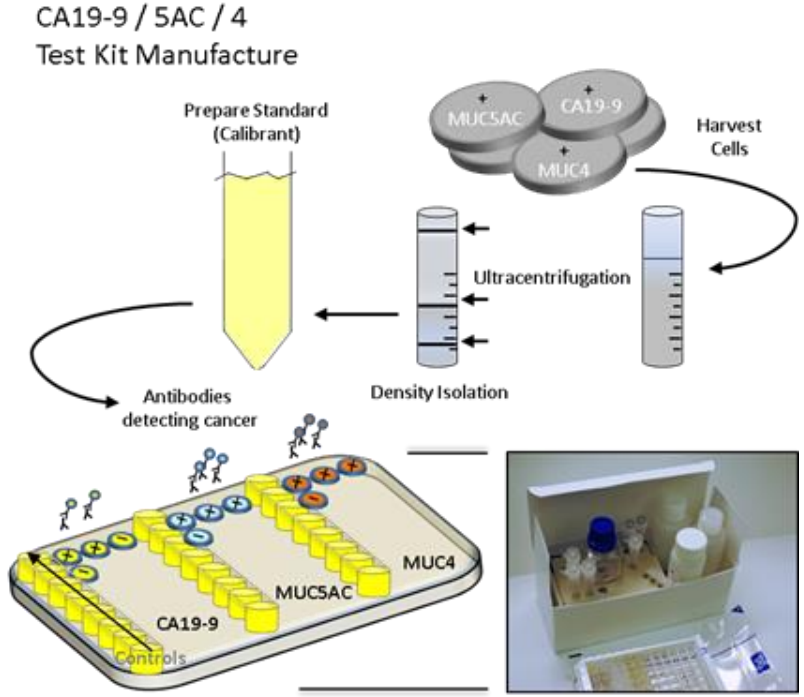


- TREFOIL FACTORS IN COMBINATION WITH MUC5AC AND CA19.9 PROVIDE ADDITIVE PERFORMANCE.
- FOUCSSING TO DEVELOP BLOOD AND RADIOMICS BASED COMBINATORIAL BIOMARKER PANEL.

SUMMARY AND CONCLUSIONS

MUC5AC IS POTENTIAL MARKER IN COMBINATION WITH CA19.9 AS ESABLISHED FROM VARIOUS COHORT STUDIES.

- **DOES EVLUATING MUC4 & MUC5AC IN LONGITUDINALLY COLLECTED SAMPLE SET HELP IN IDENTIFYING CANCER PATIENTS WITH IMPROVED DISCRIMINATORY POWER (BAKE-OFF SET 3) .**
- **HOW MUC5AC IMPROVES THE PATIENTS STRATIFICATION IN DELENIATING THE RESPONSE TO CHEMOTHERAPY AND SURGERY.**
- **DEVELOPMENT OF TEST KIT FOR COMBINATORIAL DETECTION OF MUC4, MUC5AC AND CA19.9**



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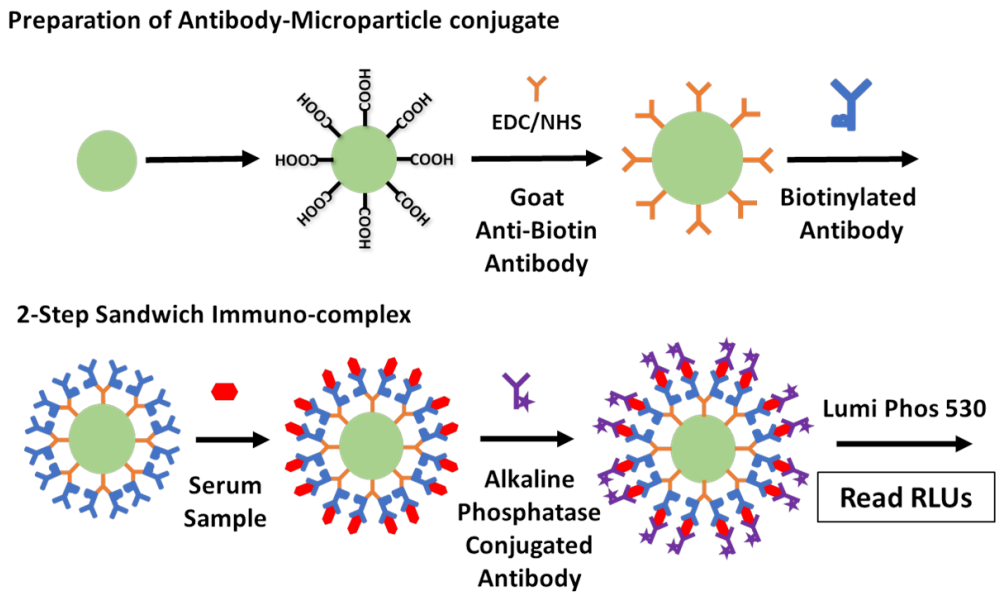
FUTURE DIRECTIONS

- Develop of sensitive and specific immunoassay for detection.
- Subtype specific circulating COMBINATORIAL signature.
- Statistical weightage based algorithm for combining biomarkers.
- Validation of biomarker panel in combination MUC5AC (using established and newly developed antibodies based assay)
- Development of multiplexed assay



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THANK YOU, QUESTIONS & SUGGESTIONS

5 minute Q&A

Chair/Co-Chair/NCI
feed Zoom Chat questions to presenter
and Track Time
NCI and Production Team
answer Chat questions not related to presentations
and use Slack