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Integration of Biomarker Signatures from Peripheral Blood for Diagnosis, Prognosis, Remission and Recurrence of Lung Cancer

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EDRN Multi PI BDL: U01 CA200495

PIs: Louise Showe, Qin Liu

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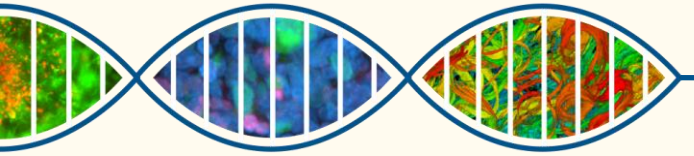

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Disclosure of Potential Conflicts of Interest

A.V. Kossenkov L.C. Showe and M.K. Showe have ownership interest as inventors on pending unpublished provisional patent application(s) WST155P1 and WST117P. G. assigned to The Wistar Institute that relates to compositions and methods of using a lung nodule classifier. L.C. Showe reports receiving a commercial subsidized research award (SRA) from OncoCyte. Oncocyte has licensed this technology.

L.C.Showe is an is a adjunct professor at the University of Pennsylvania with equity in ISOMA Therapeutics, a start-up spun out of Wistar in 2017 that is based on intellectual property jointly owned by Wistar and The University of Pennsylvania on GBM classification.

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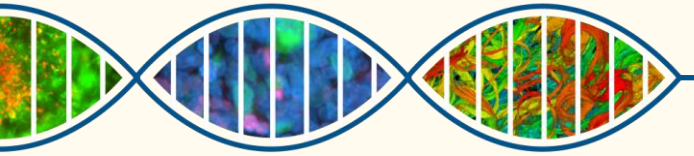
Summary

- Multi PI proposal merging complementary approaches and using the combined expertise of biologists, clinicians and bioinformaticians to develop an improved non-invasive blood-based gene expression signature for the diagnosis and prognosis of NSCL. Building on successful studies that identified a **PBMC** based we proposed to expand our study and adapt both the sample collection and our diagnostic assay our to forms more easily used in a clinical setting.
  - We proposed to **move sample collection to the PAXgene RNA stabilizing** tubes to avoid the need for rapid purification of PBMC at the collection site.
  - We also proposed to move the gene expression "classifier" developed on microarrays to the FDA approved and technically simple **NanoString nCounter platform** already FDA approved for a breast cancer prognosis signature.
- In a second project we proposed to further assess **expression of AKAP4**, a CTA, as a single gene marker for the presence of a malignant lung nodule in the same samples



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Specific Aims

Project 1, Aim 1: Identify a Biomarker Panel with high sensitivity for distinguishing benign from malignant lung nodules using PAXgene blood RNA .

- Analyze **mRNA** expression on Illumina Microarrays and
- Analyze **miRNA** expression on the ABI PCR Array System.
- Combine **mRNA and miRNA** data
- Translate to a clinically viable FDA approved platform (NanoString)

Project 2, Aim 2: Akap4 expression in PBMC derived RNA as a single gene marker for the presence of a NSCLC

- Expand study to new samples
- Reassess the **nested PCR assay**
- Test assay in **PAXgene** samples
- Identify the source of the AKAP4 signal

AIM 3 Integrate results from 1 and 2 for combined classifier

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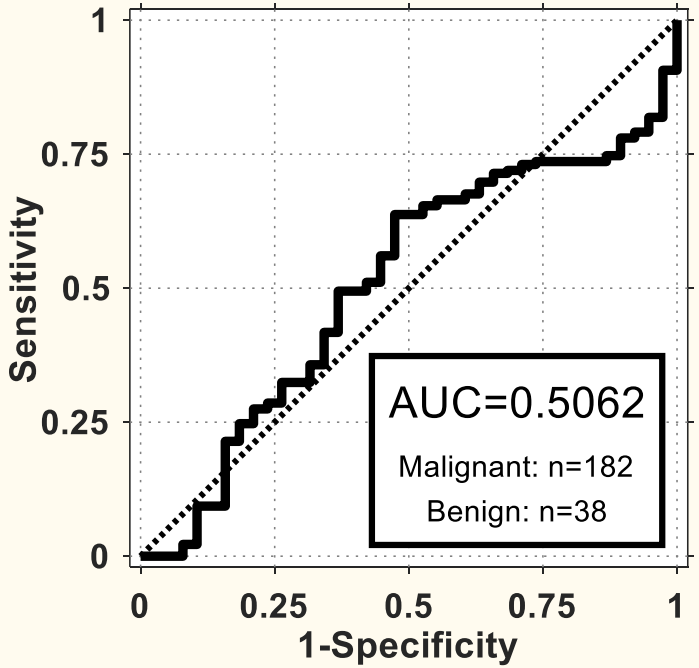
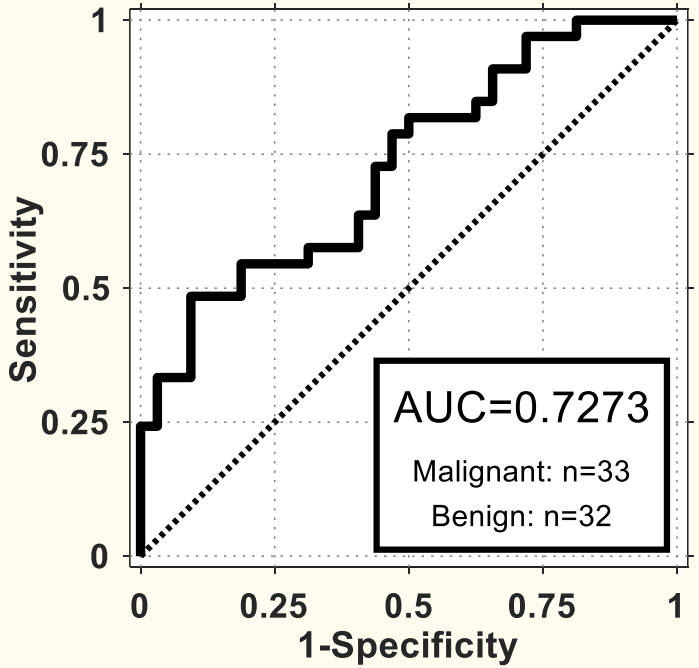
Aim 2: AKAP4 Expression In PBMC RNA As A Single Gene Marker For The Presence Of A NSCLC

site	blood	malignant	benign	normalization	AUC
ALL	PBMC	182	38	ACTIN	0.535
ALL	PBMC	182	38	RPLPO	0.506
ALL	PBMC	182	38	both	0.531
ALL	PAXGENE	33	35	RPLPO	0.702
CCHS	PBMC	28	20	ACTIN	0.643
CCHS	PBMC	28	20	RPLPO	0.611
CCHS	PBMC	28	20	both	0.652
CCHS	PAXGENE	33	32	RPLPO	0.727
NYU	PBMC	118	18	ACTIN	0.498
NYU	PBMC	118	18	RPLPO	0.424
NYU	PBMC	118	18	both	0.462

PBMC, ALL



PAXGENE, CCHS



*ALL= NYU+PENN+CCHS

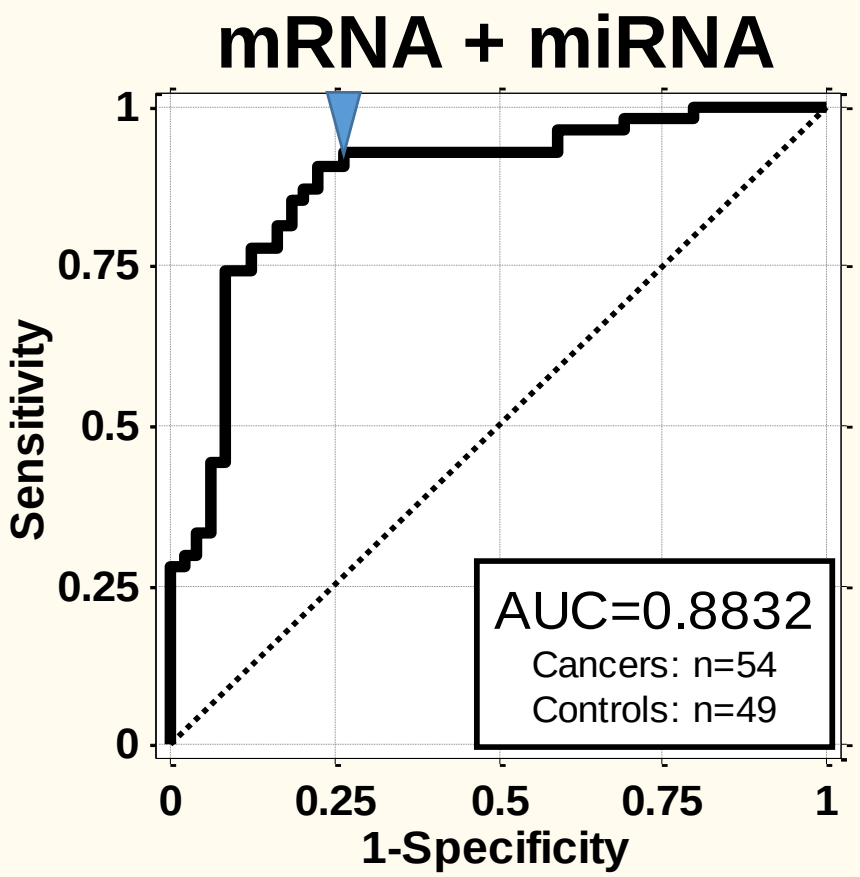
RPLPO Normalization

Aim 1: Identify A Biomarker Panel Distinguishing Benign From Malignant Lung Nodules Using Paxgene Blood RNA (mRNA+miRNA)

- 242 PAXgene training samples
- 103 PAXgene test samples

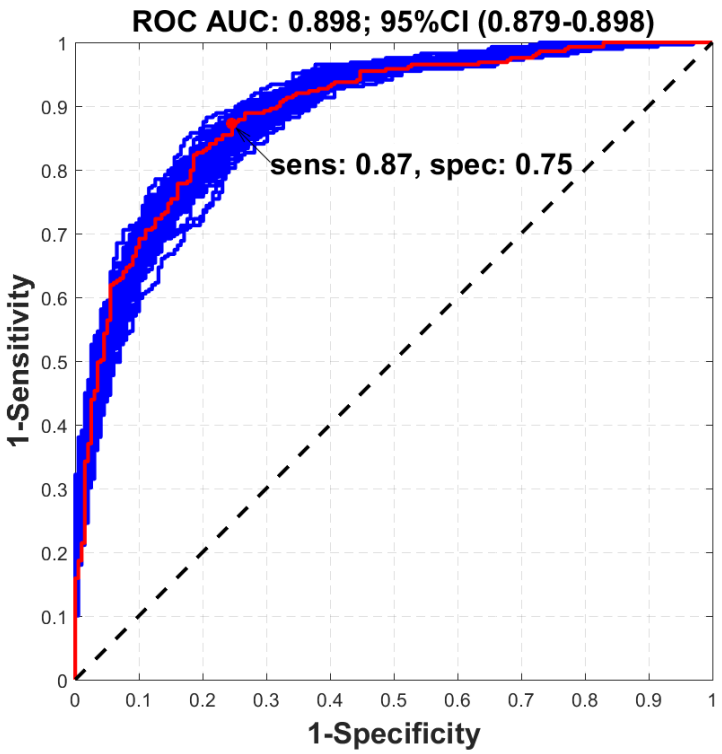
- MN vs BN
- Accuracies comparison
  - mRNA only: 79%
  - miRNA only: 71%
  - mRNA+miRNA: 83%

- 125 mRNAs + 20 miRNAs
- Sensitivity: 76%
- Specificity: 88%
- ROC AUC: 0.88

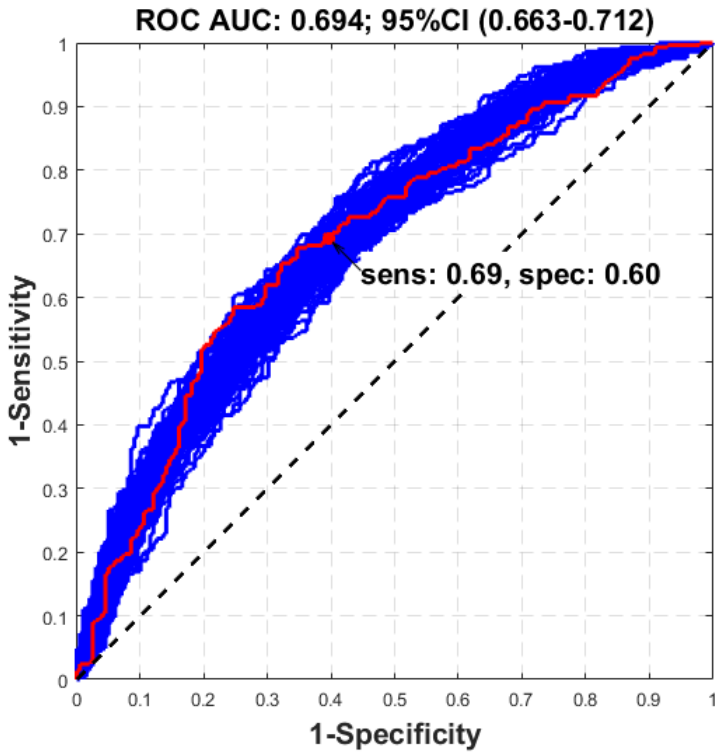


❖ Initial Performance: ROC curve: ATS 2015
-PAXgene samples can replace PBMC
-miRNAs add to the classifier accuracy

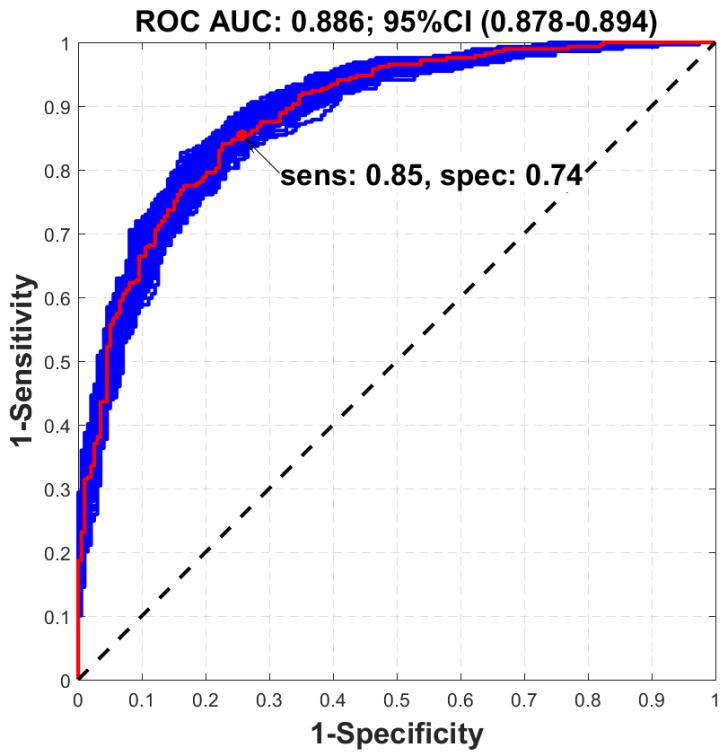
PAXgene Microarray



PAXgene OpenArray (219 miRNAs)



Combined



Classification of malignant (n=289) vs. benign nodule (n=199) using 10-Fold CV SVM. LEFT: Using the top expressed Illumina probes. MIDDLE: Using all the miRNAs detected on OpenArray. Right: Classification using the combined data.

miRNA on the NanoSTRING

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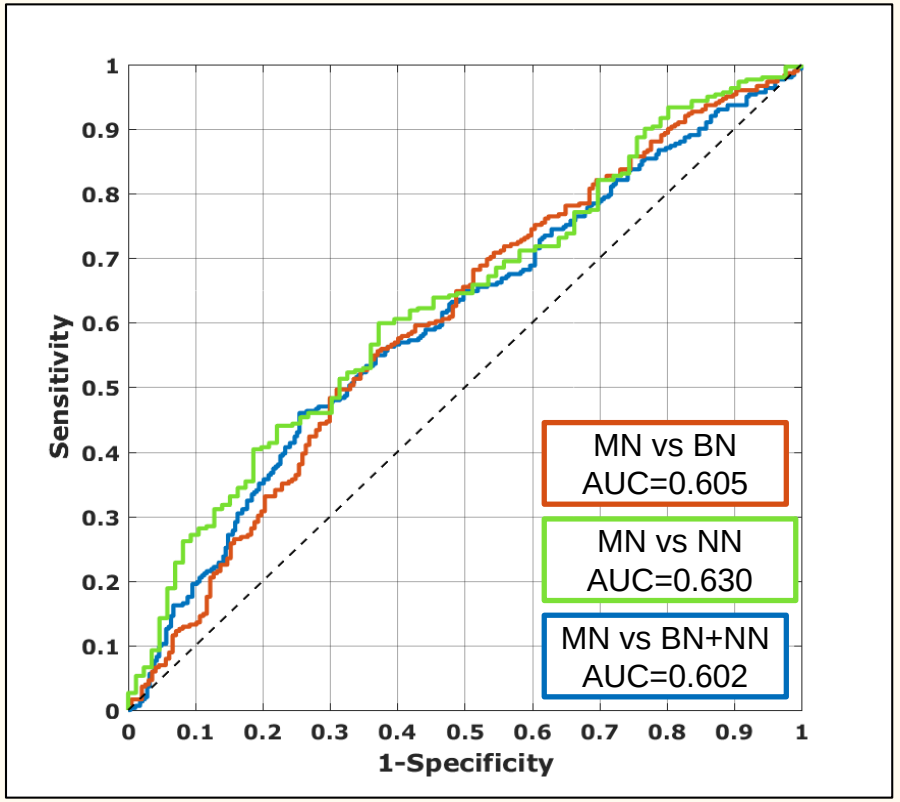
192 PAXgene samples



Pulmonary Nodules

MN= Malignant

BN= Benign

NN= Similar age and smoking histories –No nodules by LDCT

*Dropped miRNA from further studies

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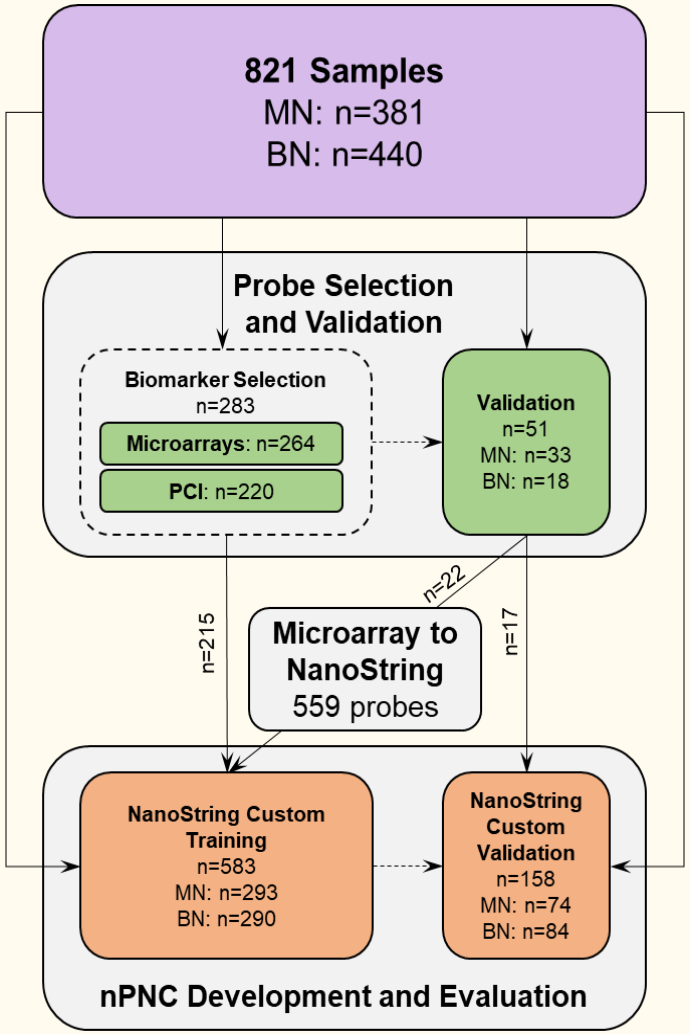
Aim1B: Transitioning to the NanoString (mRNA)



Feature Selection

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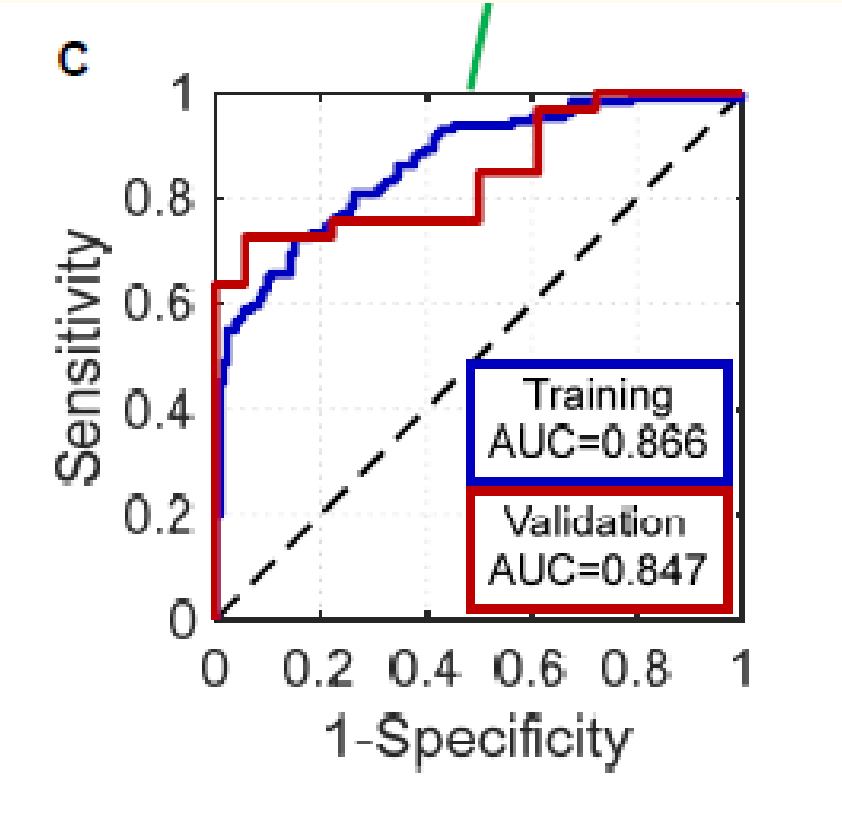
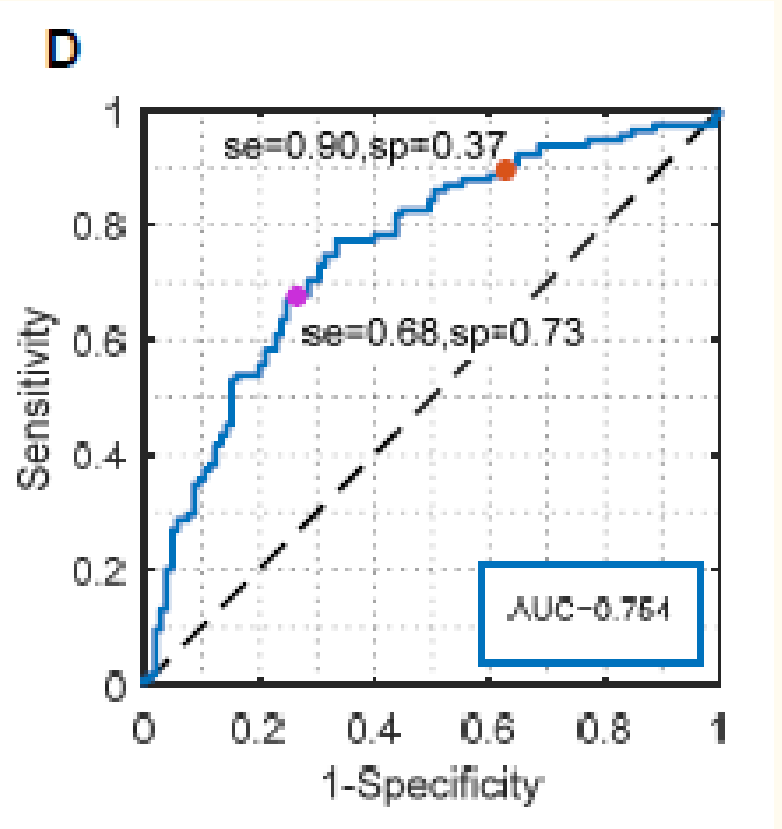
Microarray: 330 samples



NanoString PCI Panel:220 samples



311 probes selected by SVM-RFE**. 138** by p-value and fold-change, **90** from the PCI panel and **20** housekeeping genes Total **559** to be analyzed on a Custom NanoSTRING panel.

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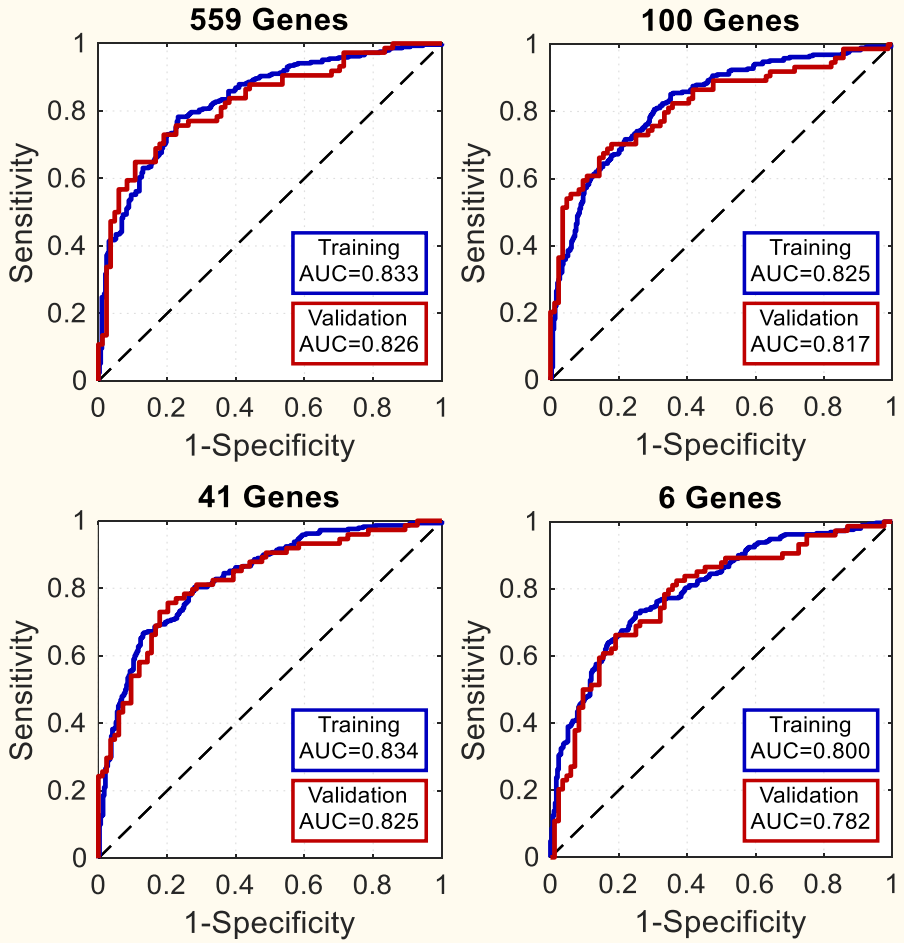
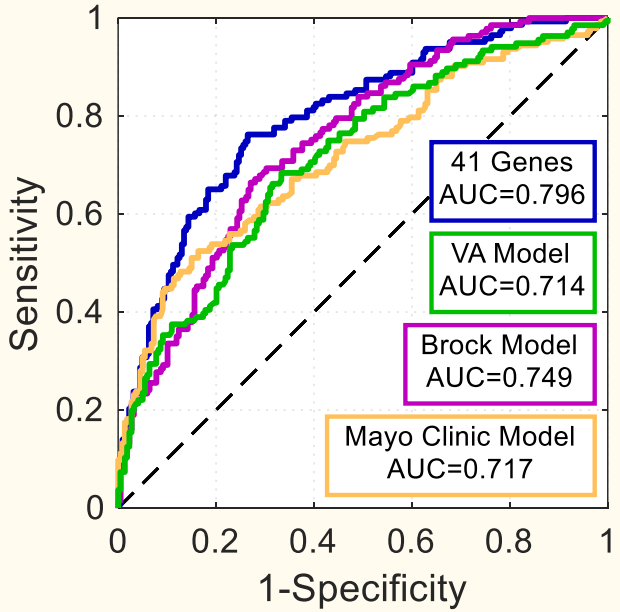
Sucessful Transition from Microarrays to NanoString

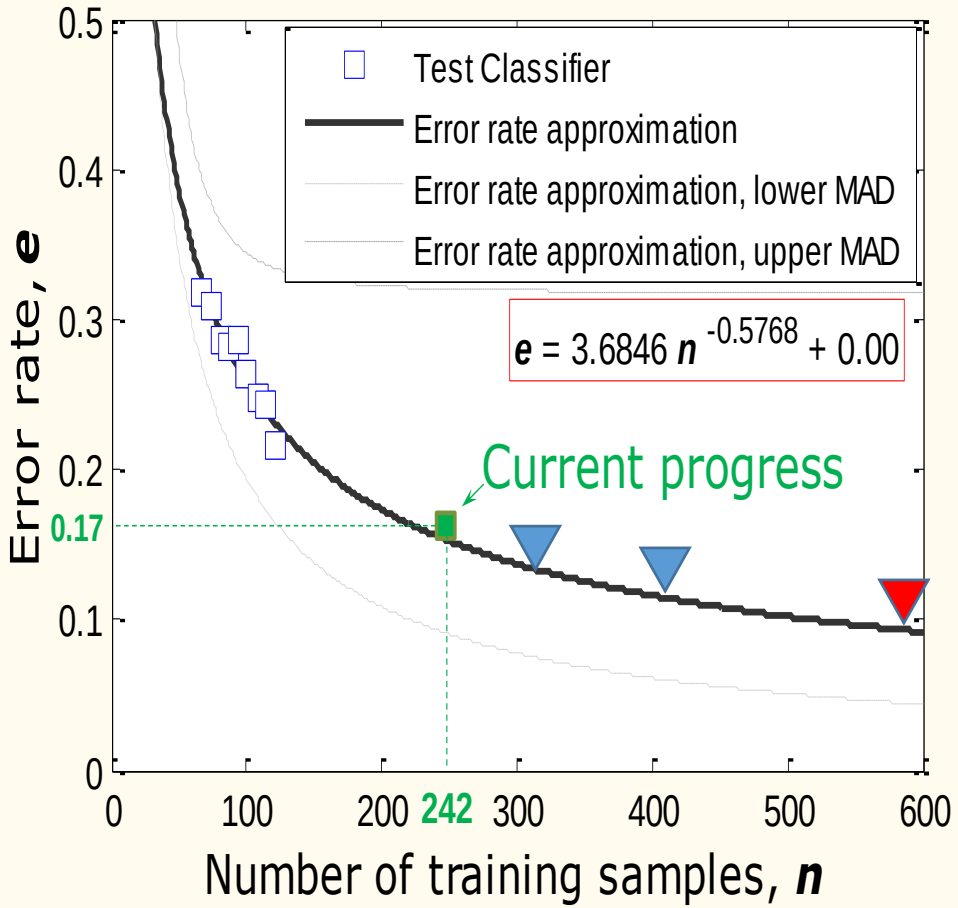


- 741 (583+158)patient samples (4 sites)
- Accuracy as on microarrays
- Don't need 559 genes
- Outperform clinical models
- Cancer Research Publication



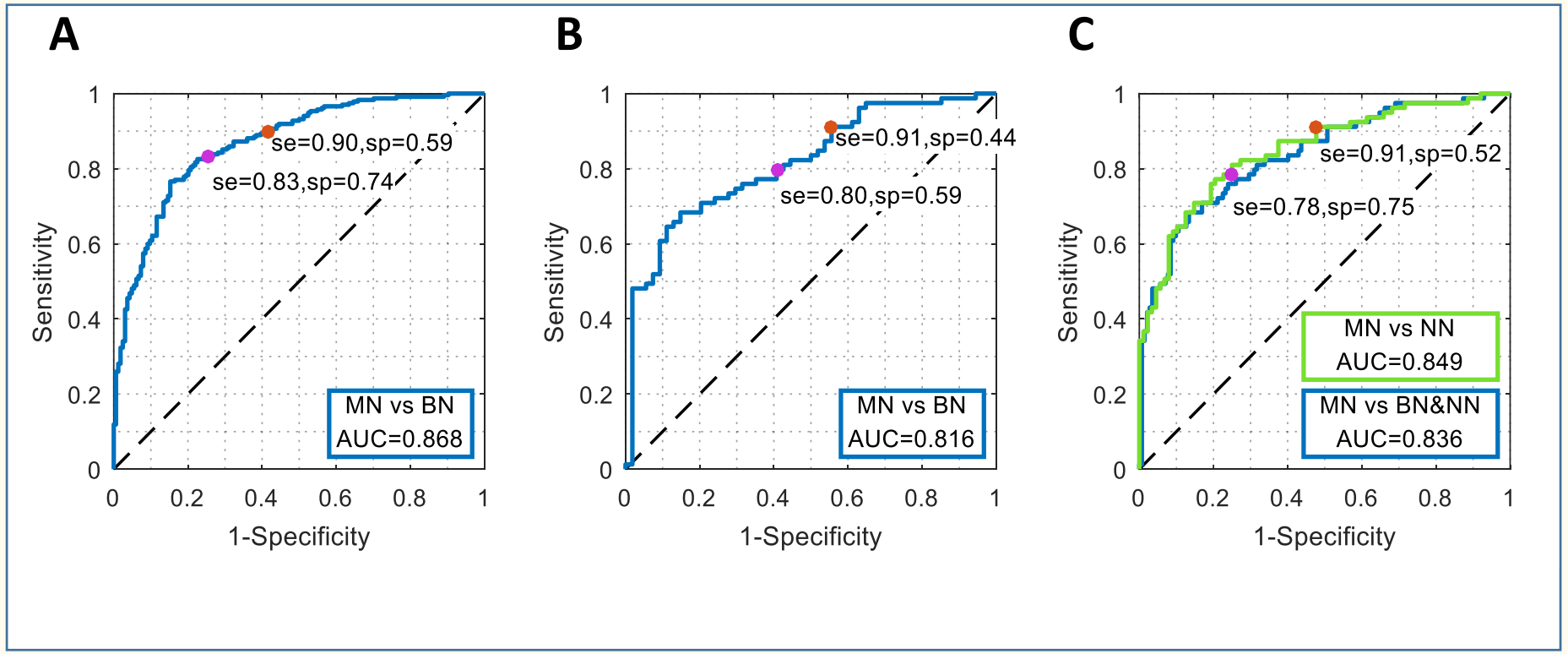
*Kossenkov, A.V., R. Qureshi et al. 2019. Cancer Research. A Gene Expression Classifier from Whole Blood Distinguishes Benign from Malignant Lung Nodules Detected by Low-Dose CT.

MicroArray Discovery Platform

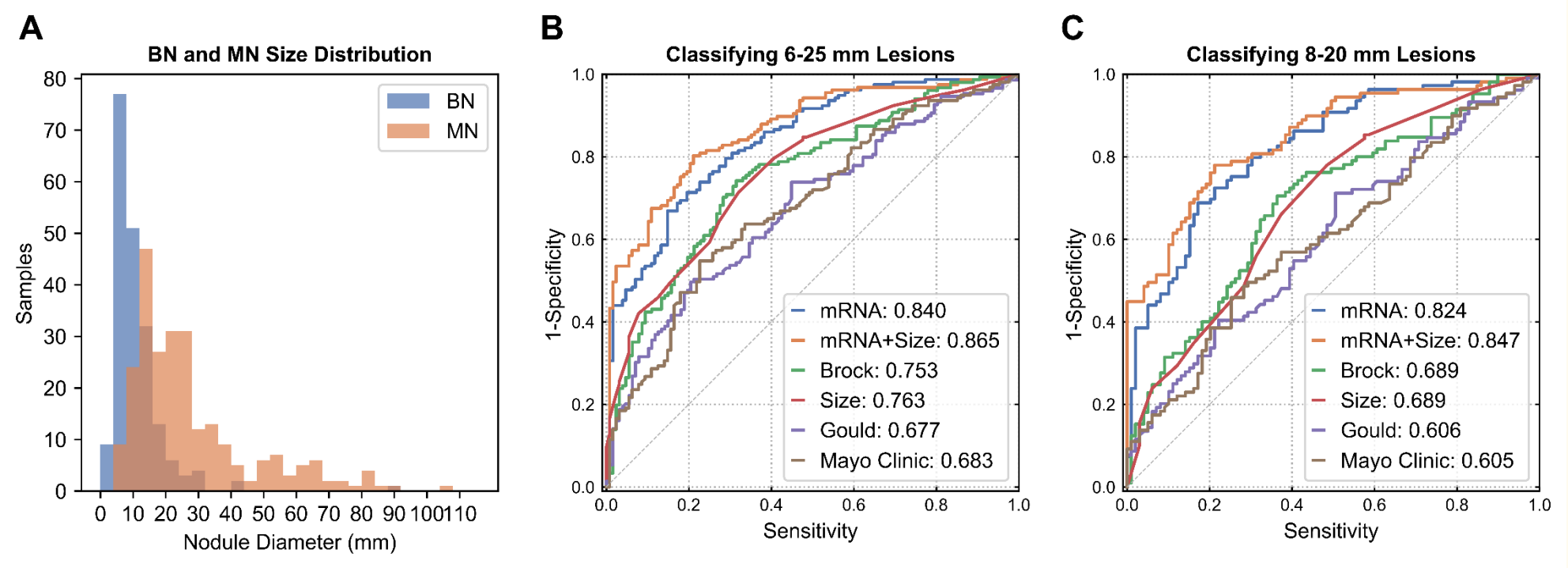


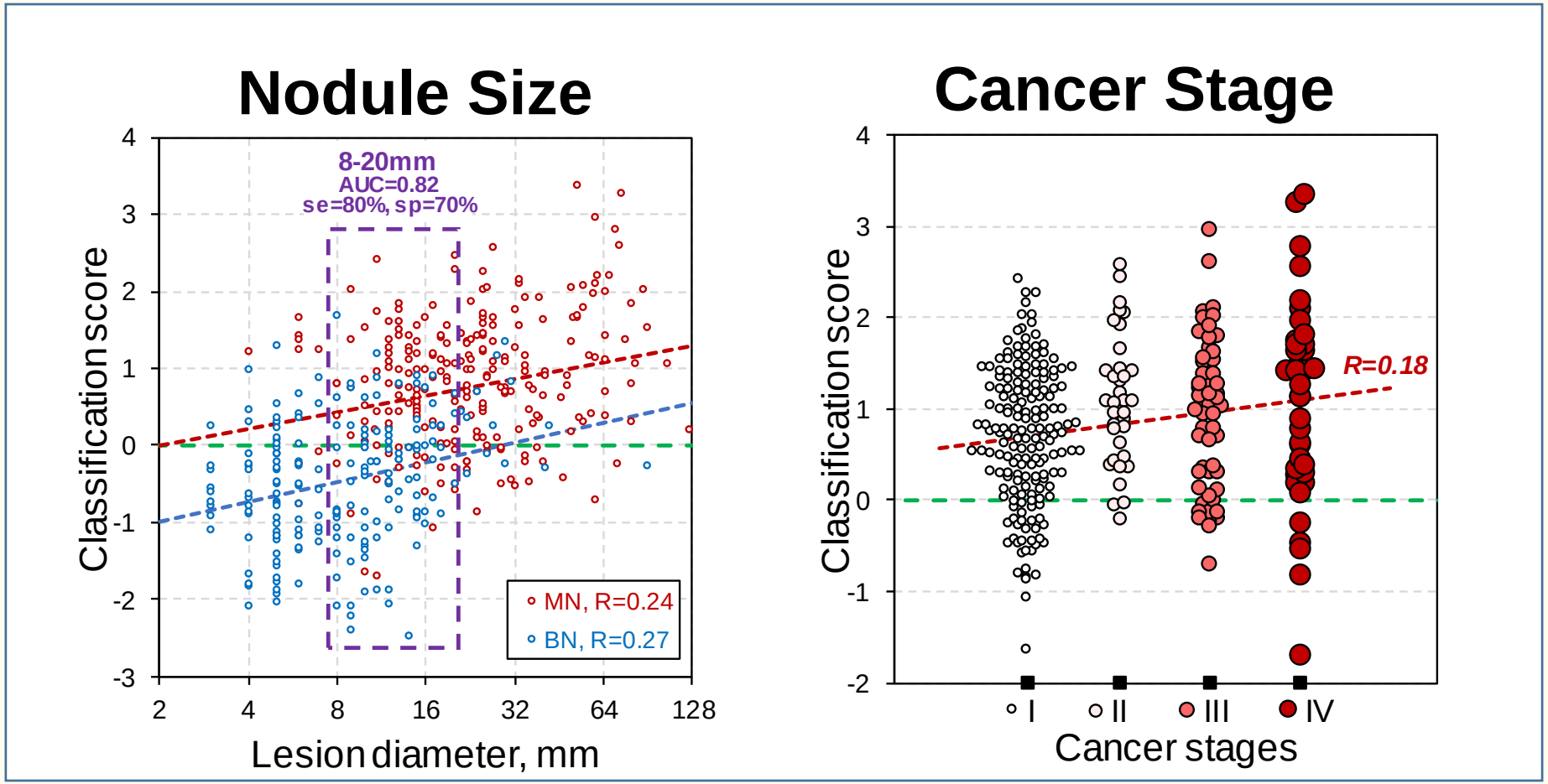
New Microarray Lung Nodule Classifier

- More Samples: 5 Clinical Sites 399 Training+ 133 Testing (88NN testing only)
- More Diversity: 11% AA : (Hope to increase this)
- More Robust Gene Selection: ≥ 3 times Microarray background (8900 probes)

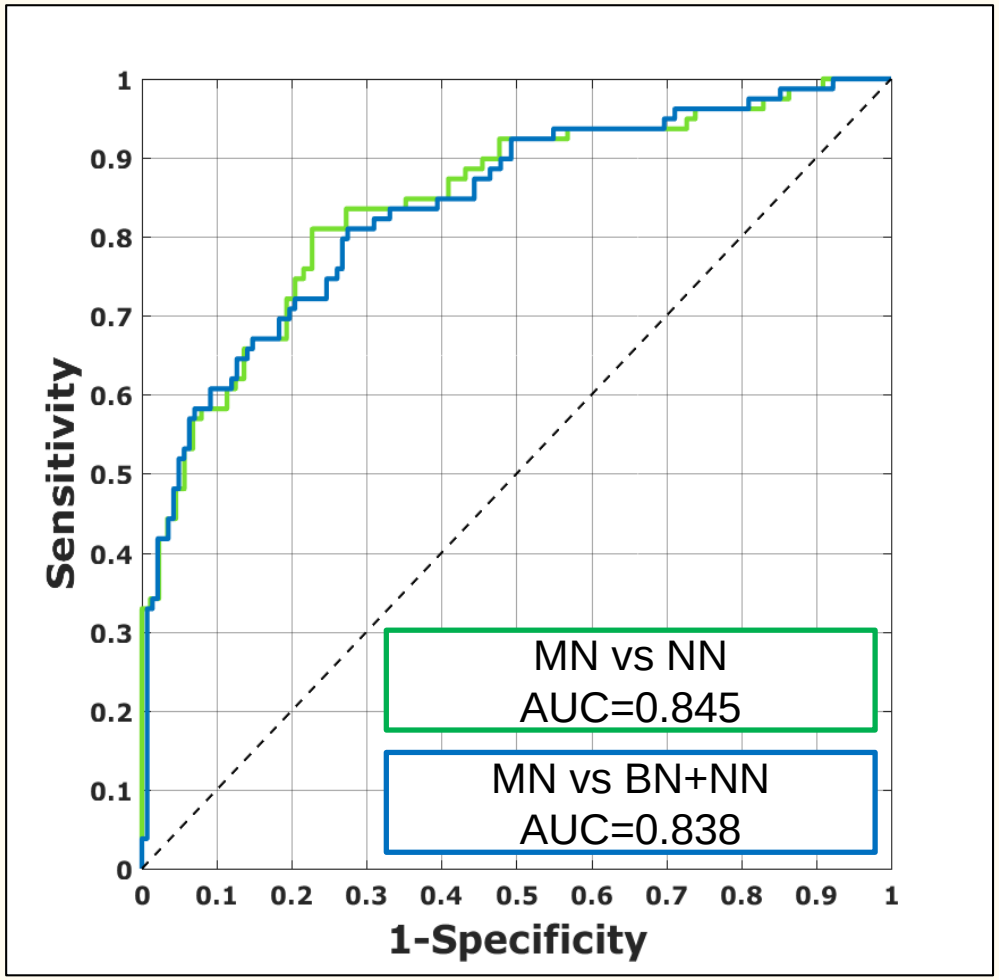


Comparing to Clinical Risk Estimators

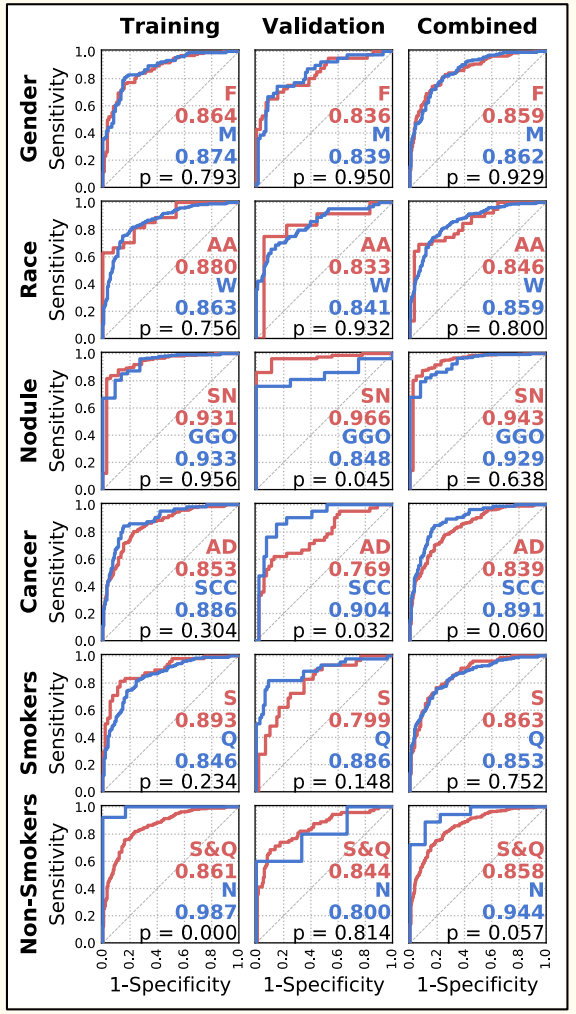



Nodule Size
8-20mm
AUC=0.82
se=80%, sp=70%
MN, R=0.24
BN, R=0.27
Classification score
Lesion diameter, mm
Cancer Stage
R=0.18
Classification score
I
II
III
IV
Cancer stages

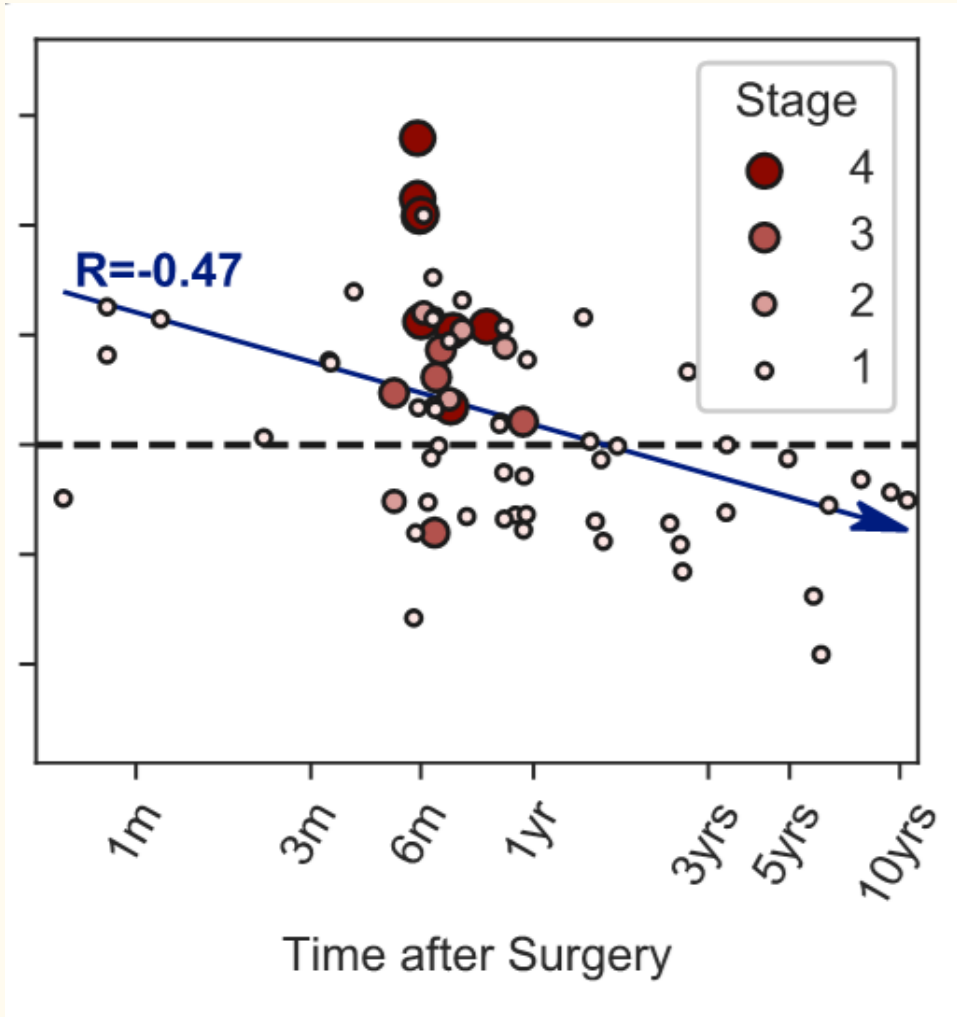
Patients with no nodules



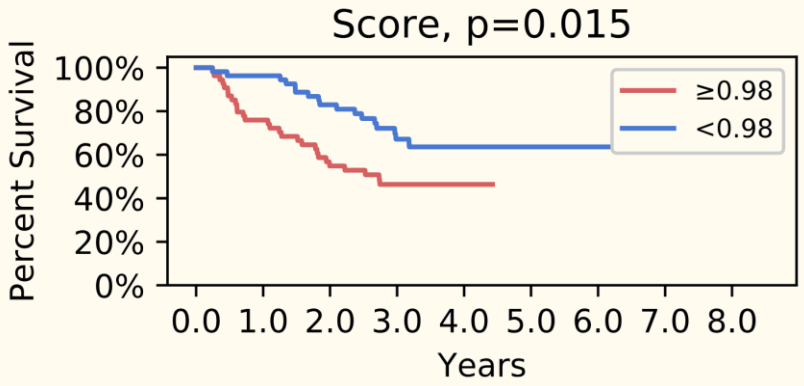
Other classes

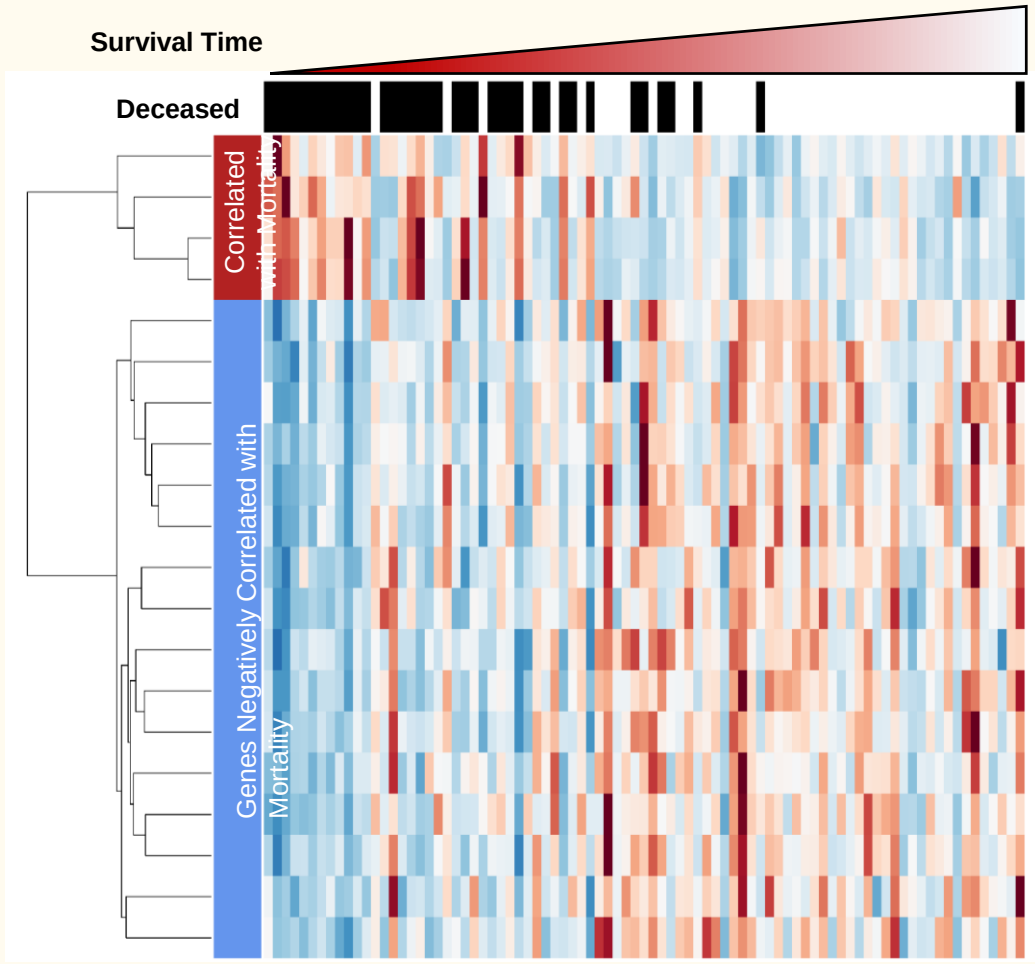


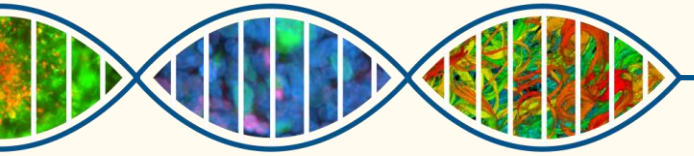
SVM Scores Post Resection & Survival



Survival Prediction Microarrays: 20 Probes







New NanoString Classifier

- 420 Probes (380 diagnostic, 20 Housekeeping, 20 prognostic)
  - Probe IDs to be standardized in all batches of reagents as for a clinical test
  - Artificial internal standard (Single-Patient Reference-Based Strategy for Batch Effect Correction: PLoS ONE 2015) duplicates run on each cartridge
- 410 samples for Stage 1 development (selecting best probes)
  - 50% MN and 50% BN
  - 250 samples for Training (Done) 160 samples for Testing (in progress)
  - Select the most informative probes
- Stage 2: Finalize a classifier with smallest number of probes returning highest accuracy
  - Order new custom panel
  - Test 1400 samples including LTP2 samples

Since we are the only group without a clinical affiliation I have been supporting James Tsay and an assistant to recruit patients for the LTP2 project for the past 2 years and that will continue in 2019

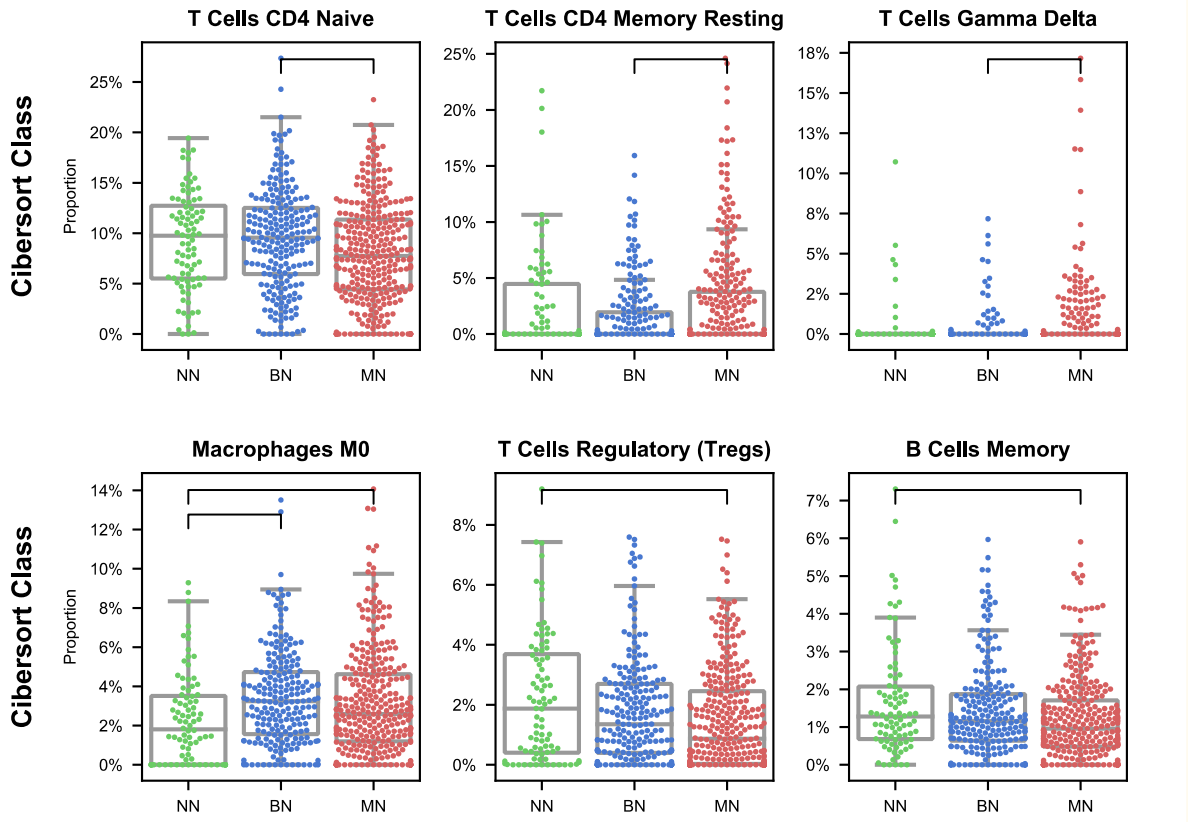
Set-aside Funds

- **Set-Aside 1 and 2**
  - Technical support for James Tsay
    - LTP2 collection and annotation
    - Updates on NYU screening cohort
  - Support for new collection sites: CCHC in Delaware and Meridian Health New Jersey.
  - First NanoSTRING panel (Cancer Research Publication)
- **Set-aside 3 and 4**
  - Continued support for NYU, CCHC and Meridian
  - Development of second generation NanoSTRING nodule classifier
  - Increased Bioinformatics support
- Core Funds
  - PAXgene work station from Qiagen

Future Studies

- **Effects of a malignant lung nodule on peripheral immune cells that might be important for:**
  - Successful immune therapy
  - Correlated with better survival
- **What distinguishes smokers without lung nodules from those with benign or malignant lung nodules?**
- **Addressing outcome differences in African Americans.**
- **Can the functional differences we detect in patient with MN and BN be reversed/corrected?**

Peripheral Immune Cell Populations And NSCLC



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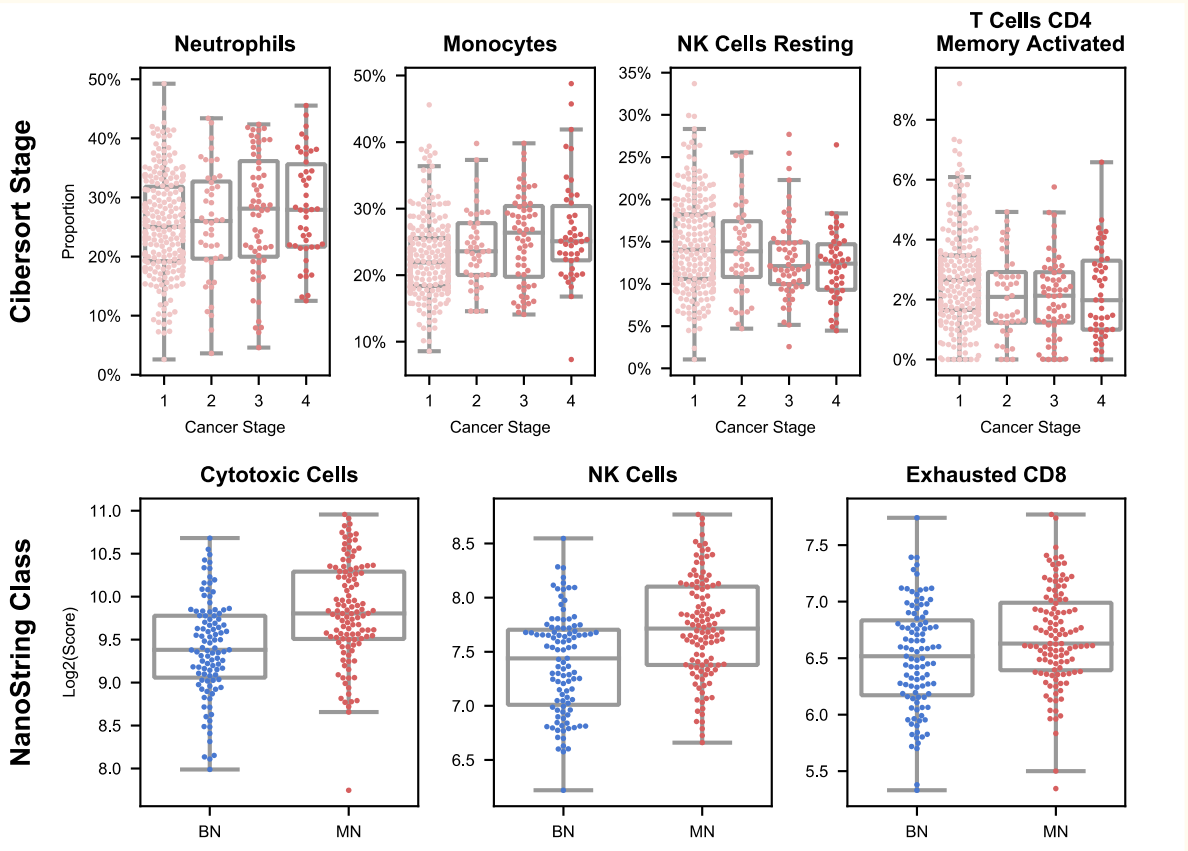
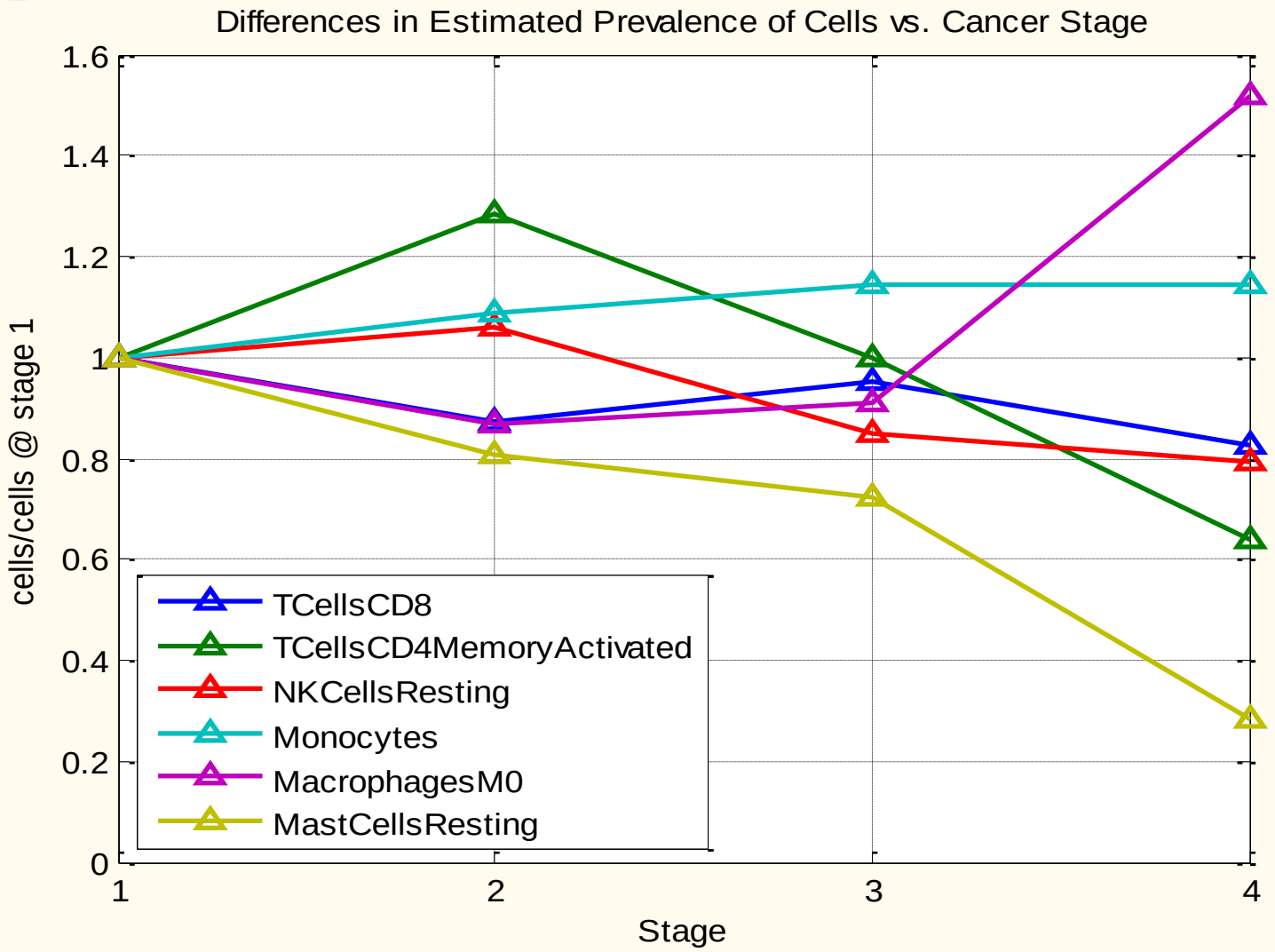
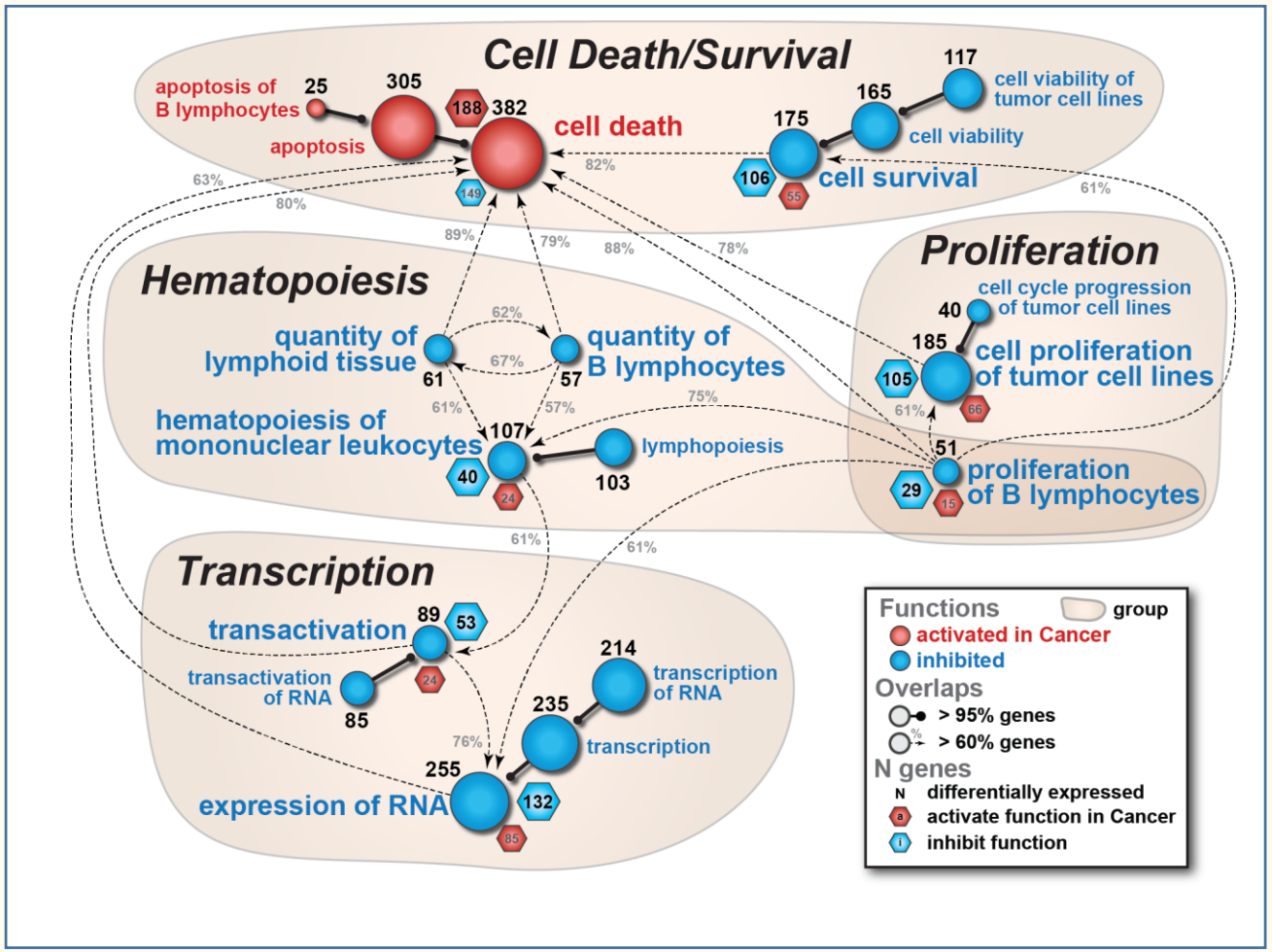


*

Fractions of immune cell populations in peripheral blood were estimated from gene expression using Cibersort. Significant alterations in CD4 T cells, and Gamma Delta T cells were detected when comparing BN to MN patients. Macrophages, T Regs, and Memory B cells were differentially expressed in MN vs NN. Peripheral immune alterations were also significantly associated with cancer stage. NanoString analysis indicates that Cytotoxic Cells, NK cells, and Exhausted CD8 cells are upregulated in MN samples.


Differences in Estimated Prevalence of Cells vs. Cancer Stage
cells/cells @ stage 1
Stage
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
TCellsCD8
TCellsCD4MemoryActivated
NKCellsResting
Monocytes
MacrophagesM0
MastCellsResting

Gene functions:Immunotherapy



Other Related Projects

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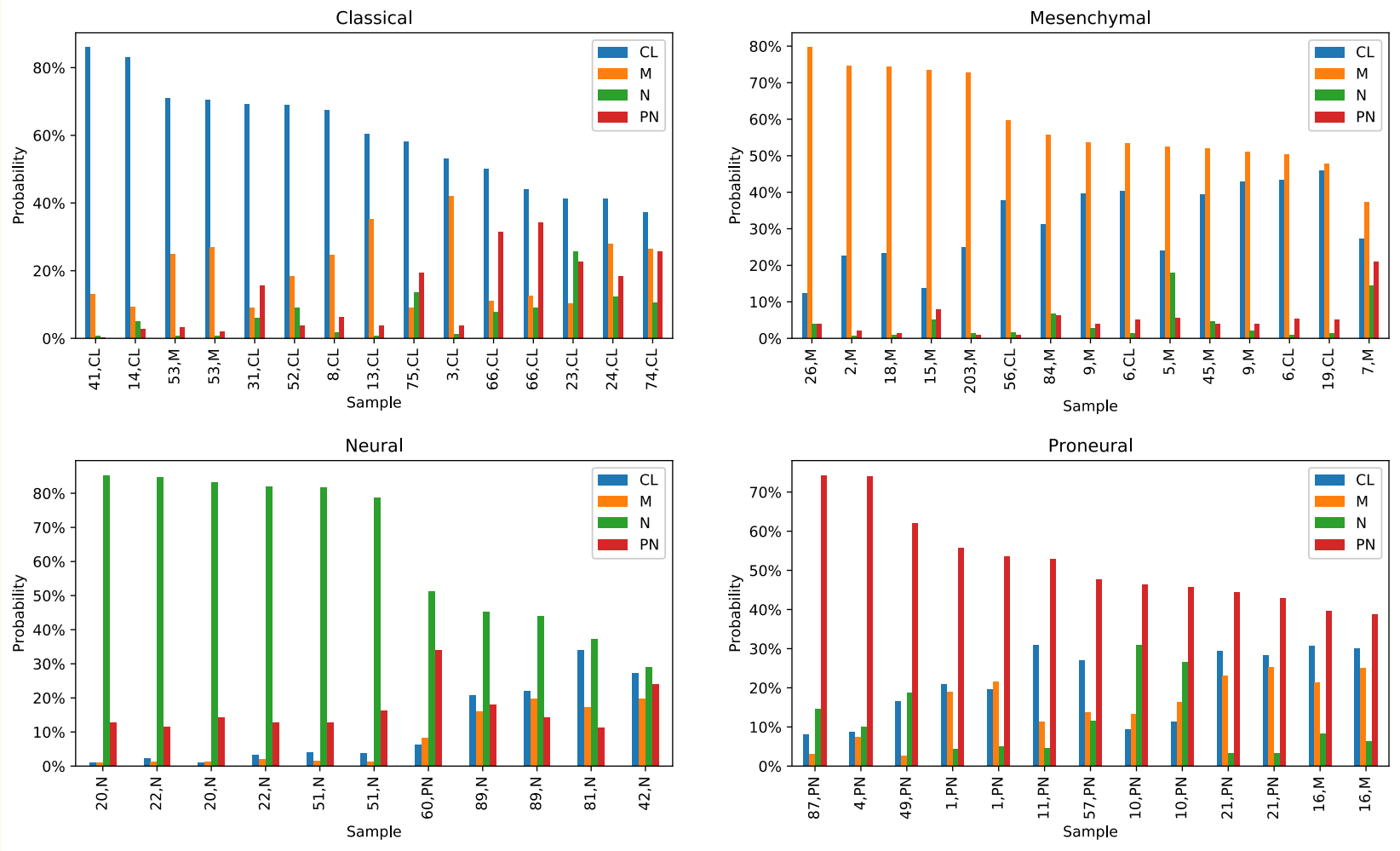
GBM : Random Forest Class Probability Predictions On A Custom NanoSTRING Panel For Alternately Spliced RNAs



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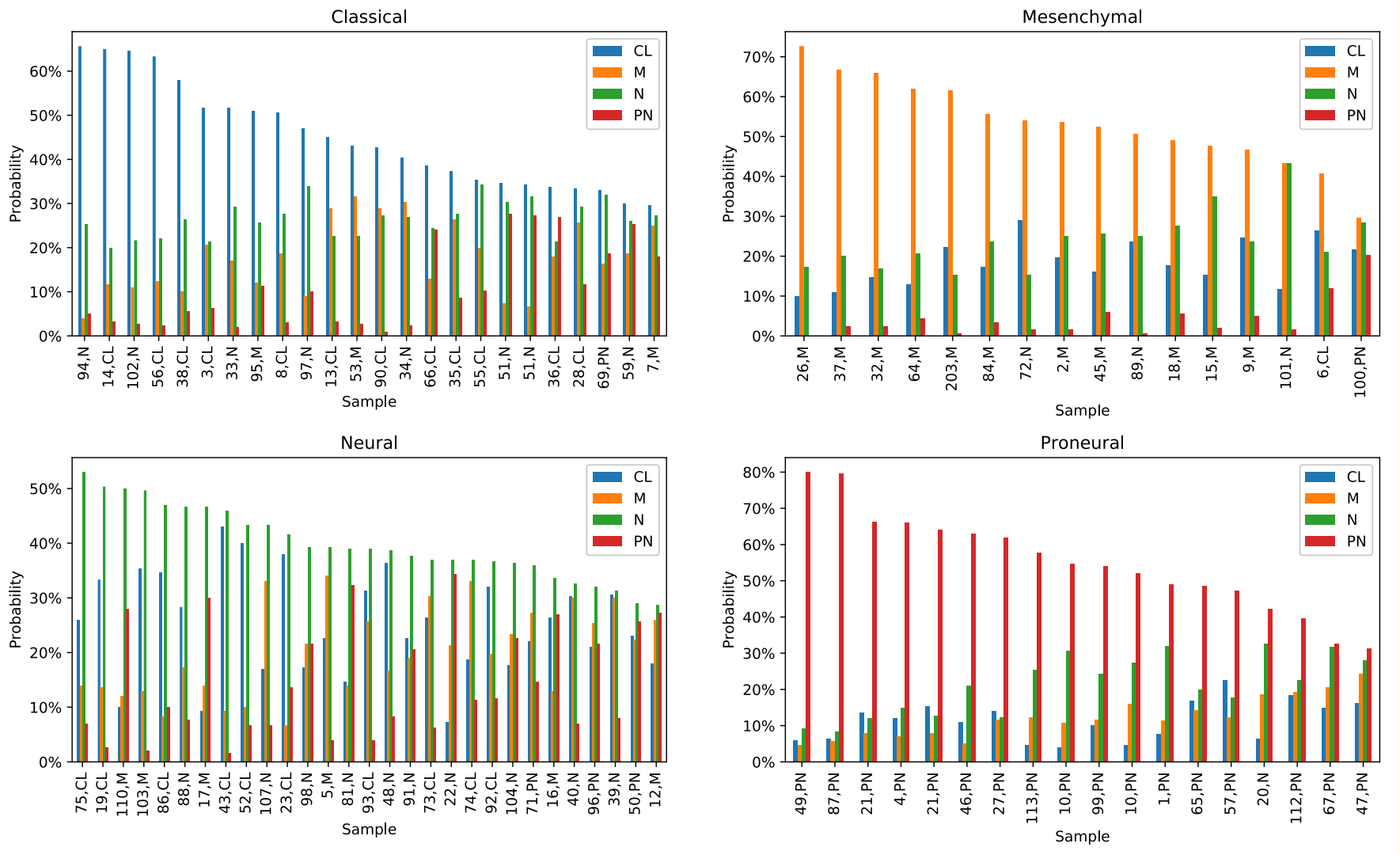
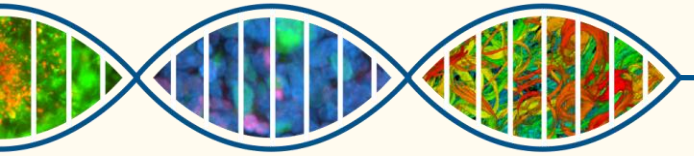
FFPE Sample Random Forest Class Probability Predictions



A Fusion Between Two lncRNAs Codes For A 33 amino acid peptide In Malignant T cells Is Also Associated With Other Cancers

Cancer	Tested Cancer	Chimera Found Cancer	Chimera Found Cancer %	Tested Normal	Chimera Found Normal	Chimera Found Normal %
DLBC	48	26	54.17	0	0	0
OV	223	76	34.08	0	0	0
SKCM	102	30	29.41	1	1	100
UCEC	102	24	23.53	35	1	2.86
LIHC	102	21	20.59	50	0	0
THYM	120	24	20	2	0	0
BLCA	102	18	17.65	19	0	0
UCS	57	9	15.79	0	0	0
COAD	102	8	7.84	41	0	0
SARC	102	7	6.86	2	0	0
ESCA	184	3	1.63	13	0	0



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- **Showe Lab**
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  - Michael Showe,
  - Kiran Gumireddy, Sonali Majumdar
  - Andrew Kossenkov
- **Genomics Core**
  - Celia Chang
  - Sonali Majumdar
  - Sandy Widura
- **Statistics**
  - Qin Liu
- **Bioinformatics**
  - Andrew Kossenkov
  - Rehman Qureshi
  - Wistar Bioinfo
- **Collection Sites**
  - NYU: William Rom, James Tsay, Harvey Pass
  - CCHC: Brian Nam
  - Meridian: Thomas Bauer
  - Temple U: Gerard Criner
  - UPenn: Anil Vachani
  - Roswell Park: Sai Yendamuri

SUPPORT: NCI EDRN BDL UO1, NCI R21, Wistar CCSG Core Support, PA DOH Tobacco Settlement CURE Funds

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