

# Potential Blood-Based Biomarkers for the Early Detection of Lung Cancer

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Prevent Cancer Meeting

Dialogue for action on cancer screening and prevention



# Disclosures

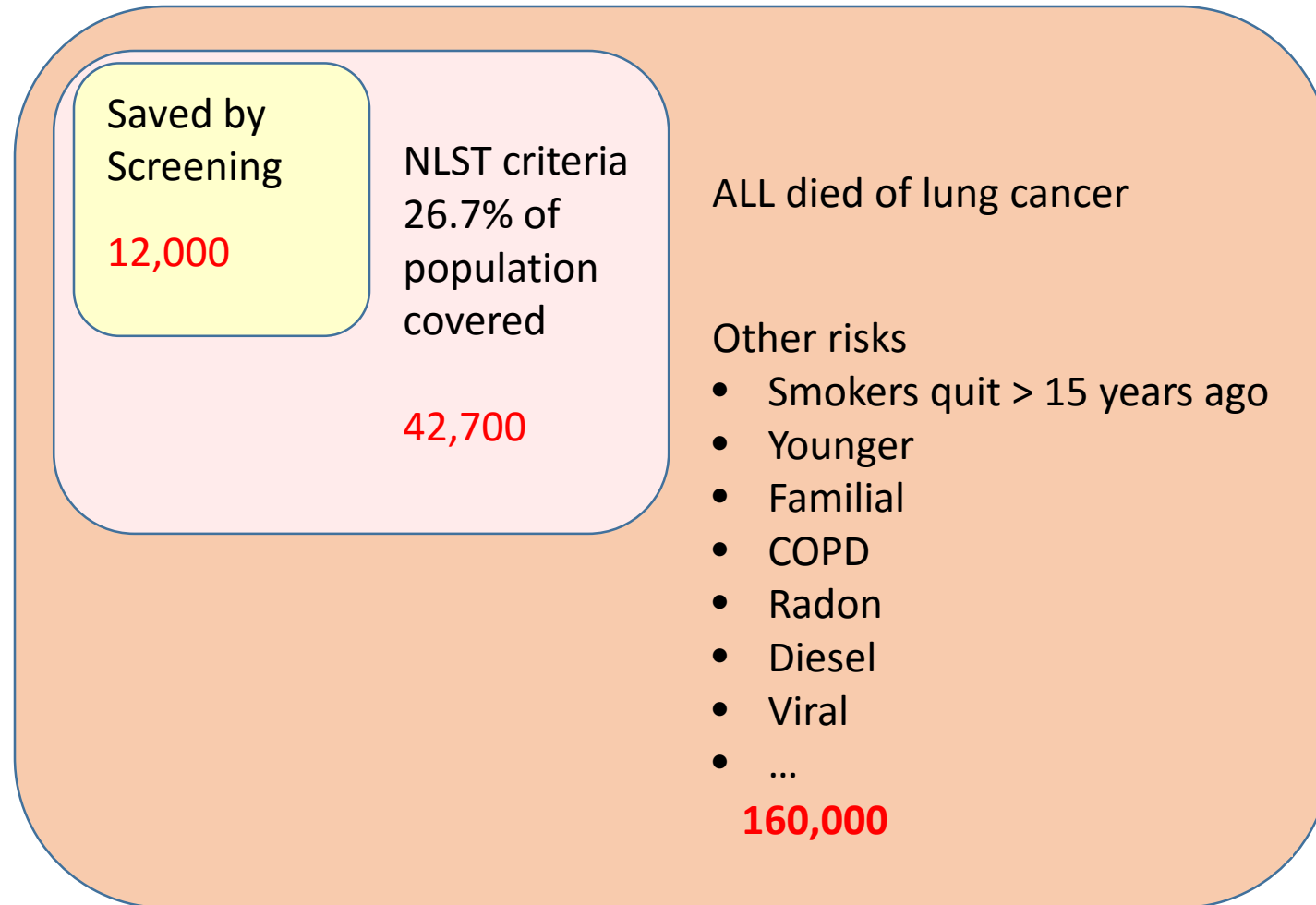
- I am an unpaid advisor to Oncimmune, Integrated Diagnostics, Ajinomoto, Natera, Optellum, Veracyte and Nucleix.
- I am the PI on studies at VUMC sponsored by Ajinomoto and Veracyte. The sponsors do not support my salary.

# RATIONALE: Early Detection of lung cancer saves lives

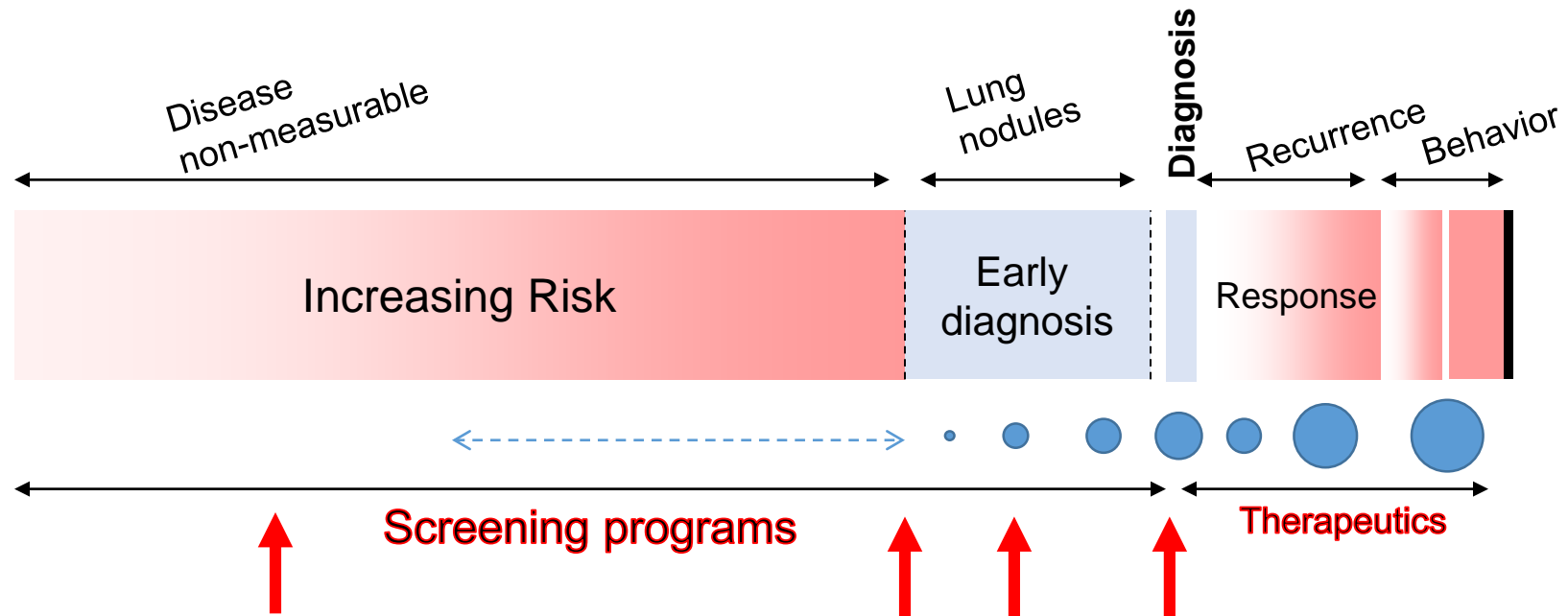
- Saves lives; >10 x more than non-surgical therapeutics
- Increases the chances for successful treatment
- Reduces cost of cancer care
- Results in the decline in U.S. cancer deaths
- Enjoys only 15% of cancer research funds

Fulfilling the Potential for Cancer Prevention and Early Detection.  
National Research Council , 2003

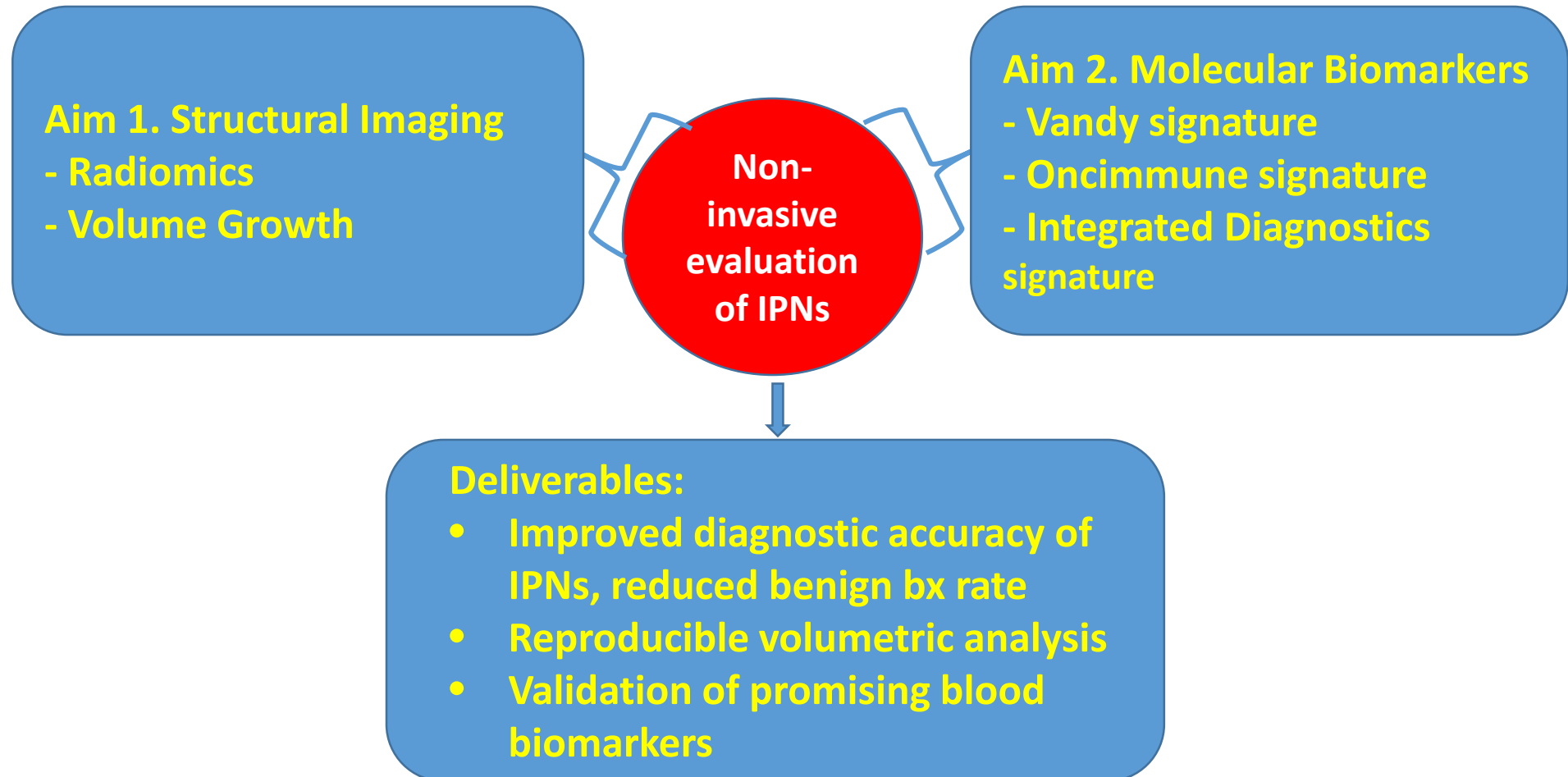
# Rationale for the use of biomarkers in the CT screening era

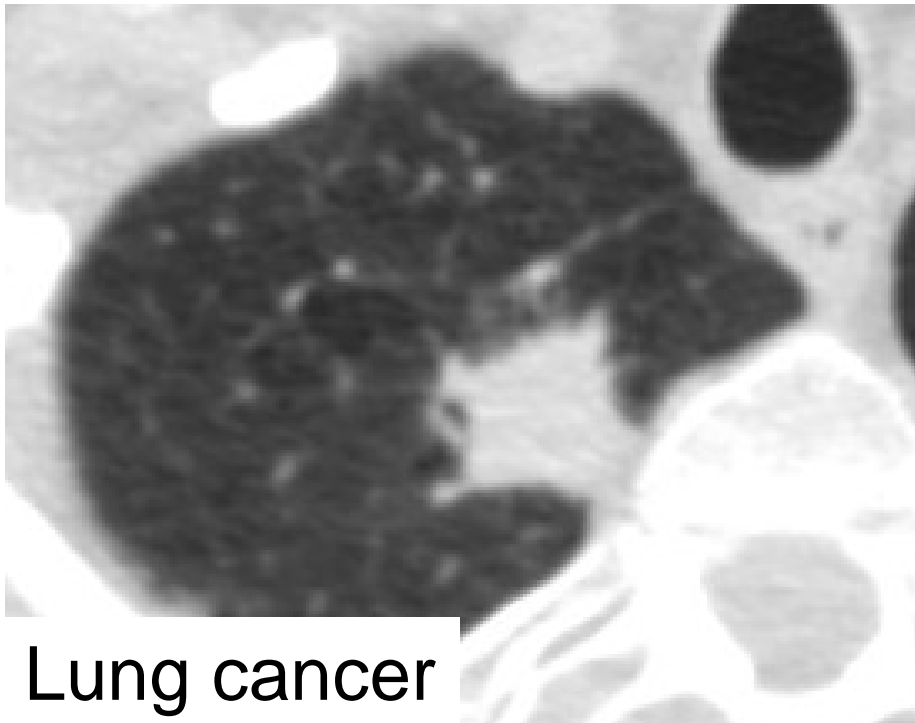


# Molecular biomarkers in lung cancer management

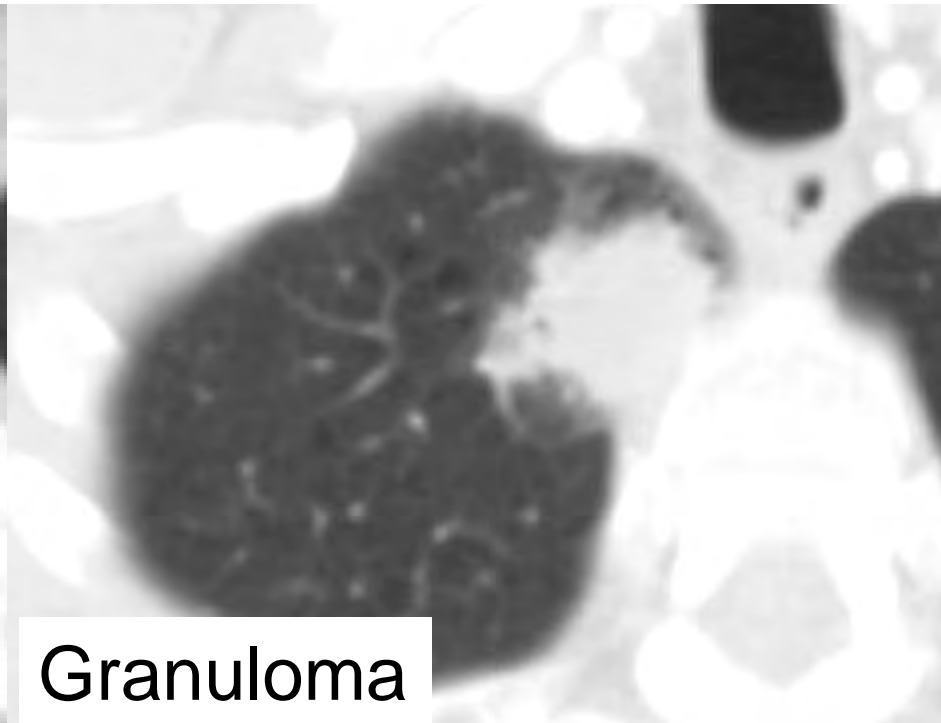


# A 3 pronged approach

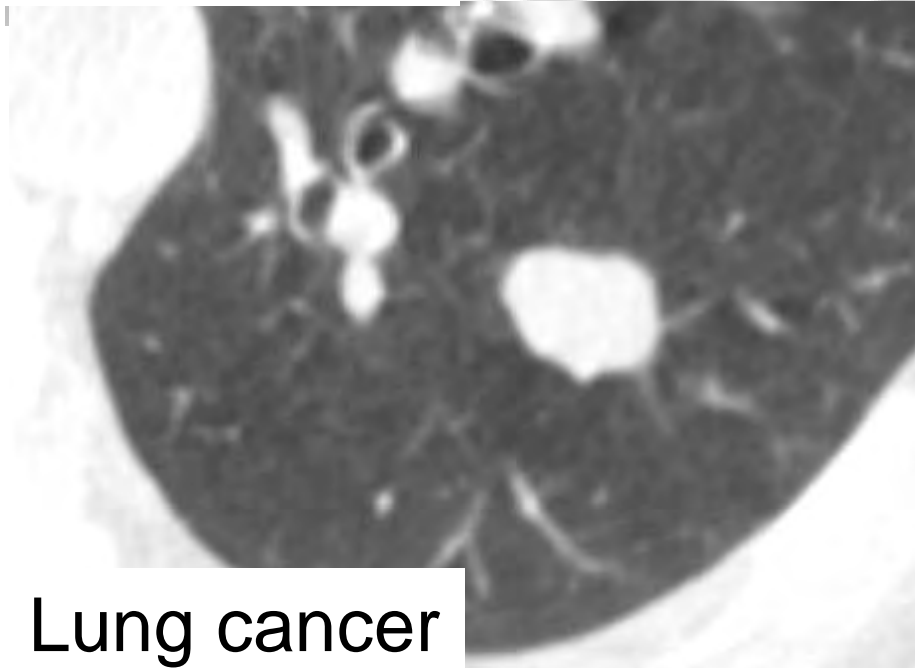




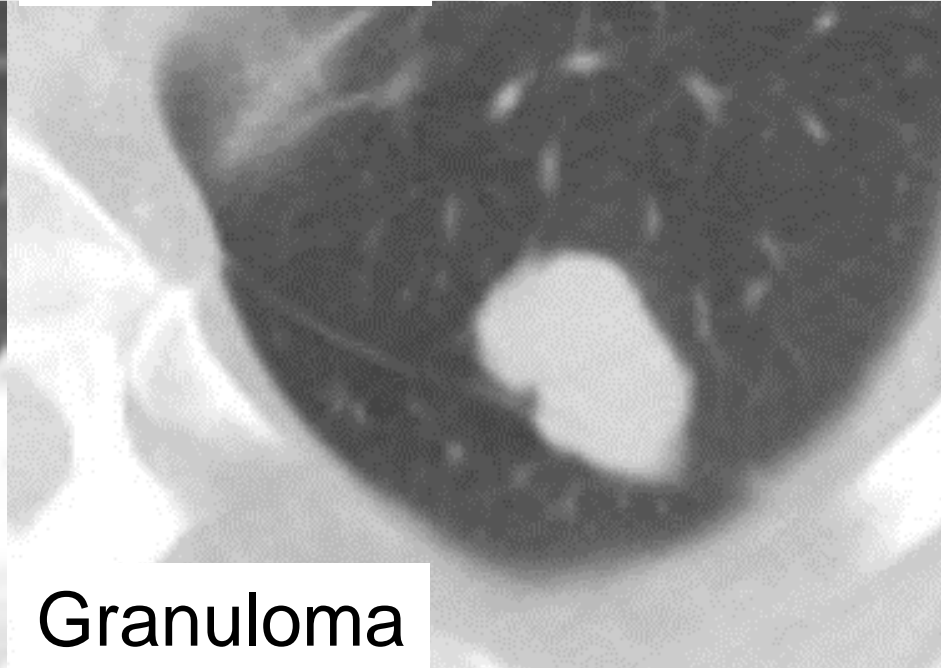
Lung cancer



Granuloma



Lung cancer



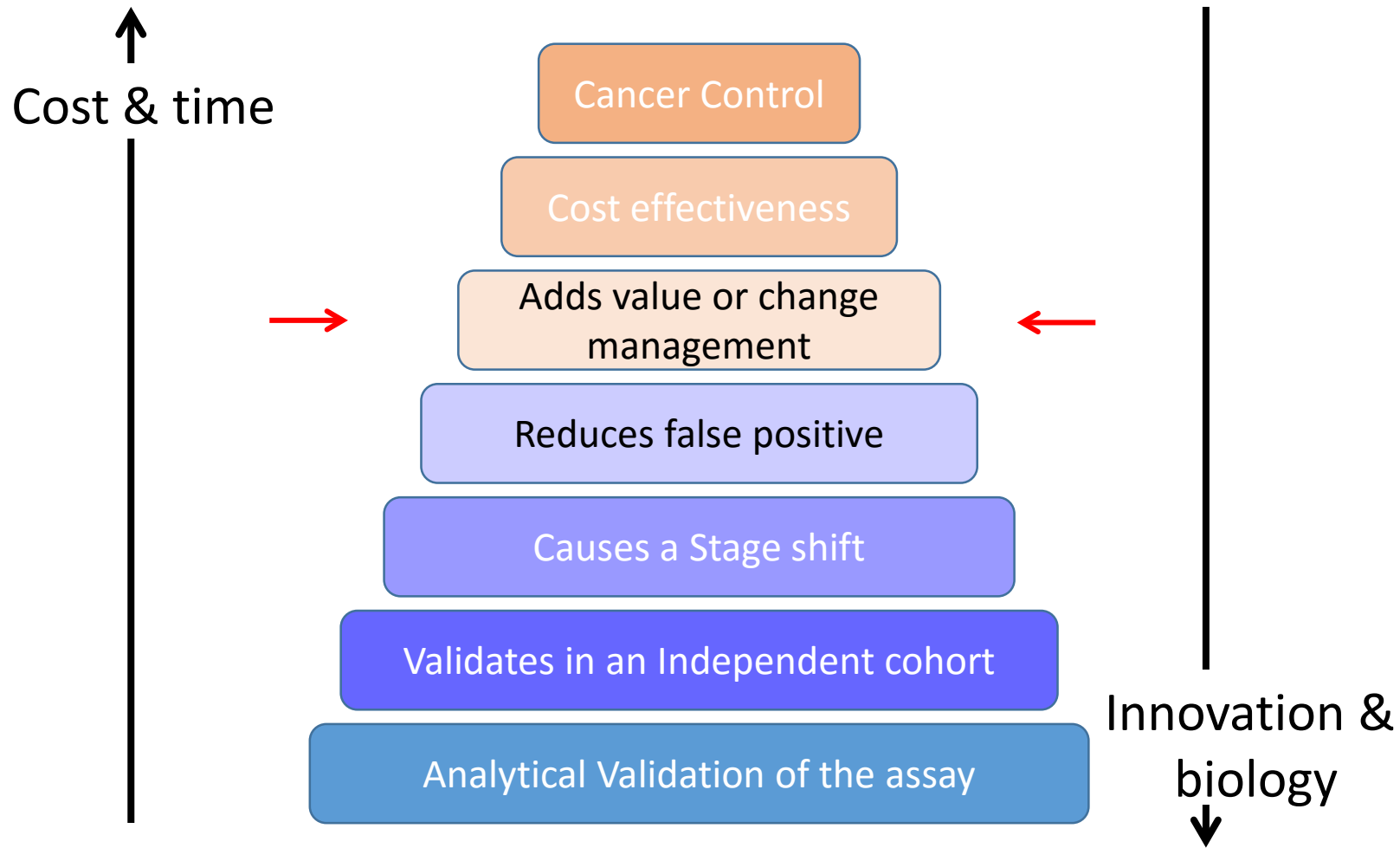
Granuloma

# Diagnostic biomarkers for lung cancer

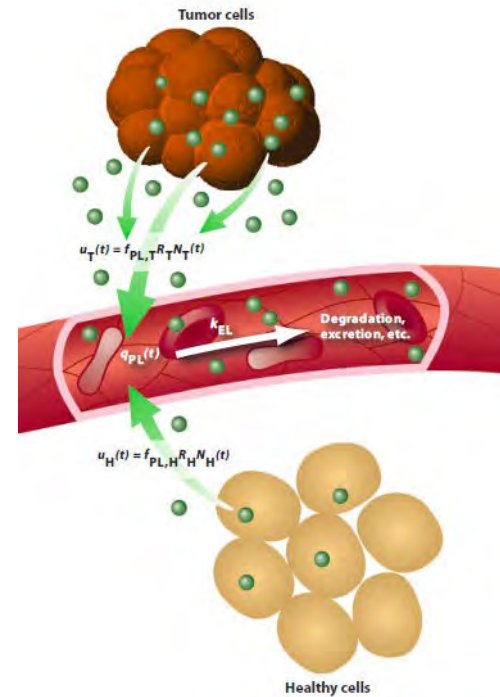
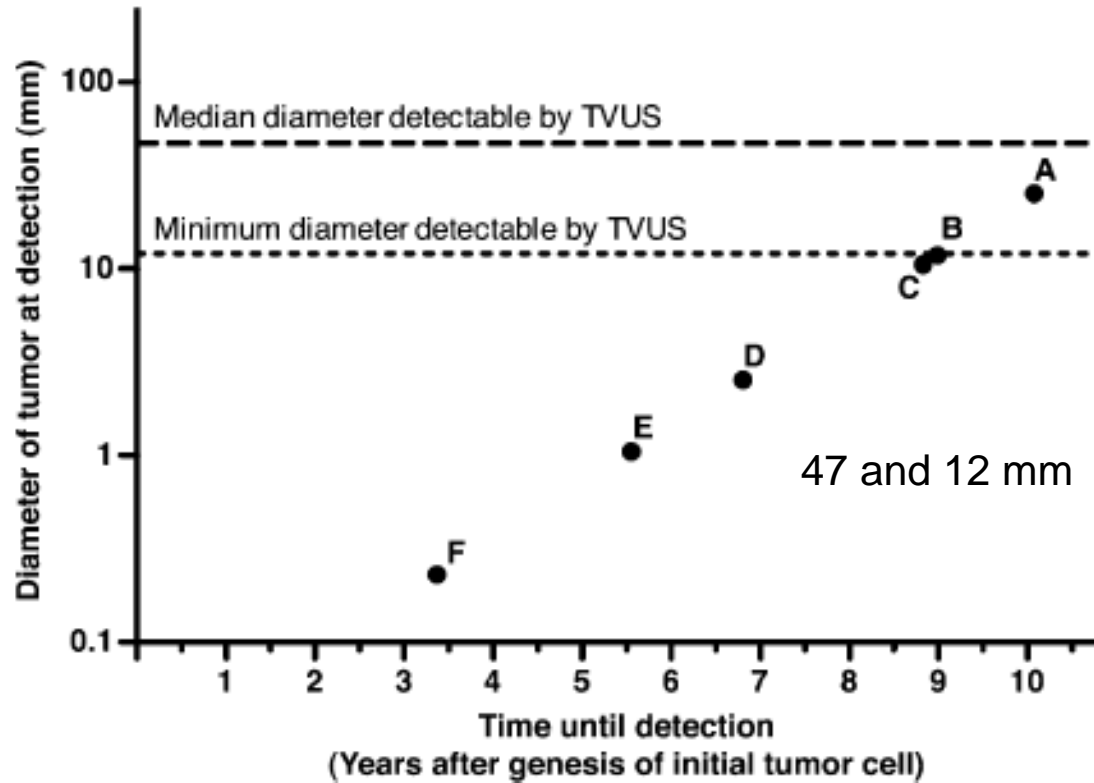
Candidates	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
	Discovery, Prediction	Assay validation	Retro-longitudinal	Prospective screening	Cancer Control
<b>SERUM/PLASMA</b>					
Proteomic profiling	x	x	x		
Autoantibodies	x	x	x	x	
Specific antigens /proteins	x	x	x		
miRNA	x	x	x	x	
DNA methylation Blood	x	x			
Circulating Tumor DNA	x	x	x		
<b>TUMOR/airway epith</b>					
Preinvasive histo/cytology	x	x	x		
DNA methylation	x	x	x		
RNA airway signature	x	x	x	x	
Proteomic profiling	x	x			
Chromosome aberrations	x	x			
<b>SPUTUM/EBC</b>					
DNA Methylation Sputum	x	x	x		
DNA CN -FISH	x	x			
VOCs	x	x			



# Criteria for clinical use of biomarkers



# Sensitivity Matters



A: ELISA

B: 100% vasc permeability

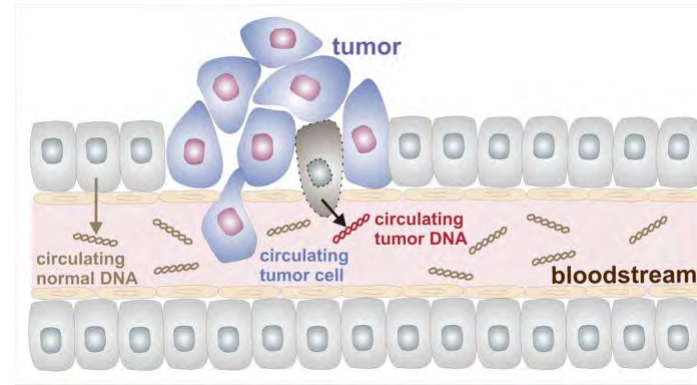
C: not shed by healthy cells

D: shedding is 1000x nl

E: not shed in nl and improved 1,000 x assay sensitivity from baseline

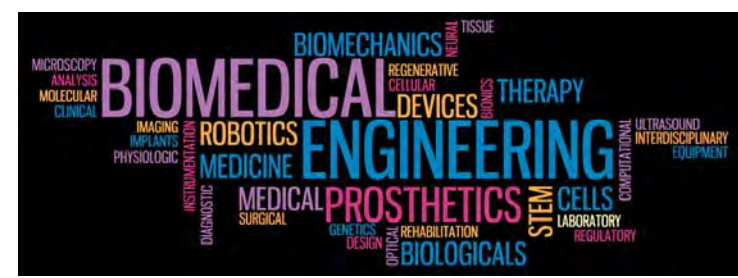
F: not shed in nl and improved 100,000 assay sensitivity from baseline

**ctDNA:** Outstanding specificity  
We still lack sensitivity!

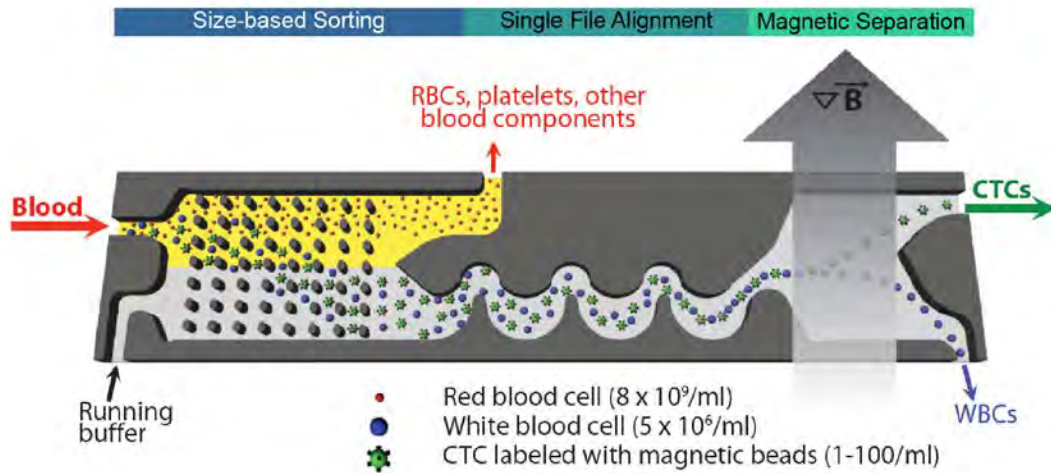


Zeus; Le Louvre

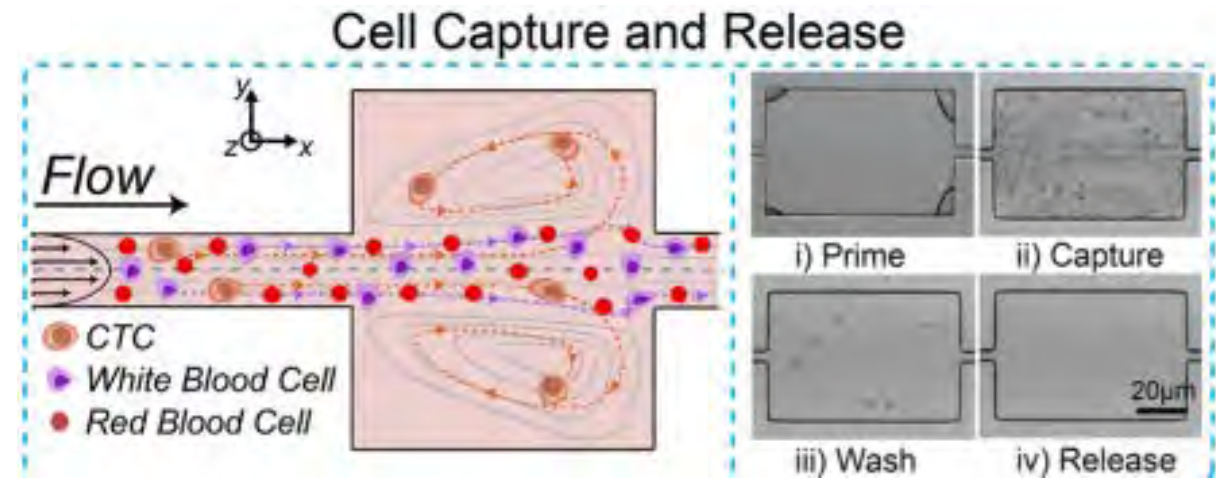
- Variable input 1-20 ng of cell free DNA
- Detection of a mutation in  $< 1: 100,000$  cells-- One diploid cell= 6.6pg
- Size of ctDNA is around 166 bp (nucleosome + linker)
- 1 ng of DNA fragment of 166 bp =  $5 \times 10^9$  molecules
- ctDNA is 0.01% to  $>90\%$  fraction of circulating DNA
- CAPP seq detects 2.5 ctDNA molecules in  $10^6$  -- Safe-Seq has sens 9 in  $10^6$
- Detection limits of mutant allele fractions in ctDNA is 0.1-0.01% , or  $\sim < 1$  mutant template molecular in 1 mL of plasma
- Need coverage depth as high as 10,000 $\times$  to uncover rare ctDNA mutations. Coverage depth competes with sequencing errors



# CTCs We lack sensitivity! Ilie, PLoS One 2014

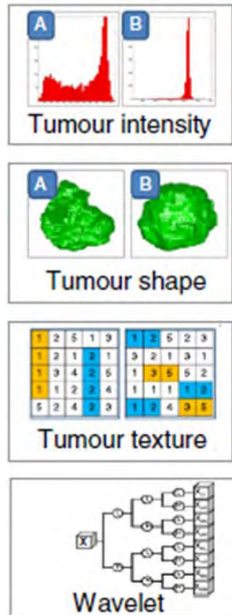


Murat Karabacak et al.  
 STM, 2013.

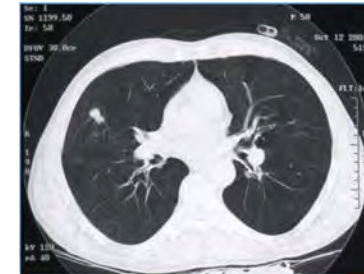
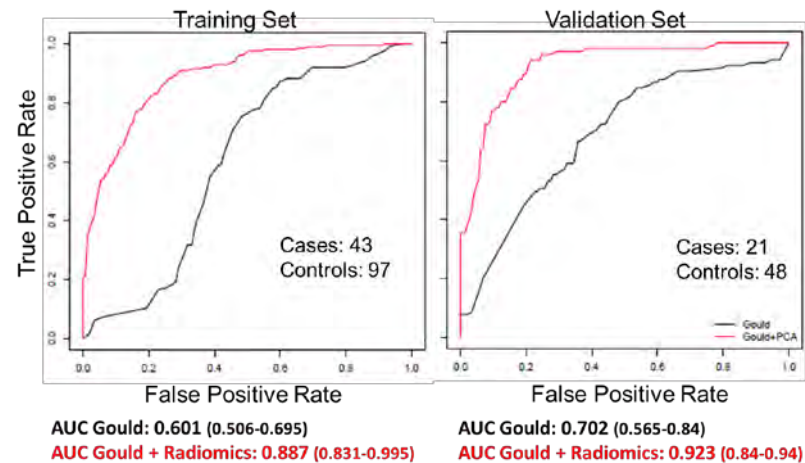


Dhar et al.  
 Sci Rep. 2016; 6: 35474  
 Renier et al.  
 Precision oncology 2017

# Thoracic Imaging Repository- XNAT Radiomics



209 nodules ; 221 radiomics features (7 components)



**Funding**  
U01 CA196405

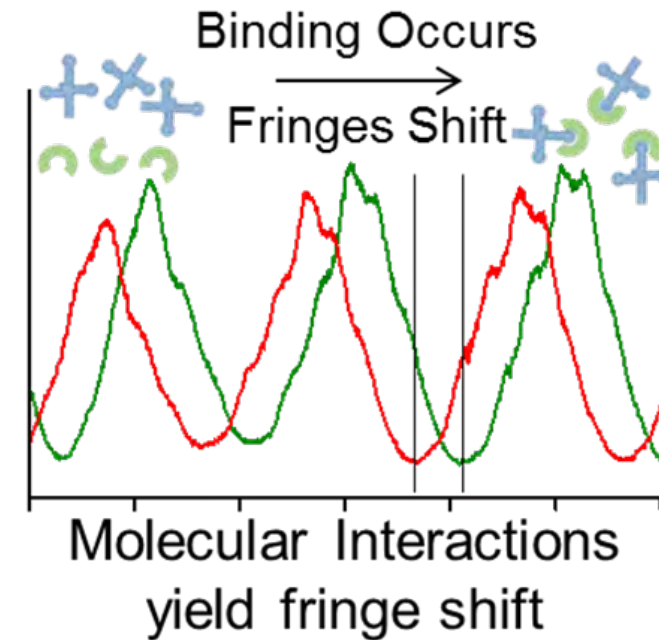
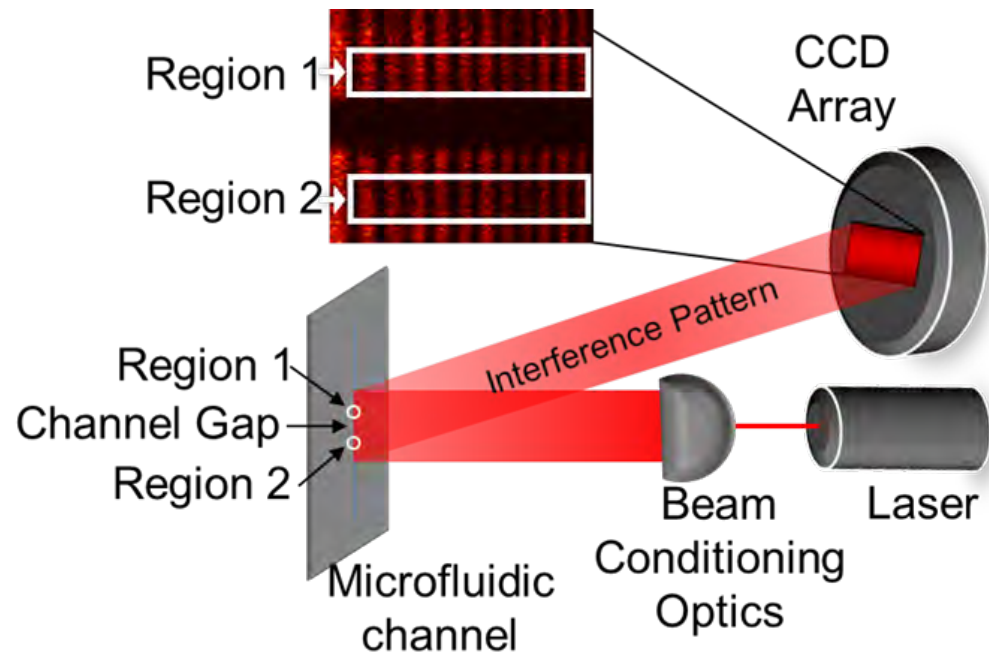
**Publications**  
Maldonado AJRCCM 2015  
Cytometry B Clin Cytom. 2017  
Gillies, Radiology. 2016  
Diehn, Nature Med 2014

**Shared Resources**  
Genomic Sciences  
Quantitative Sciences  
Flow/Mass Cytometry core  
Innovative Translational Research

# CYFRA 21-1

- Fragment of Cytokeratin 19.
- CYFRA 21-1 epitope is a polypeptide, which is most likely released following cell death (Stieber et al, 1993; Sheard et al, 2002).
- Serum fragments of cytokeratin-19 can be detected using anti-CYFRA 21-1 antibody (Pujol et al, 1993).
- Patients with nonmalignant disease showed almost no elevation of serum CYFRA 21-1, except in cases of cirrhosis, renal failure, or infectious lung disease
- Stability data demonstrated that CYFRA 21-1 is stable in serum for a minimum of 48 h at ambient temperatures and 14 days at 4 °C.

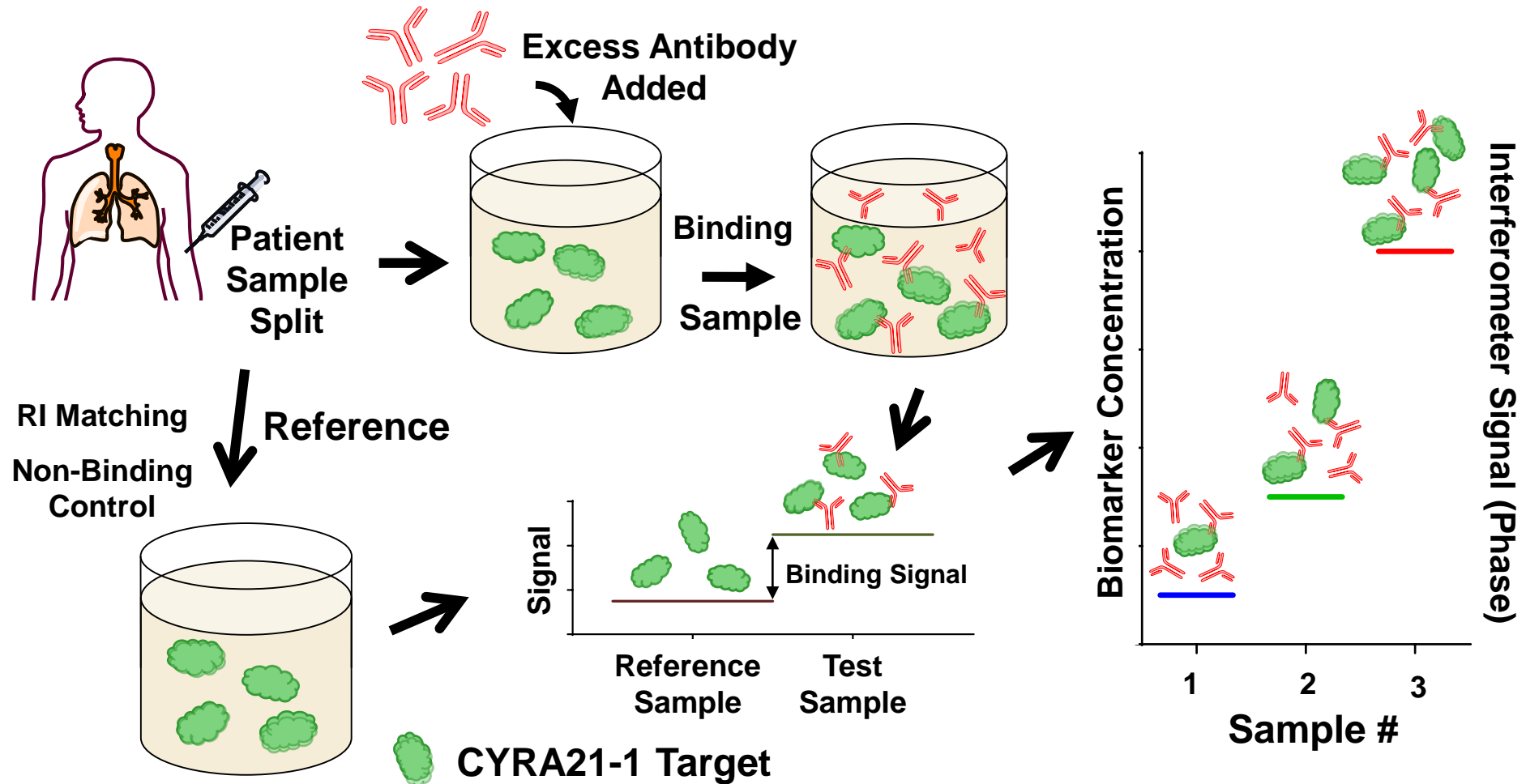
# Compensated Backscatter Light Interferometry (CBSI)



*Olmsted et al. Anal Chem 2014*

*Kammer, et.al., Anal. Chem., Revised (Feb. 2017)*

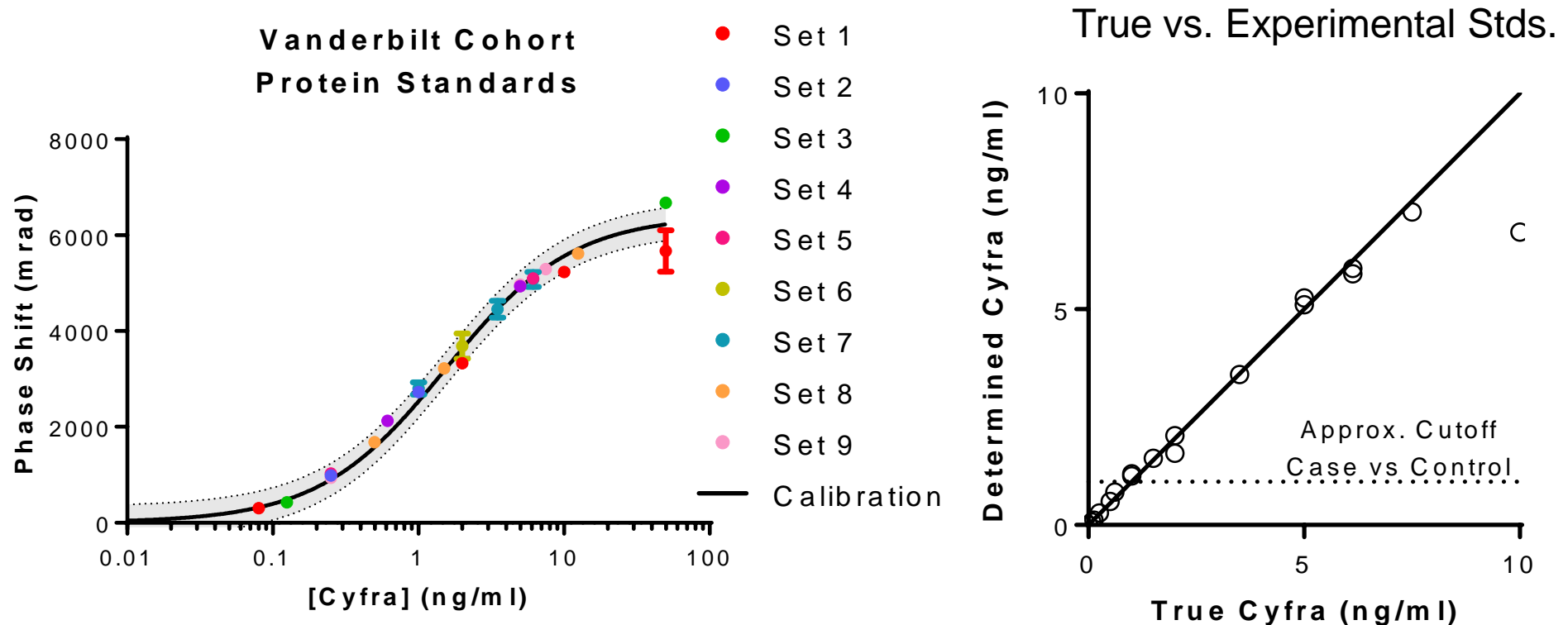
# Mix-and-Read Free-Solution Assay



- Determine if probe produces quantifiable signal.
- Calibration done using spiked serum samples.
- Non-specific binding tested by titrating antibody with serum.



# Daily Standards of Spiked Serum Ensures Accurate CYRFA Quantification



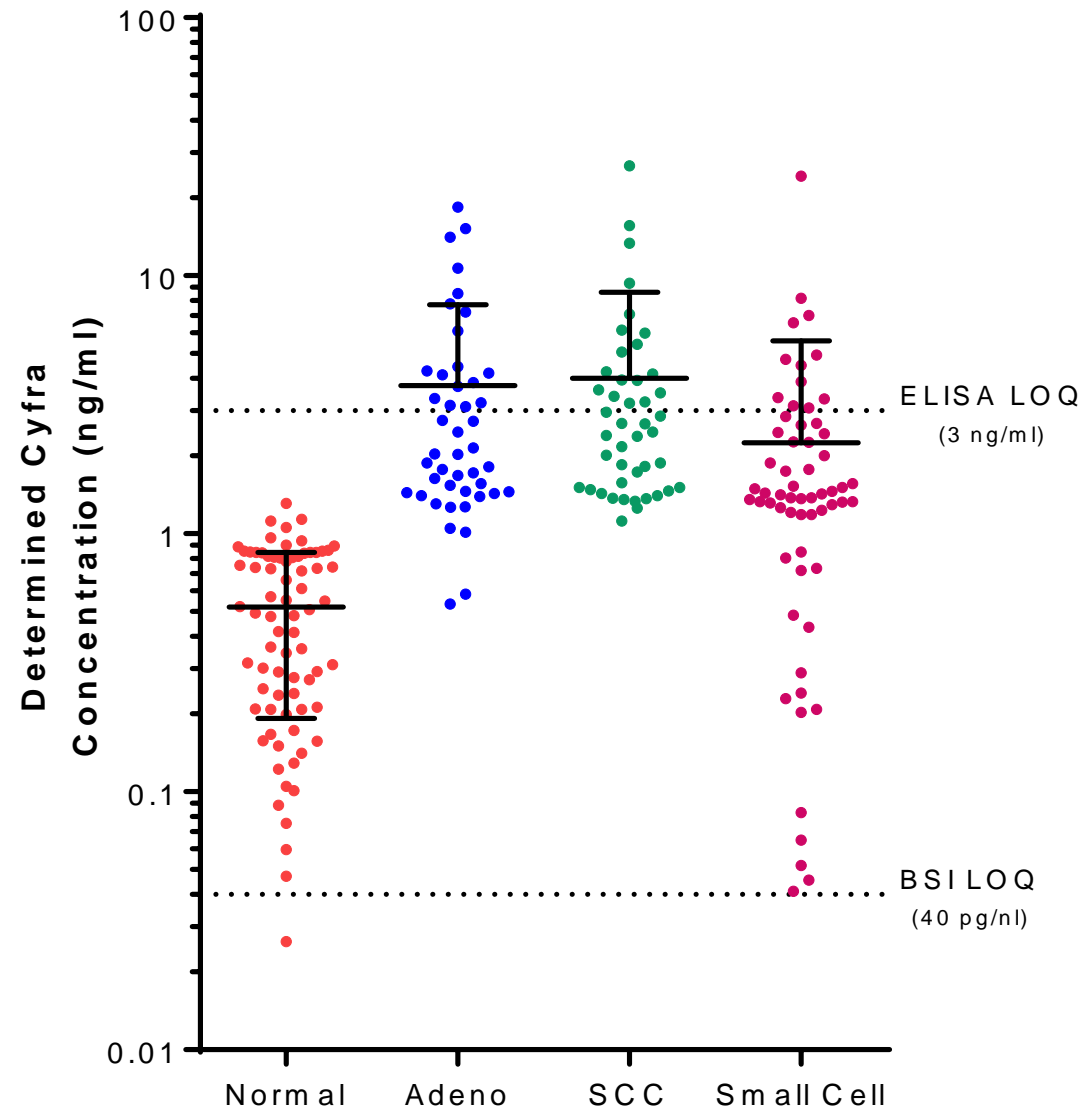
- Black line = Pooled calibration curve
- Grey area = 95% confidence interval
- Colored dots = Protein standards run on each day
- **Minimum of 6 replicates at each concentration**

# Patients Characteristics

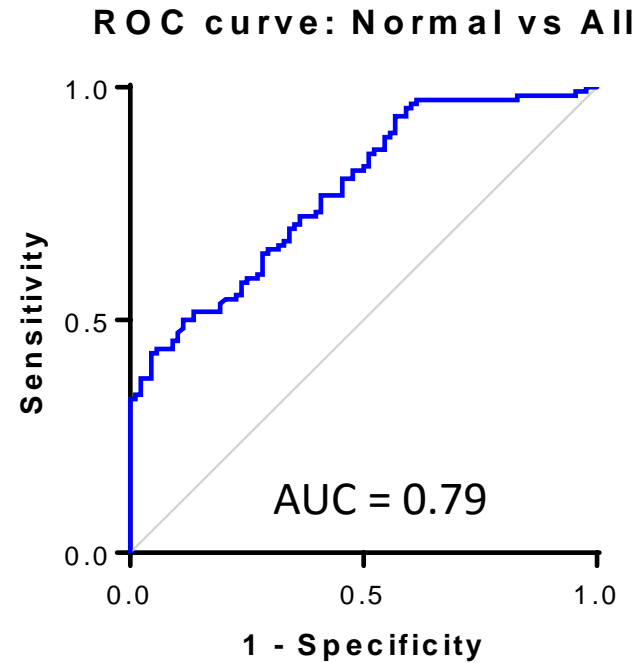
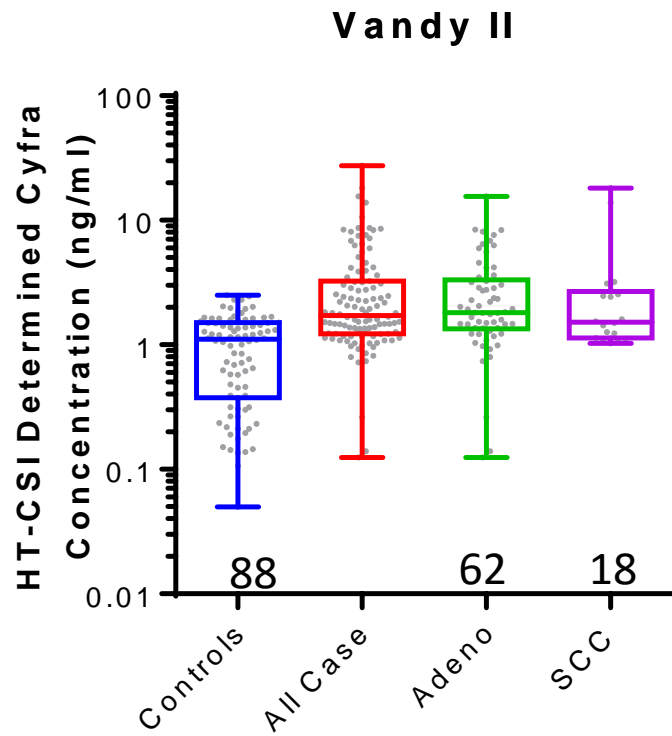
Assembled cohorts	Preliminary data VUMC n=225			
	ADC (%)	SCC (%)	SCLC (%)	No Cancer (%)
Patients	N= 45	N= 44	N= 61	N= 75
Age ± SD	65.2 ± 8.0	65.8 ± 7.8	63.9 ± 8.9	59.2 ± 12.7
Gender				
Male	26 (58)	29 (66)	36 (59)	40 (53)
Female	19 (42)	15 (34)	25 (41)	35 (47)
Nodule Size (cm) ± SD	2.7 ± 1.7	2.7 ± 2.0	3.6 ± 2.6	2.5 ± 1.6
Smoking Status				
Never Smoker	0 (0)	0 (0)	3 (5)	1 (1)
Ex-Smoker	26 (58)	23 (52)	18 (30)	38 (51)
Current Smoker	19 (42)	21 (48)	40 (65)	36 (48)
Pack Years ± SD	50.1 ± 31.3	53.9 ± 23.5	63.7 ± 32.8	41.1 ± 30.3
Cancer Path Stages				
IA-IB	33 (73)	34 (77)	0 (0)	0 (0)
IIA-IIIA	12 (27)	10 (23)	0 (0)	0 (0)
IIIB-IV	0 (0)	0 (0)	0 (0)	0 (0)
Limited	0 (0)	0 (0)	33 (54)	0 (0)
Extensive	0 (0)	0 (0)	28 (46)	0 (0)
Cancer histologies				
Adenocarcinoma	45			
Squamous carcinoma		44		
Large cell carcinoma				
Small cell carcinoma			61	
Other malignant				
Benign Histologies				
TB				6 (8)
Fungal Infection				7 (9)
Bacterial Infection				10 (13)
Inflammation				45 (60)
Fibrosis				5 (7)
Hamartoma				2 (3)



# Results from Vanderbilt 225 Patients Cohort

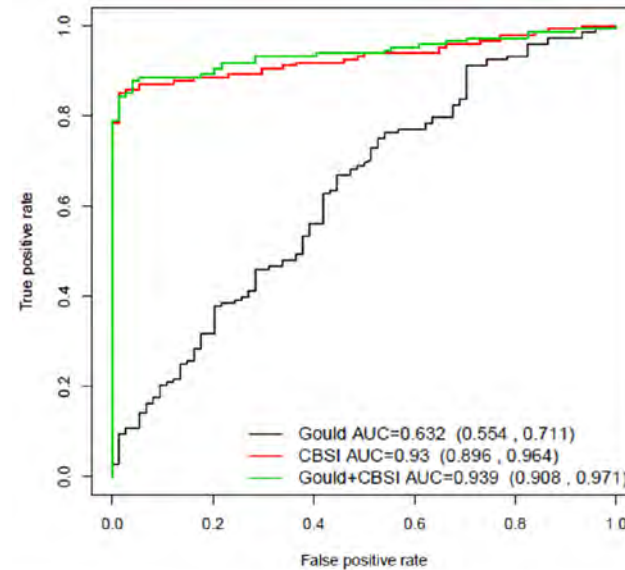


# Validation cohort Vandy 2 IPNs 6-30 mm

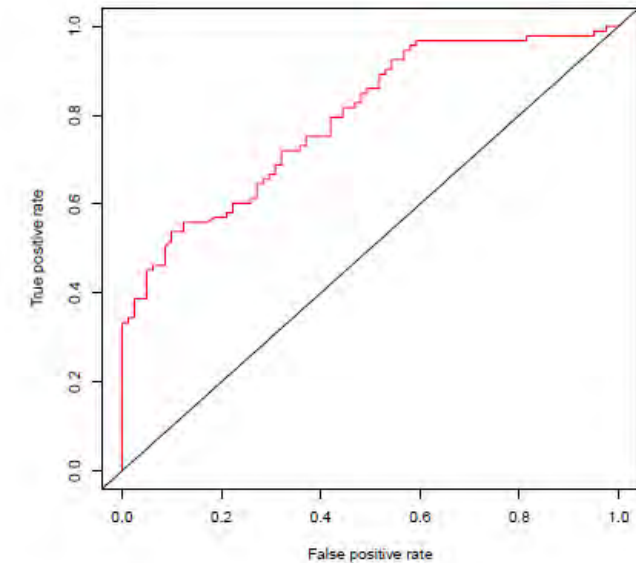


# Validation of CYFRA 21-1 IPNs 6-30 mm

	CYFRA 21.1- BSI	
	Vandy 1	Vandy 2
Sample size	129	174
cutoff	0.116	0.567
Sensitivity	0.87	0.53
Specificity	0.96	0.9
PPV	0.97	0.86
NPV	0.82	0.63
<b>DLR.Positive</b>	21	<b>5.4</b>
<b>DLR.Negative</b>	0.13	<b>0.51</b>
Cancer Prev	0.66	0.46
Nodule size mm	18.1	15.7

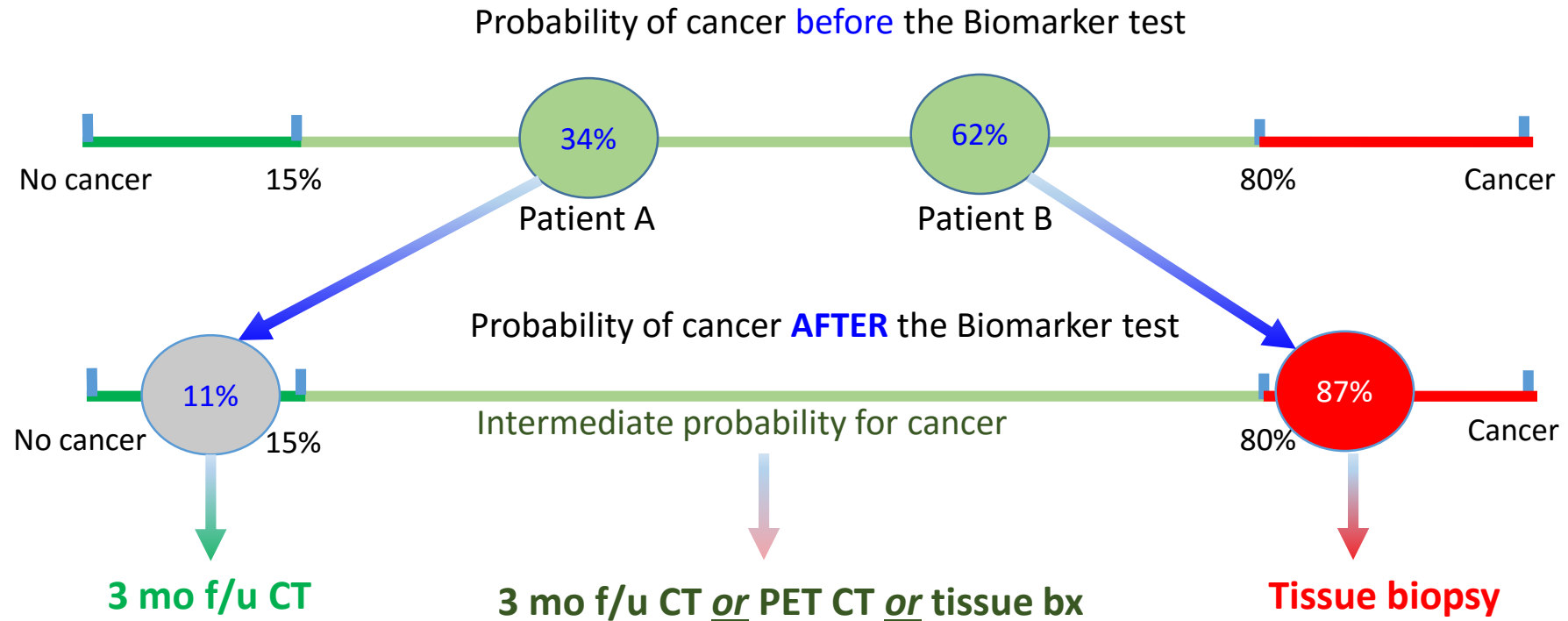


Vandy 1 dataset  
AUC 0.93

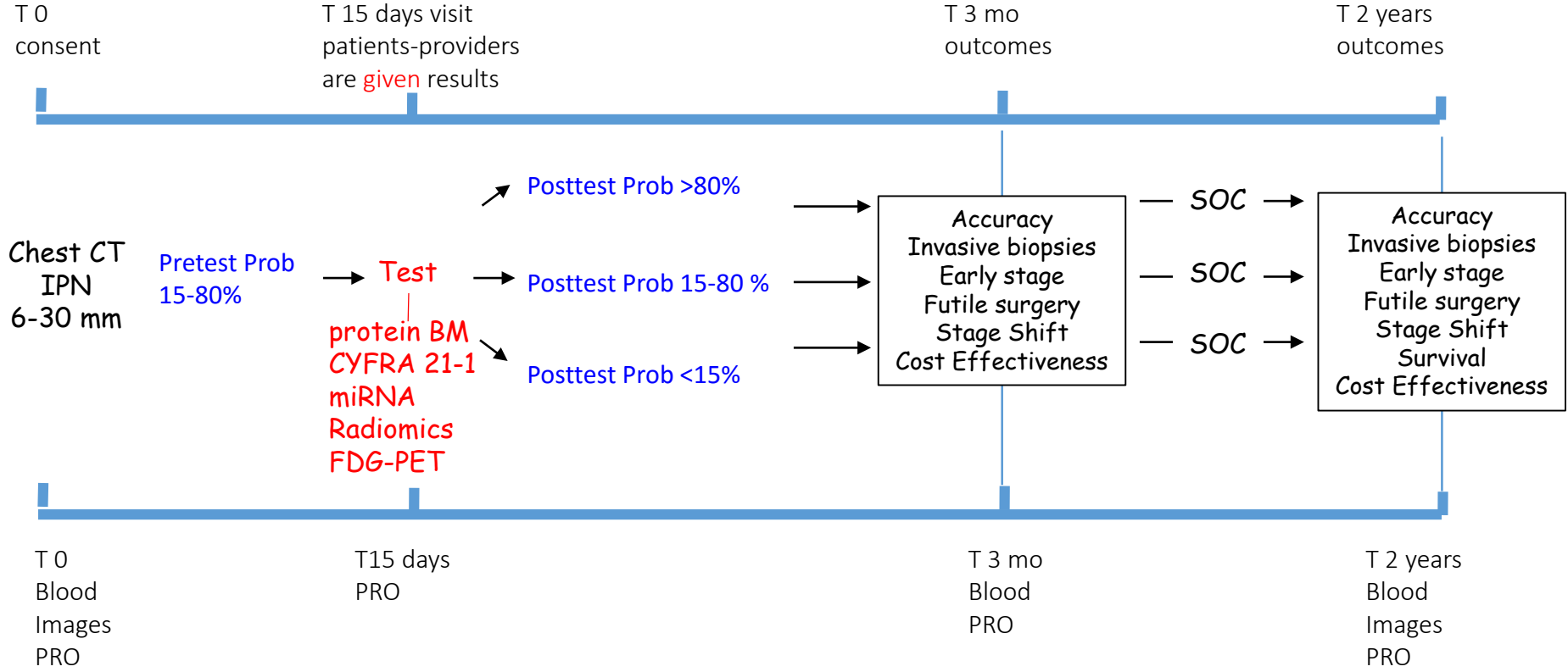


Vandy 2 dataset  
AUC 0.79

# Patient Provider report



# Biomarker driven trial for the management of IPNs



# Acknowledgments

## Massion Lab

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- Bob Gillies, Matt Schabath
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- Oncimmune
- Integrated Diagnostics
- Natera
- Veracyte

•Patients

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