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How to Complement Biomarker Testing with Diagnostic Imaging

Catherine R. Sears, M.D., ATSF

Associate Professor, Indiana University School of Medicine
Director Pulmonary Oncology and Lung Cancer Screening- Richard L. Roudebush VA Medical Center
Indianapolis, Indiana

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3. Some trade names are used in this talk

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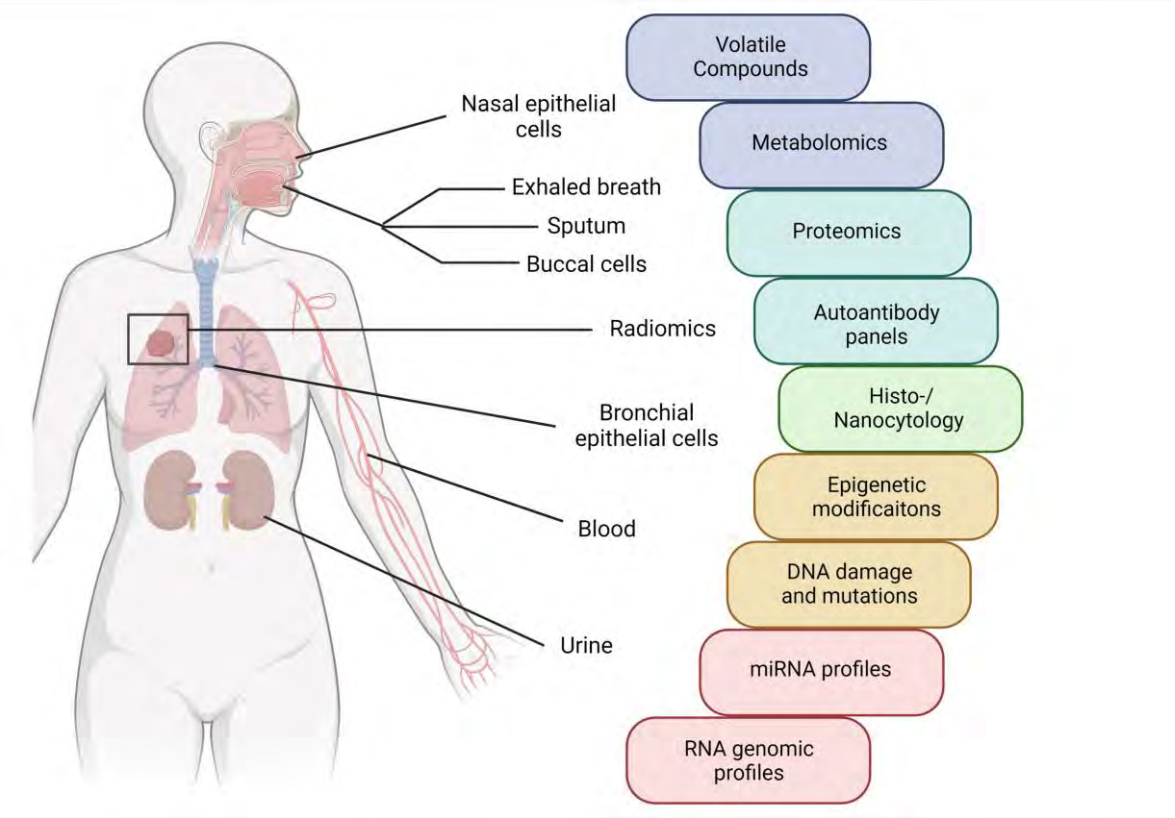
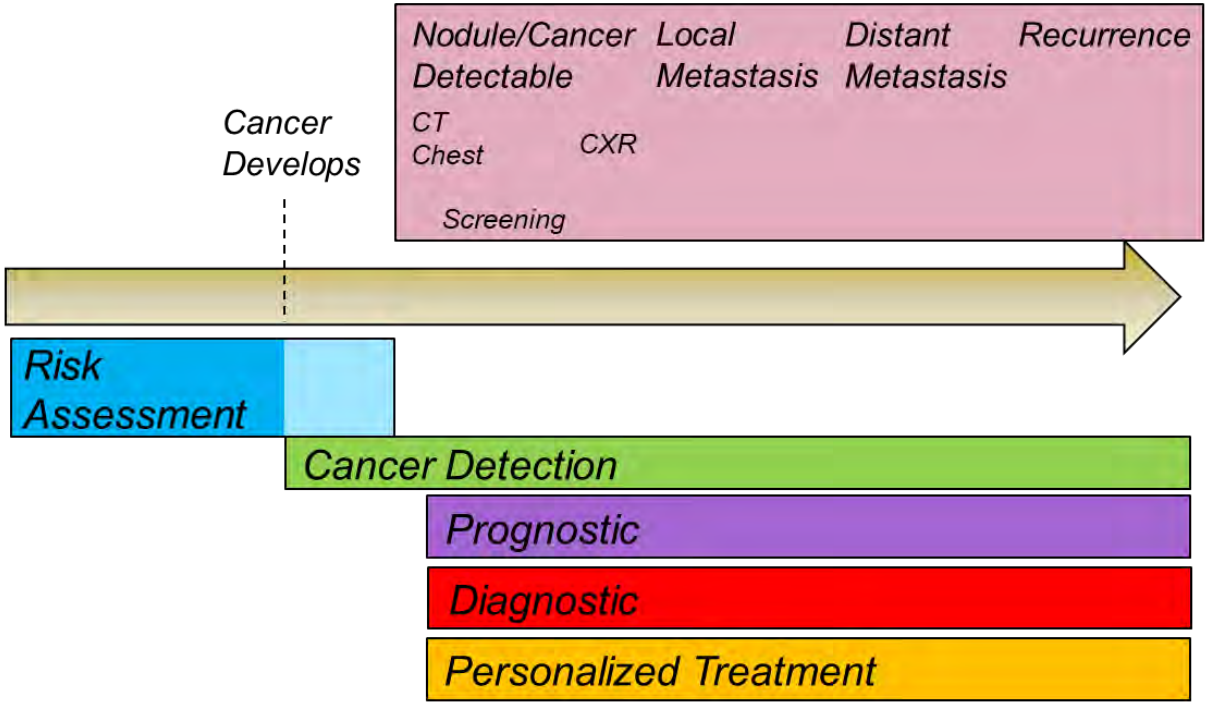
The contents of this presentation do not represent the views of the U.S. Department of Veterans Affairs of the United States Government.

Biomarkers



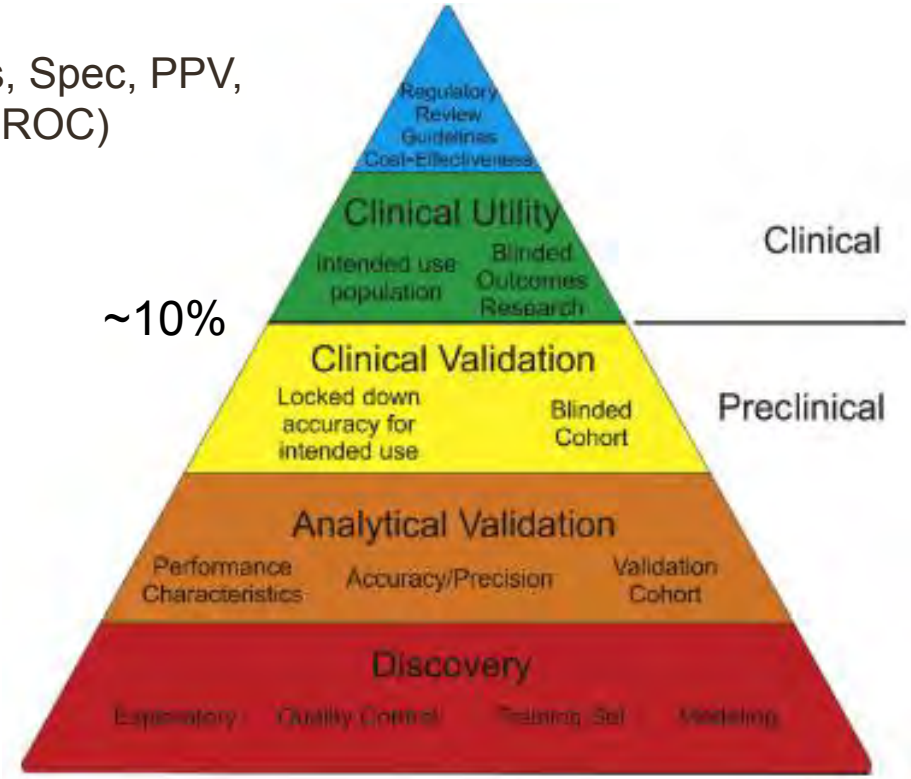
Vedachalam, Tanner and Sears. Chapter 4: Approach to Lung Nodules. Respiratory Medicine (Ed. MacRosty and Rivera). *In Review.*

Biomarkers in lung cancer continuum



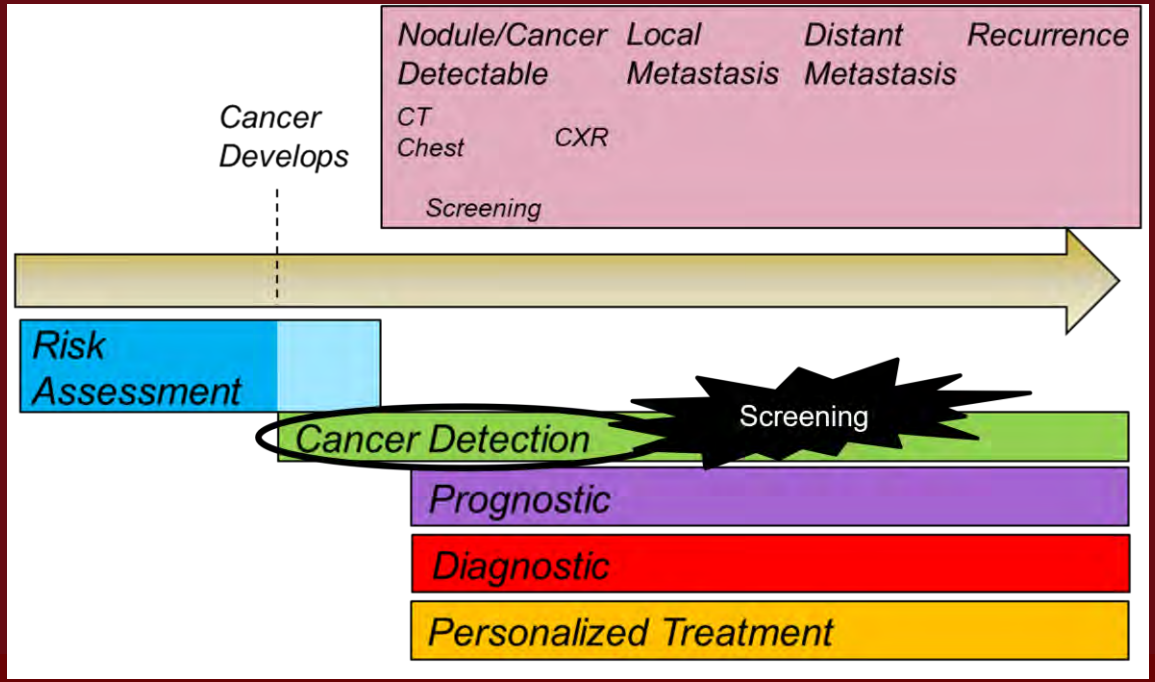
Ideal Lung Cancer Biomarker

1. Favorable Performance Metrics (Sens, Spec, PPV, NPV, ROC)
2. Easily Accessible Material
3. Small amount needed
4. Little/simple sample preparation
5. Inexpensive/Cost Effective
6. Applicable to Large Target Population
7. Clinically Useful
   - Advantage over/with standard of care
   - Easy to interpret / act on results



Sears and Mazzone. Clin Chest Med. 2020;41(1):115-127

Biomarkers for Lung Cancer Screening



Lung Cancer Biomarkers - Screening

Biomarker	Measurement	Validation Cohort	Sensitivity Specificity*	Proposed Use	Availability/ Clinical Utility
Nodify CDT (Biodesix)	Blood auto-antibody panel (7): ELISA	Patients: 1613 Cancers: 61	Sens: 37% Spec: 91%	**Screening** risk assessment (outside LCS criteria, more frequent LDCT)	CLIA/ **US (**NCT01700257 – completed 2020)/ **UK** (ECLS) – Stage Shift with more frequent screening
miR-Test	Blood: miRNA	COSMOS Patients: 1115 Cancers:48	Sens: 78% Spec: 75%	Enrichment of high-risk screening cohort	No/**Clinical utility trial** (COSMOS II)
MSC signature (miRNA)	Blood: miRNA	MILD pts: 1085/939 Cancers: 85	*Sens: 95% *Spec: 78%	Enrichment of high-risk **screening** cohort	No/ **Clinical utility trial completed 3/2022** (BIOMILD) – More lung CA dx, supports longer duration btwn scans if negative
PAULA's (Protein Assay Using Lung cancer Analytes) (Genesys)	Blood antigen / protein panel: ELISA	Patients: 150 Cancers: 75	Sens: 71% Spec: 88%	Enrichment of high-risk **screening** cohort	CLIA / **Recent new clinical validation trial using 5th biomarker**
4-MP	Blood auto-antibody panel (4): immunofluorescence bead/flow cytometry + PLCO2012	PLCO pts: Patients: 2,745 Cancers: 552	Sens: 83.5%*/88.4% Spec: 71.6%*/56.2% (*$PLCO_{2012} \geq 1.7\%$ $PLCO_{2012} \geq 1\%$)	Enrichment of high-risk **screening** cohort/ Early nodule diagnosis	No
Lung EpiCheck (Nucleix)	DNA methylation	European/Chinese Patients: 361 Cancers:209	Sens 78%-90% (Stage I-IV). Spec: matched control 64%, unmatched controls: 93%	**Screening** / Early diagnosis	No / clinical investigations
DELFI-LUNG FirstLook-Lung (Delfi Diagnostics, Inc)	Blood: cfDNA fragmentation pattern	Enrolling prospective study- 15,000 LCS pt	Varies based on multiple analytic cohorts	Enrichment of high-risk **screening** cohort / symptomatic lung cancer / Rule-in nodule biomarker	No / NCT05306288 (CASCADE-LUNG for LCS) / NCT04825834 (DELFI-L101 for Nodule Clin validation)
RespiraGene (Synergenz)	Oral swab: 20 SNPs + clinical			Enrichment of high risk **screening** cohort Smoking cessation for high risk (GeTSS)	**No**

Not being used for screening

Sears, Mazzone. Clin Chest Med. 2020

Fahrmann et al. J Clin Oncol. 2022

Nichols et al. BMC Res Notes. 2017

Mathios et al. Nature Communications. 2021

In development: Lung-CLiP (ctDNA mutations), Freenome (cfDNA-multiomics), Multicancer screening biomarkers: Galleri (cfDNA methylome, GRAiL), Adela MRD (cfDNA methylome), Cancer-SEEK (cfDNA mutations+proteins, Exact Sciences), SPOT-MAS (cfDNA methylome+fragmentation), BlueStar Genomics (cfDNA 5hmC), ELISA-Seq (cfDNA methylome, Burning Rock Dx),, Verita (EV proteins, Biological Dynamics), Caris Life Sci (cfDNA/RNA NGS), Early Diagnostics (cfDNA mC-NGS), Freenome (cfDNA-multiomics), LungLifeAI (CTC FISH+AI), Natera (cfDNA NGS+Protein), 20/20 Gene Sytems (blood Ag)

Current lung screening guidelines

	USPSTF (2021)	CMS (2022)	NCCN (10/2024)
Age	50-80 yo	50-77yo	≥50*
Smoking history	≥ 20 PY	≥ 20 PY	≥ 20 PY[1] or ≥ 20 years[2B]
Smoking Status	Current or quit ≤ 15yrs	Current or quit ≤ 15yrs	Current or quit
Secondary criteria	None	None	Additional risk factor(s) (race, exposure to radon, risk calculator, etc)

*-77 yo or older if healthy and likely to benefit

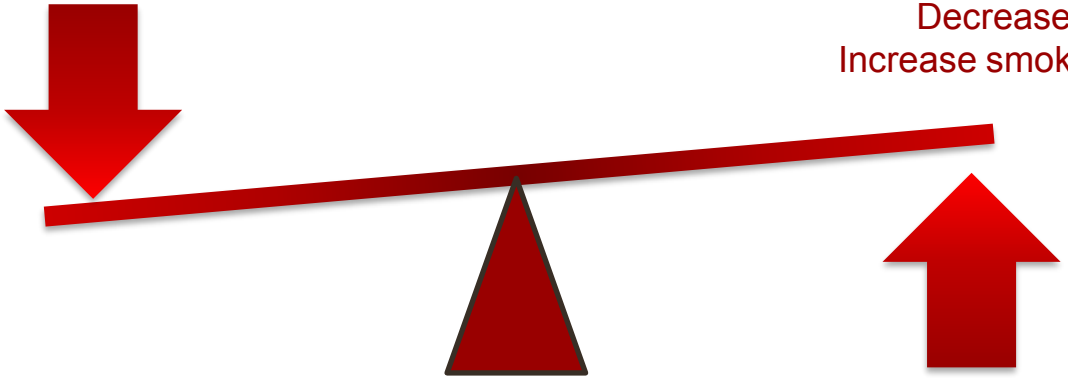
Biomarkers for LCS: Optimize Benefit to Risk Ratio

RISKS:

Nodules: False positives (high, ~96%)
Overdiagnosis Psychological stress
Procedure complications
Radiation exposure
+/- Cost

BENEFITS:

Decreased lung cancer mortality
Decreased anxiety
Increase smoking cessation

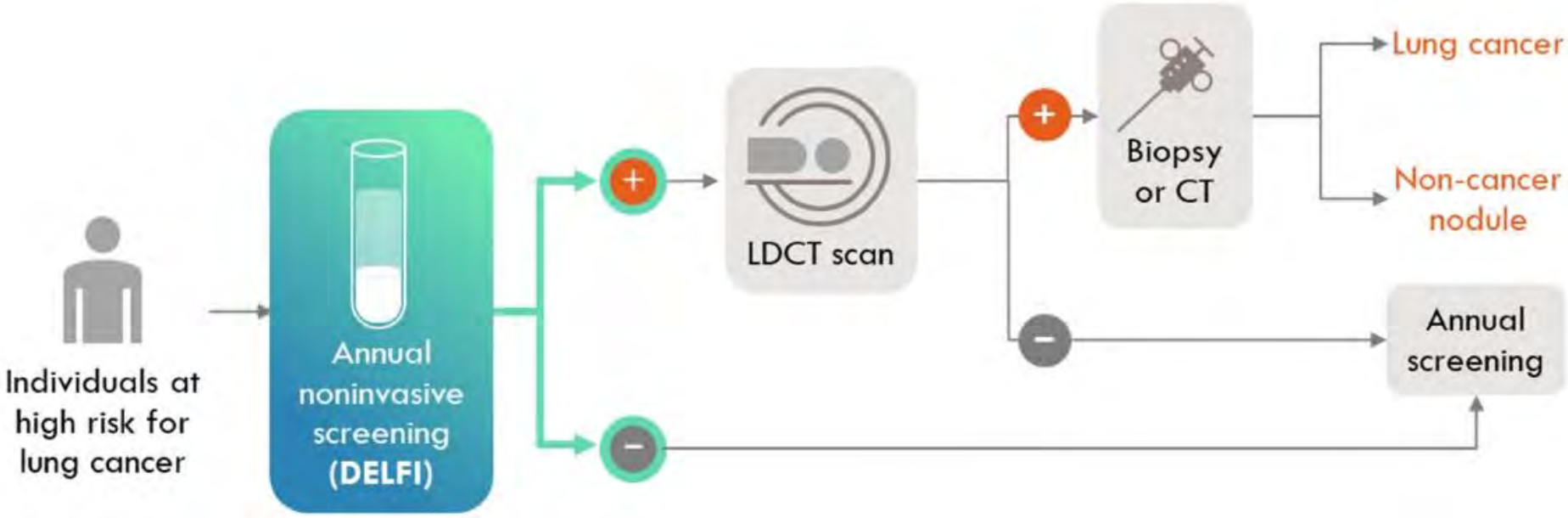


Clinically Useful Biomarker for Lung Cancer Screening

Group #1 Currently Screen Eligible

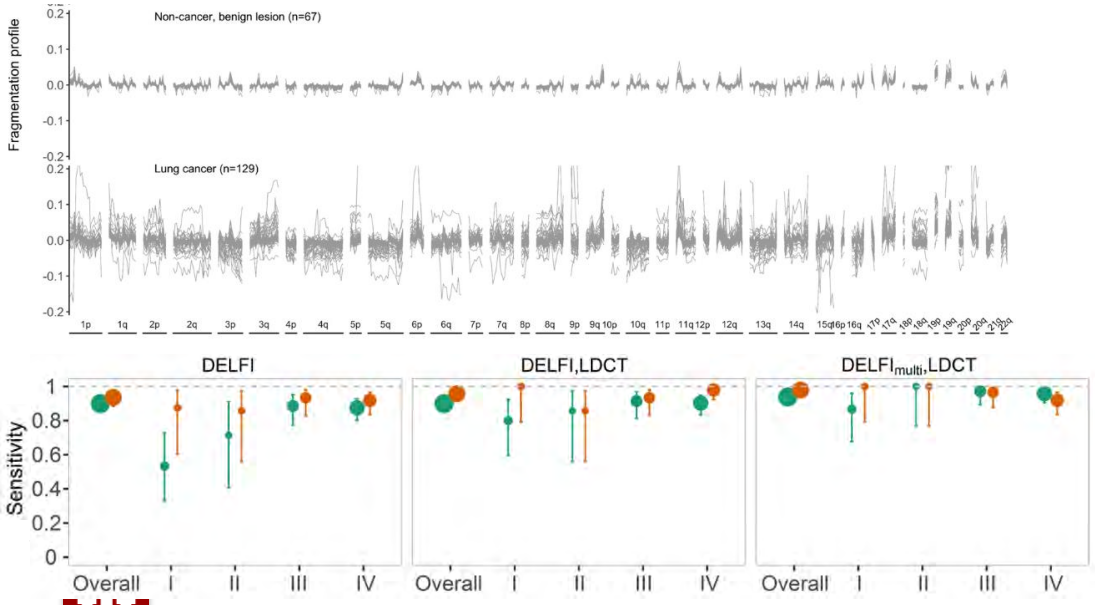
- Increase cost-effectiveness of LCS
- Patients with comorbidities: highest benefit
- Appropriate duration to follow-up LDCT (negative)
- Duration to LDCT f/u (positive LDCT)
- Increase LDCT uptake in those not getting screened (Low Resource or Disadvantaged Groups)
  - Rural/Geographic, Socioeconomically disadvantaged, *non-compliant*

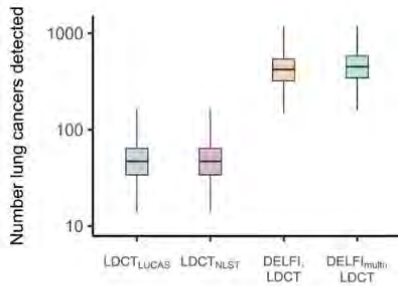
Sample Utility Schema

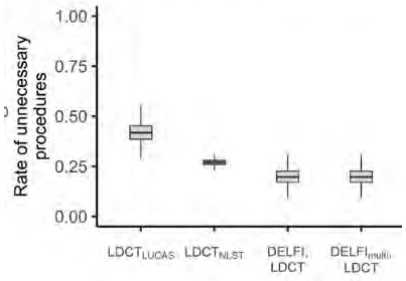


Combining Biomarkers with LCS Estimates and Risk Stratification Models

DELFI-LUNG







Mathios et al. Nature Communications. 2021

Lung Cancer Risk Assessment Models (Gold Standard?)

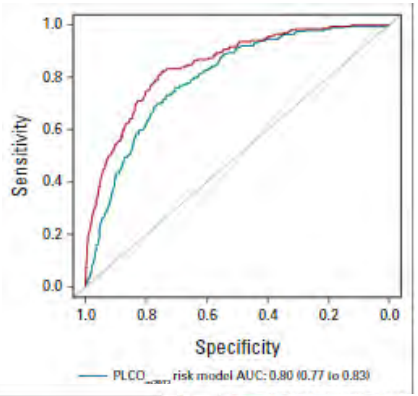
	PLCOm2012 *	Bach Model	LLP*	LCDRAT	Kovalchik Model	TSCE Models	Knote Model	Hunt Model
Source	PLCO	CARET	LLP	PLCO	PLCO	NHS, HPFS	CPS-I/II (ACS) +/- NHS	HUNT2
Factors	Age* Race/ethnicity BMI Education Prior CA Smoking* Family History COPD	Age Sex Smoking	Age* Sex* Prior CA Smoking*	Age Sex Race/ethnicity BMI Education Smoking FH Emphysema	Age BMI Smoking FH Emphysema	Age Sex Smoking	Age Race/ethnicity Smoking	Age BMI Smoking Daily cough

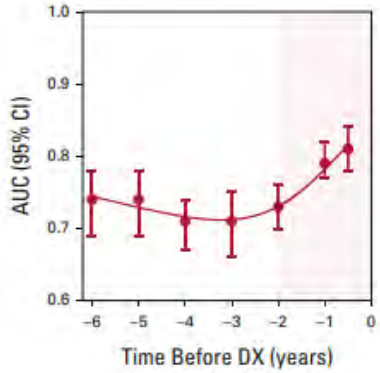
*Included in Simplified models

Lung Cancer Screening Biomarker Needs to be Better than Clinical Risk Factors

Combining Biomarkers with LCS Estimates and Risk Stratification Models

4MP + PLCO2012





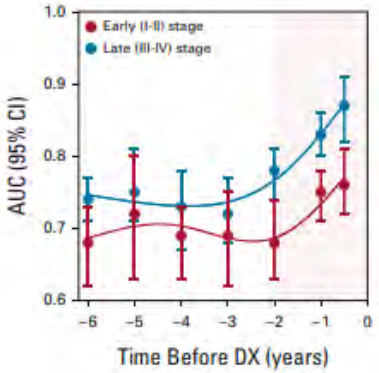


TABLE 2. Accuracy Performances in the Validation Set for the 4MP, $PLCO_{m2012}$, and the Combined Model of 4MP Plus $PLCO_{m2012}$ at Fixed Thresholds of > 1.7% and > 1% 6-Year Risk, to be Comparable With USPSTF2013 and USPSTF2021 Criteria in ESIA10+

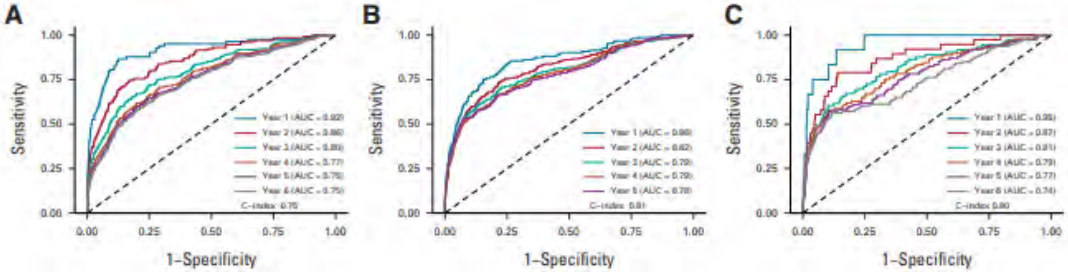
Criteria	N1[a]	N0	1-Year Sensitivity[b]	Specificity	1-Year TP[c]	FP[c]
> 1.7% risk threshold						
USPSTF2013[d]	119	32,243	0.716	0.564	85	14,061
4MP[e]	119	32,243	0.824	0.632	98	11,866
$PLCO_{m2012}$[f]	119	32,243	0.776	0.654	93	11,145
Combined 4MP + $PLCO_{m2012}$ model[g]	119	32,243	0.835	0.693	100	9,905
> 1.0% risk threshold						
USPSTF2021[d]	119	32,243	0.785	0.493	94	16,356
4MP[e]	119	32,243	0.915	0.454	109	17,591
$PLCO_{m2012}$[f]	119	32,243	0.920	0.466	110	17,224
Combined 4MP + $PLCO_{m2012}$ model[g]	119	32,243	0.884	0.562	105	14,122

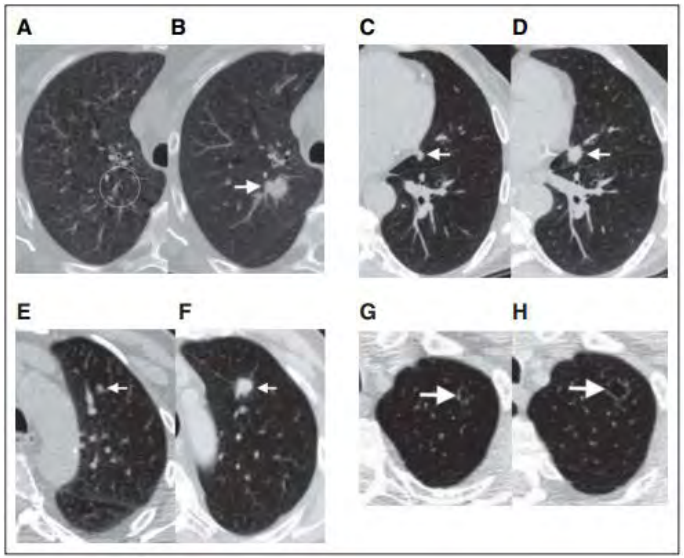
Fahrmann et al. J Clin Oncol. 2022

Trend: Lung Cancer Risk Prediction from a Single LDCT Image - Sybil

- NLST (15,000 participants)
  - Training set: 28,162 LDCTs
  - Development set: 6,839 LDCTs
  - Internal test set: 6,282 LDCTs

- Independent Testing Sets
  - MGH: 8,821 LDCTs (169 lung cancers)
  - CGMH: 12,280 LDCTs (101 lung cancers)





Mikhael PG et al. *JCO.* 2023

Clinically Useful Biomarker for Lung Cancer Screening

#2 Currently Screen Ineligible

Define High Risk Cohort who will Benefit from LCS

Refine/Combine with Clinical Risk Factors

~ 50% of lung cancer patients

- Radon Exposure
- Occupational Exposures (asbestos, chromium, coal smoke, diesel fumes, uranium, radiation, silica, soot)
- HIV+ on ART
- Lung diseases (COPD, pulmonary fibrosis)
- Family history of lung cancer (early, never-smokers)
- Prior cancer history (lymphoma, H&N cancer, smoking-related cancers)
- Heavy 2nd-hand smoke, biofuel, open stove exposure
- Populations at high risk for EGFR mutant lung cancer

Trend: Pan-Cancer Biomarkers Galleri (GRAIL)

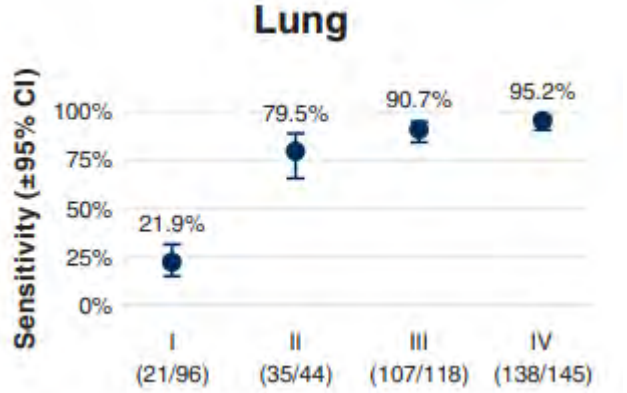
Targeted Genome Methylation Assay using cfDNA

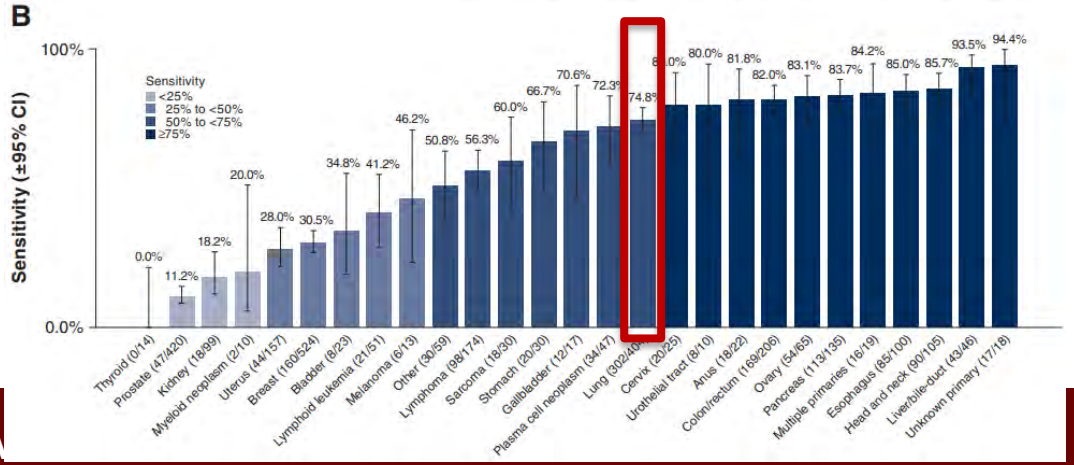
Prospective collection/retrospective analysis (CCGA)

- 15,254 patients (8584 cancer, 6670 non-cancer)
- 142 sites in N. America
- Clinical validation on 5309 participants

Sens: 51.5% (75% Lung Cancer)

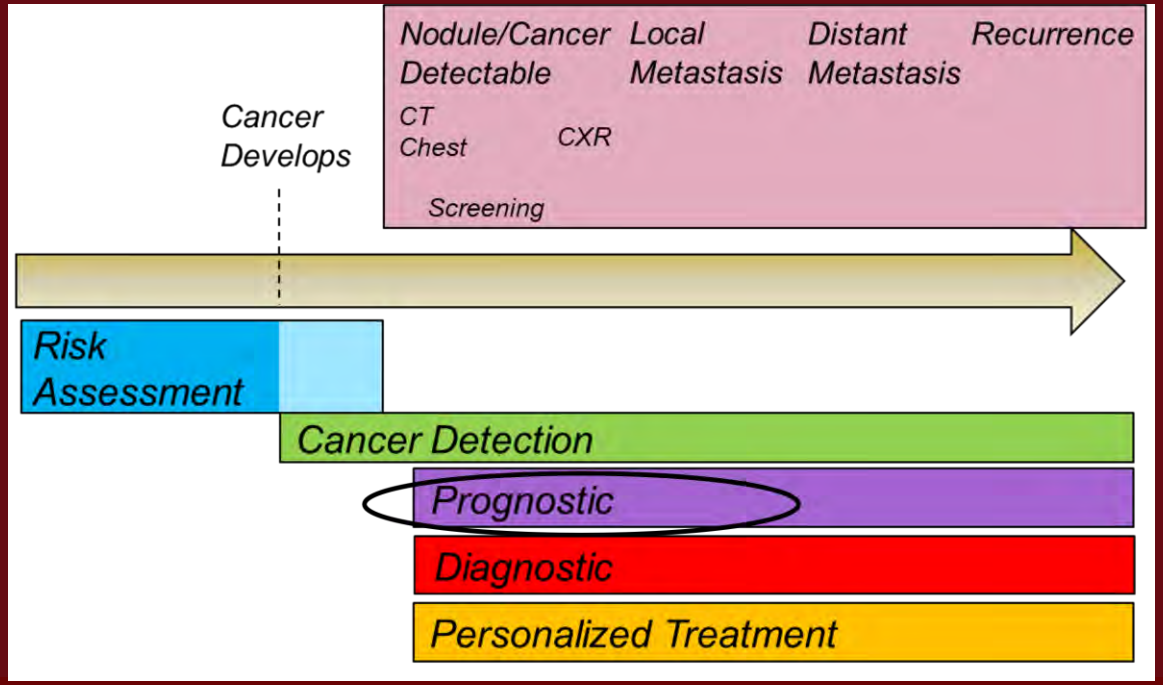
Spec: 99.5%



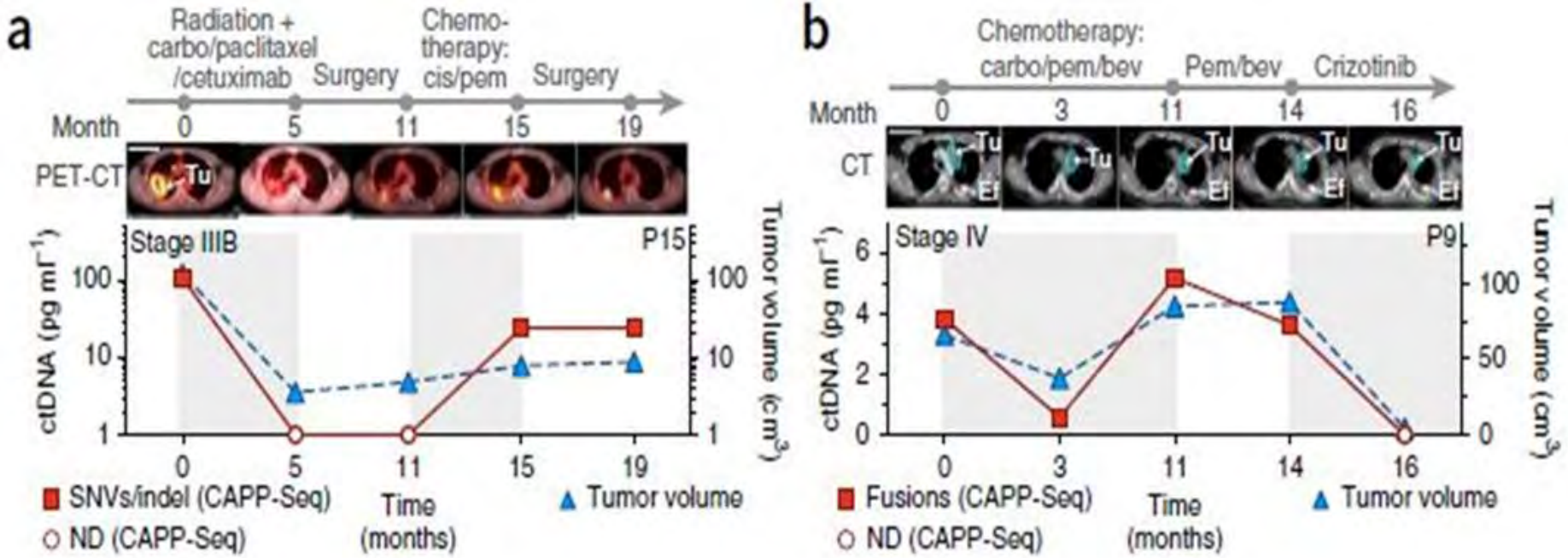


Klein et al. Annals Oncol. 2021;32(9):1167-1177.

Biomarkers for Lung Cancer Prognosis



Circulating Free Tumor DNA (cfDNA)

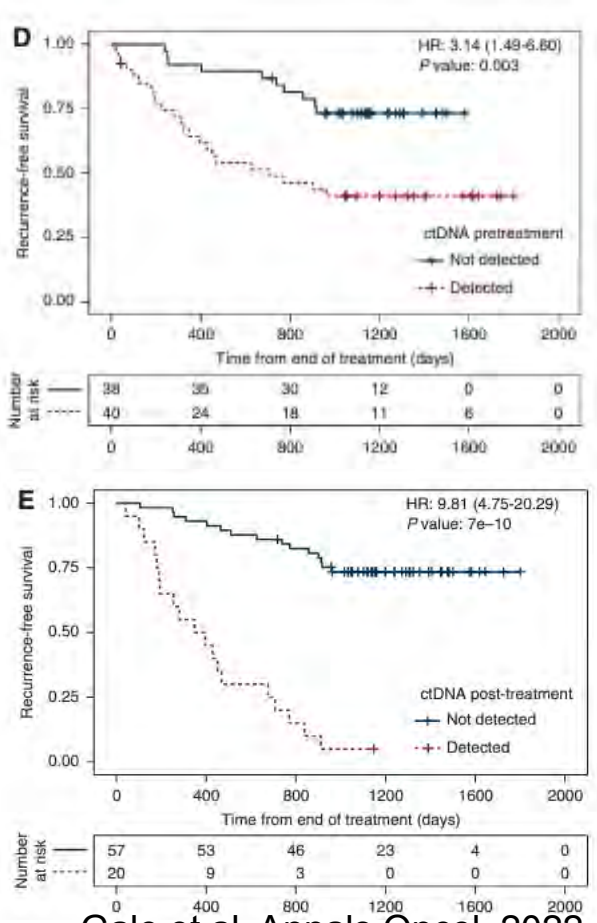


Newman et al. Nat Med. 2014;20:548-554

ctDNA detection for recurrence

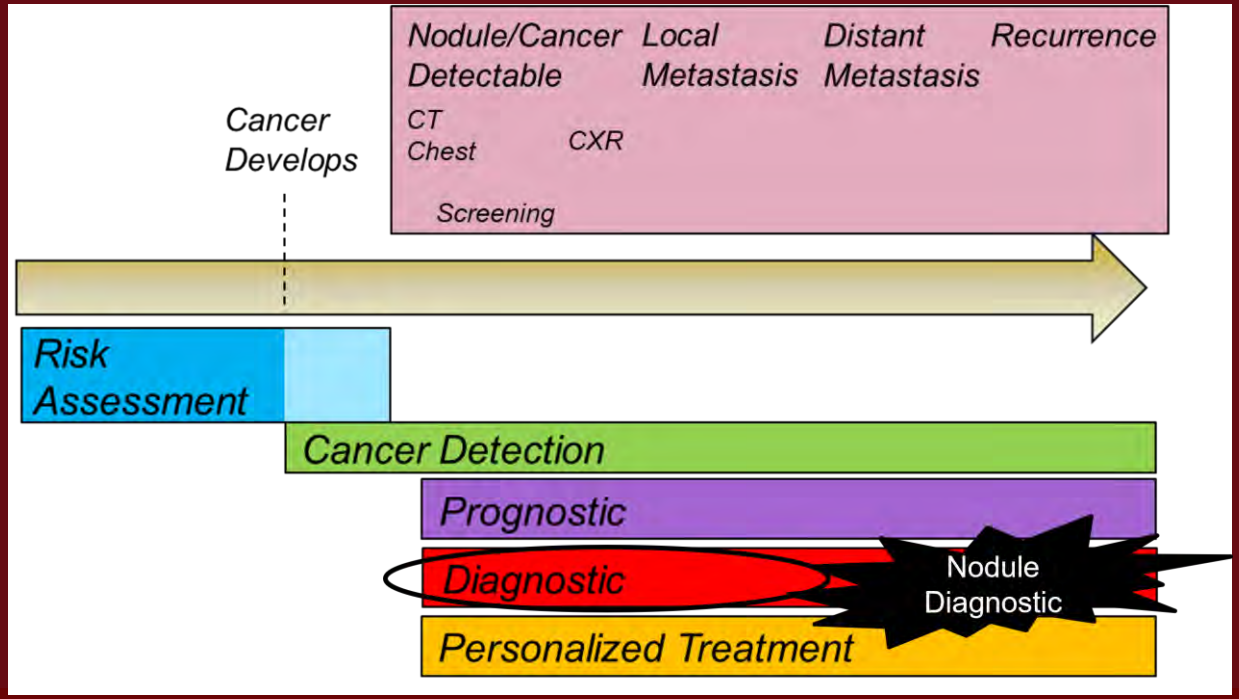
- 88 NSCLC patients
  - Longitudinal samples, 3 yrs f/u
- 17% ctDNA 2wks-4mo post-tx
  - Detected 64% clinical recurrence
  - Spec >98.5%
  - Detected median 213 days before clinical recurrence

May predict residual disease post-treatment
? helpful biomarker to select for adjuvant tx/clinical trials



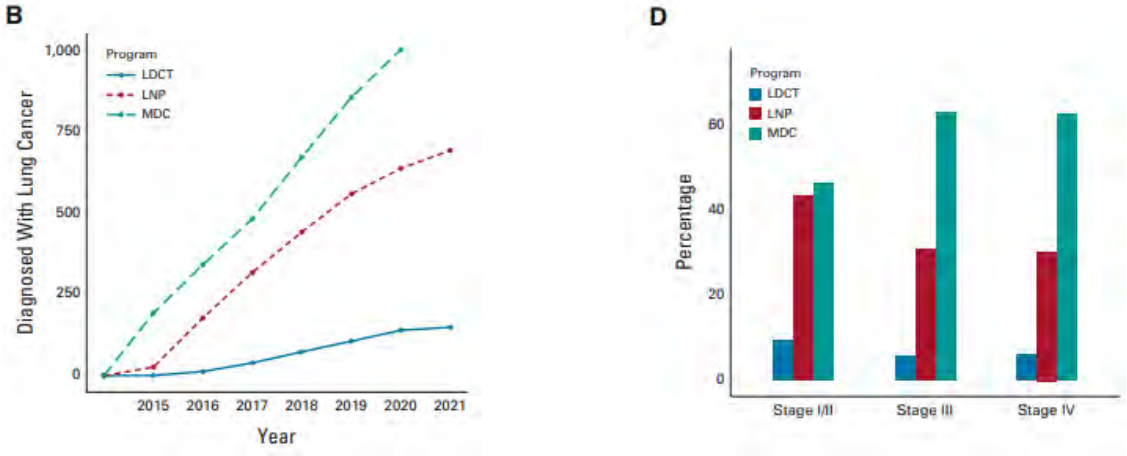
Gale et al. Annals Oncol. 2022

Biomarkers for Nodule Risk Assessment



Problem… Pulmonary Nodules are Common

>1,600,000 incidental lung nodules/year…. And growing!



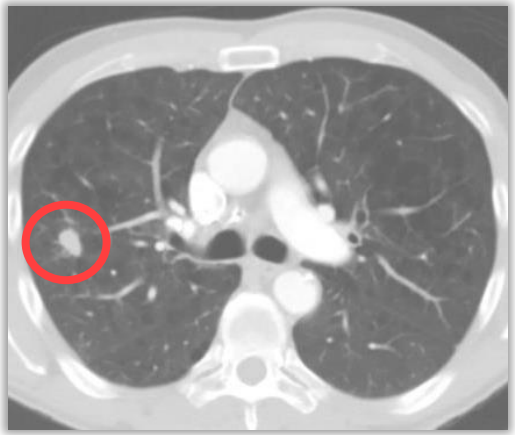
Most (Early) Lung Cancers are Detected Incidentally!

Gould et al. Am J Resp Crit Care Med, 2015;192(10)
Smith-Bindman et al. JAMA 2019;322(9):843-859

Osarogiagbon et al. J Clin Oncol 2022;40:2094-2105.

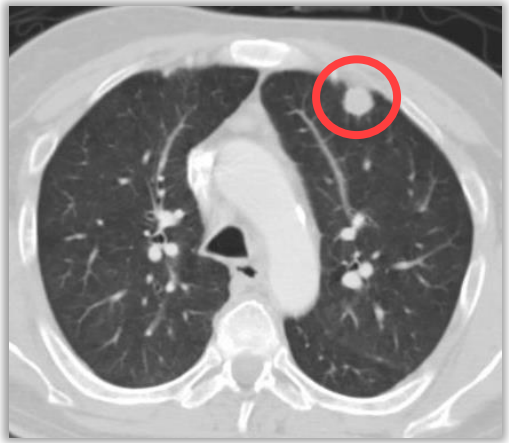
Pulmonary Nodule Diagnostic Biomarkers

62-Year-Old Male Smoker



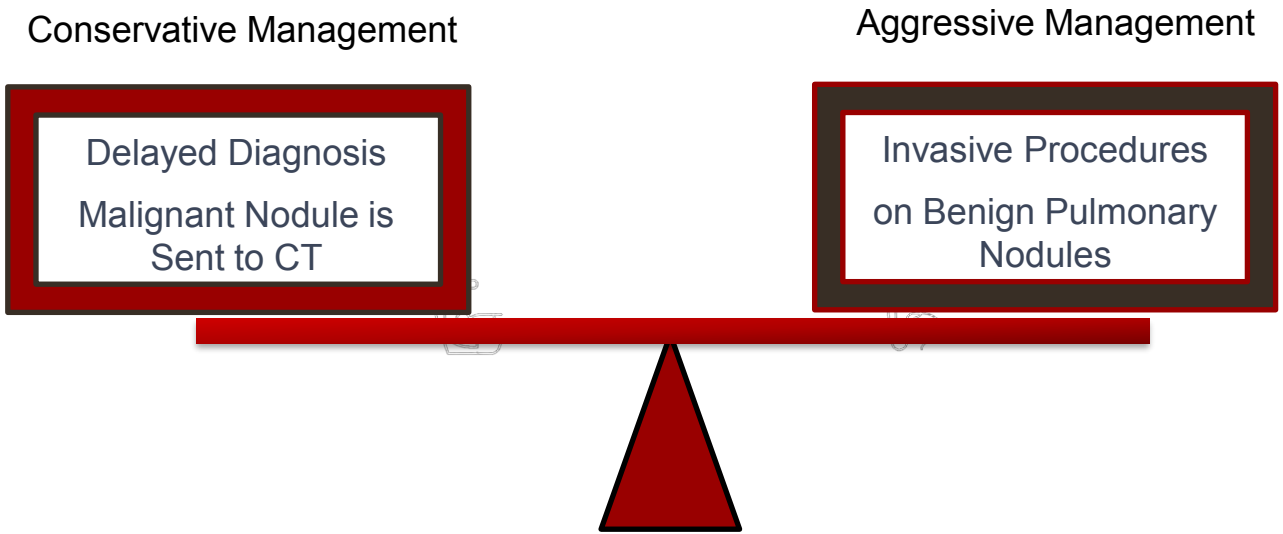
LUNG CANCER

54-Year-Old Male Smoker

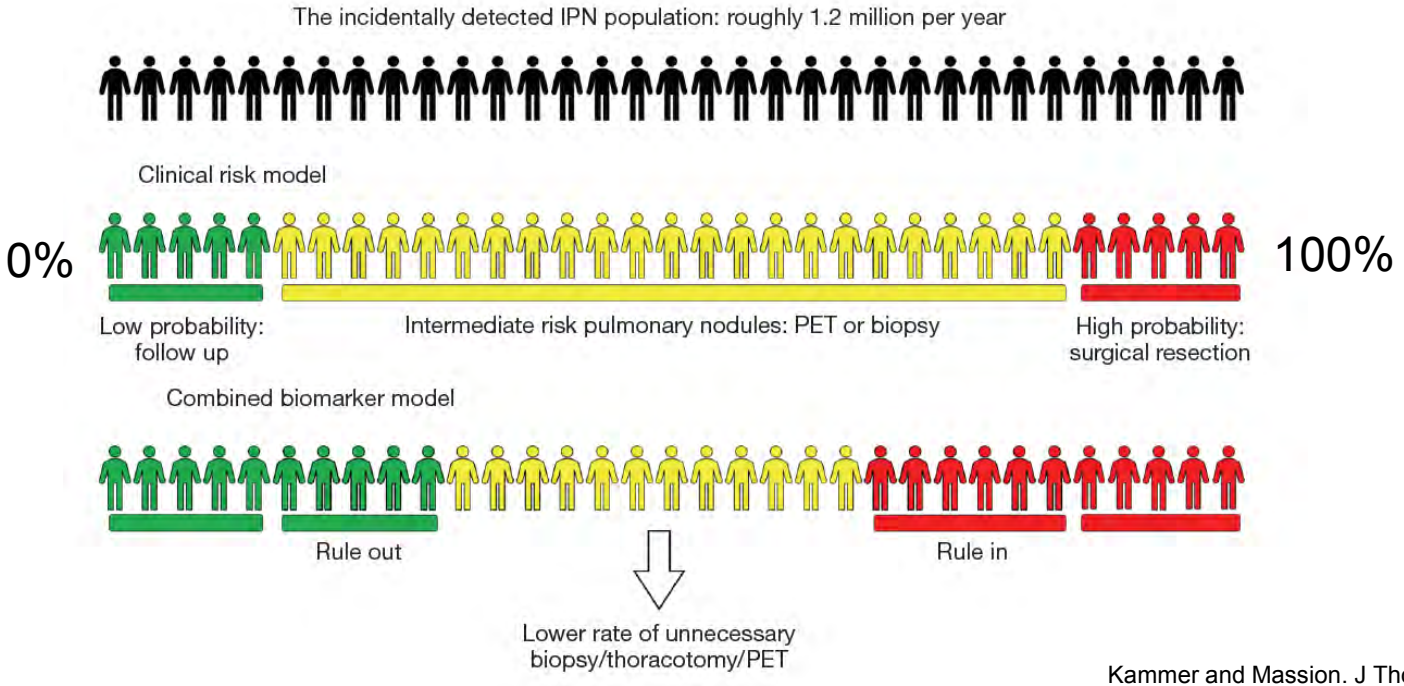


HISTOPLASMOSIS

The Lung Nodule Biomarker: Goal



Problem: Intermediate Risk Nodules



Kammer and Massion. J Thorac Dis. 2020

Nodule Management Biomarkers

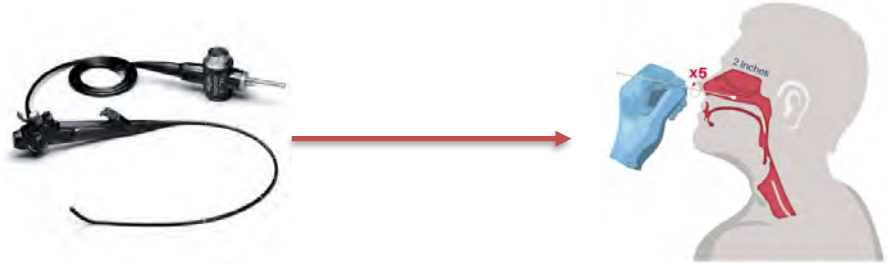
	Measurement	Validation Cohort	Sens/Spec	Proposed Use	Availability/ Clinical Utility
Nodify CDT (Biodesix)	Blood auto-antibody panel: ELISA	Patients: 1613 Cancers: 61	Sens: 37% Spec: 91%	**Nodule management** (intermed. risk) • Positive = aggressive management	Yes / No clinical utility trial
Nodify XL2 (Biodesix)	Plasma Protein: MRM Mass Spectrometry + 5 Clinical characteristics (Mayo)	PANOPTIC: Patients: 392 (178*) Cancers: 29	Sens 97% Spec: 44%	**Nodule management** (low-intermed risk) Pretest Probability Cancer < 50% • Negative = radiologic surveillance	Yes/**Clinical utility trials initiated** (ALTITUDE) NCT04171492
Percepta GSC (Veracyte)	Bronchial epithelial cells: mRNA/gene expression profile	AEGIS-1/AEGIS-2 Patients: 639 Cancers: 487	Sens: 88% Spec: 47%	**Nodules** (intermed risk) undergoing bronchoscopy • Negative = radiologic surveillance	Yes / Clinical Utility extrapolated
Percepta Nasal Swab (Veracyte)	Nasal epithelial cells: mRNA/gene expression + clinical risk factors	AEGIS-2/Lahey Patients: 249 Cancers: 134	High -> Low Risk: Sens: 58 -> 96% Spec: 96 -> 42%	**Nodules** (intermediate risk) • Negative = radiologic surveillance	Yes/ No clinical utility trial
DELFI-LUNG (Delfi Diagnostics, Inc)	Blood: cfDNA fragmentation pattern	Enrolling prospective study- 15,000 LCS pt	Varies based on multiple analytic cohorts	Enrichment of high-risk **screening** cohort / symptomatic lung cancer / **Rule-in nodule biomarker**	No / NCT05306288 (CASCADE-LUNG for LCS) / NCT04825834 (DELFI-L101 for Nodule Clin validation)

Large clinical validation/registry studies ongoing - LungLB (LungLifeAI), DELFI, DetermaDx Lung, CyPath Lung (bioAffinity), 4-MP, Radiomics and many multi-cancer platforms

Withdrawn From the Market: Paula's test (for nodule diagnosis), MagArray

Sears, Mazzone. Clin Chest Med. 2020. Trivedi et al. Biomed Research Clin Practice. 2018. Kossenkov et al. Cancer Res. 2019. Ostrin et al. J Thorac Oncol. 2021. Lamb et al. CHEST. 2023

Trend = Less Invasive Percepta Nasal



1st Gen (AEGISI/II,Lahey): Bronchial airway brush, mRNA

Non-dx bronchoscopy; Low-intermediate risk nodule "Ru

Validation cohort: 1129 (487 malignant): Sens 88%, S

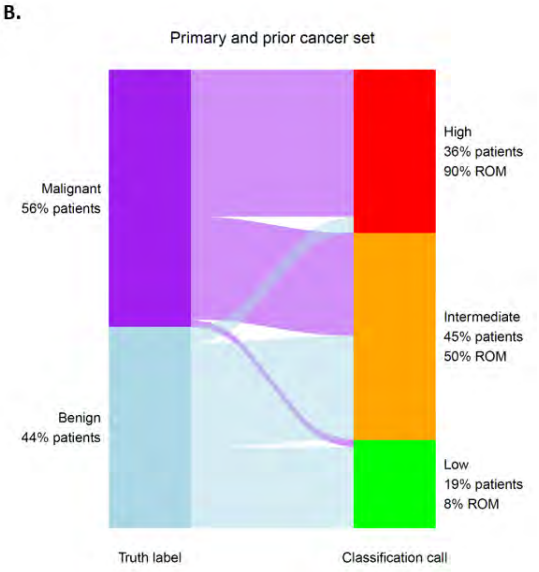
Registry=34% down-classified, 78% had change in pract

2nd Gen: Nasal epithelial swab, mRNA gene panel (1120 t

Validation cohort: 249 samples (134 lung CA), cigarette s

Low risk: Sens 96% (spec 42%); High risk: Spec 90% (se

- *included a cohort w/ prior cancers (non-lung)*

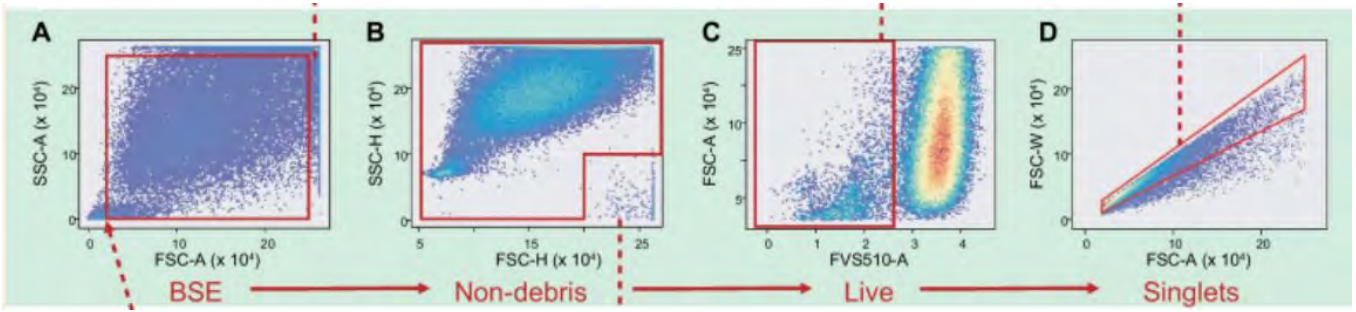


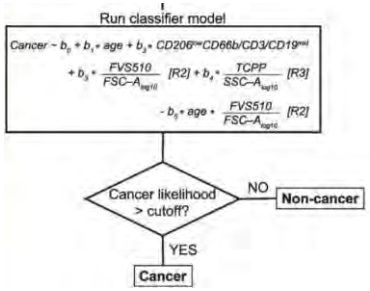
Silvestri et al. NEJM 2015; 373:243-251.
[2]Lee et al. CHEST. 2021; 159(1):401-412 3Lamb et al. CHEST. 2023.

Trend – Less Invasive CyPath Lung (BioAffinity)

Redeeming Sputum?

- Single cell suspensions from induced sputum x 3 days
- Automated flow cytometry -> Porphyrin labeled cancer-associated cells
- Prospective collection
  - Model validation (LSRII): 150 high-risk -> 28 lung cancer
  - Independent validation (Navios-EX): 32 high-risk -> 6 lung cancer
    - Unable to perform: 13/45 (29%);Technical: 5, Insuff. cells: 7, Insuff Macs:1





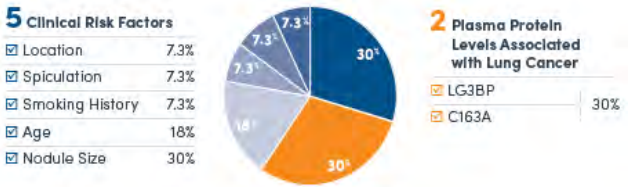
- 82-83% Sens. 77-87% Sens. 95-96% NPV. 45-61% PPV.

Lemieux et al. Resp Res. 2023.

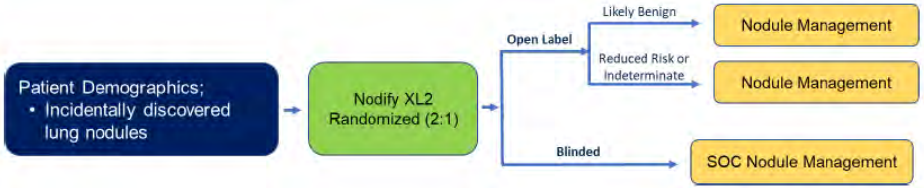
Trend: Confirming Estimated Clinical Utility
Nodify XL2 - ALTITUDE Study

Low-intermediate risk incidental nodule
"Rule-Out" Biomarker, blood, MRM proteomics

Nodify XL2 – Biomarker + Clinical Risk

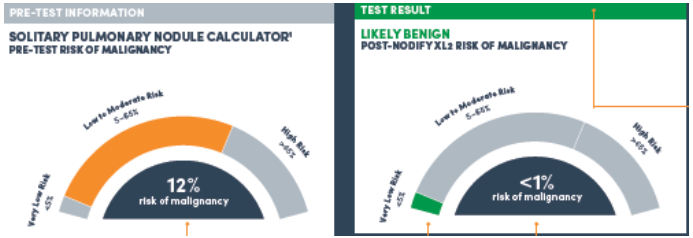


ALTITUDE – Clinical Utility Study



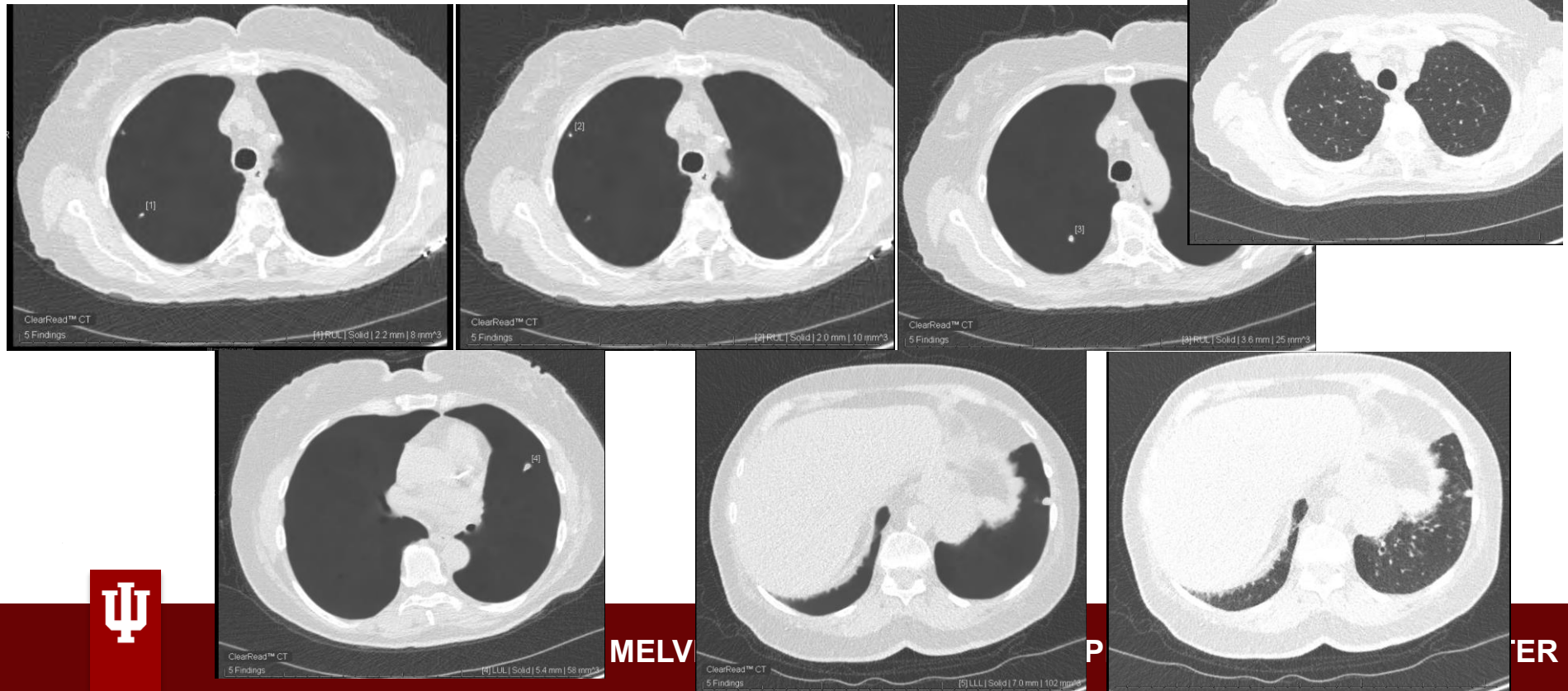
Results of Clinical Validation Study (PANOPTIC)

- Sensitivity: 97%. NPV 98%.
- Anticipated 47% fewer procedures on benign nodules (reclassified < 5% risk)

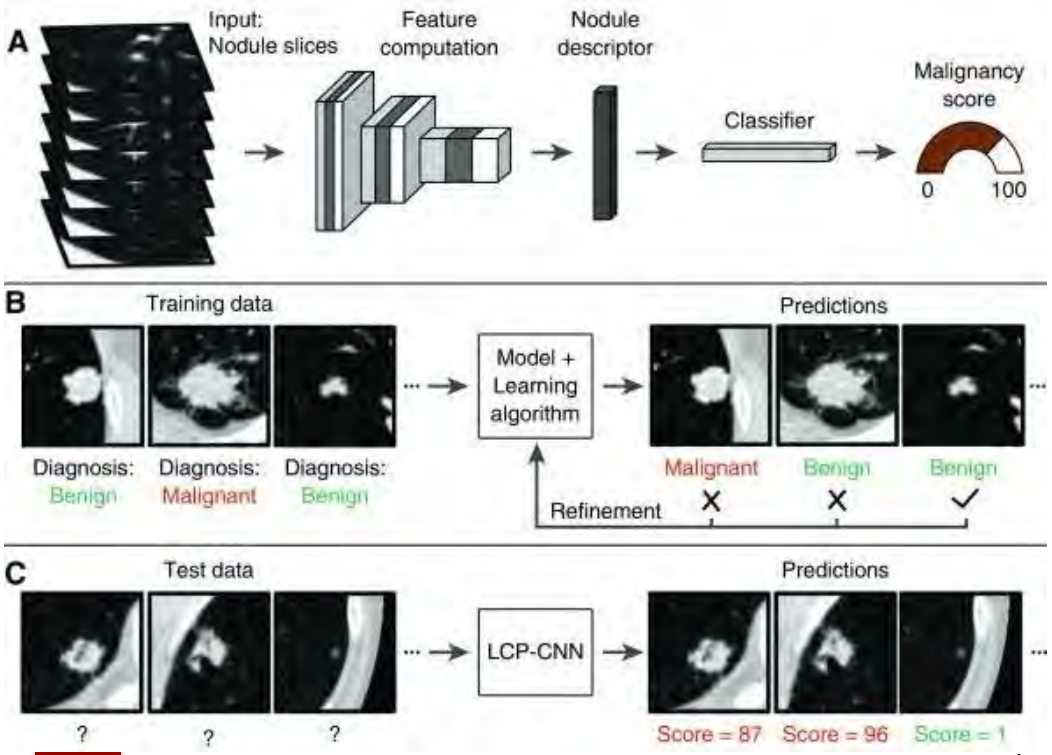


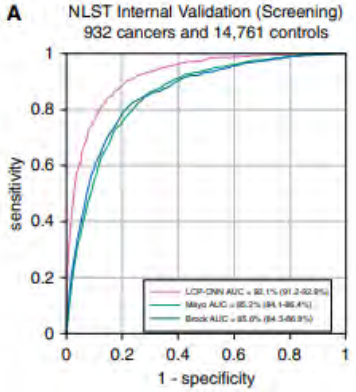
Silvestri et al. Chest 2018;154(3):491-500.

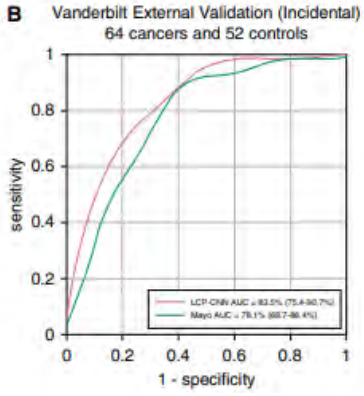
Trend: AI in practice -> Radiomics for Nodule Risk Prediction

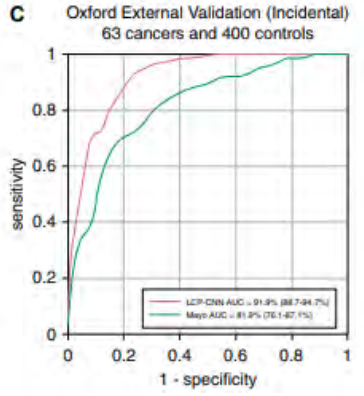


Deep Learning Modeling - LCP-CNN for Nodule Risk Assessment



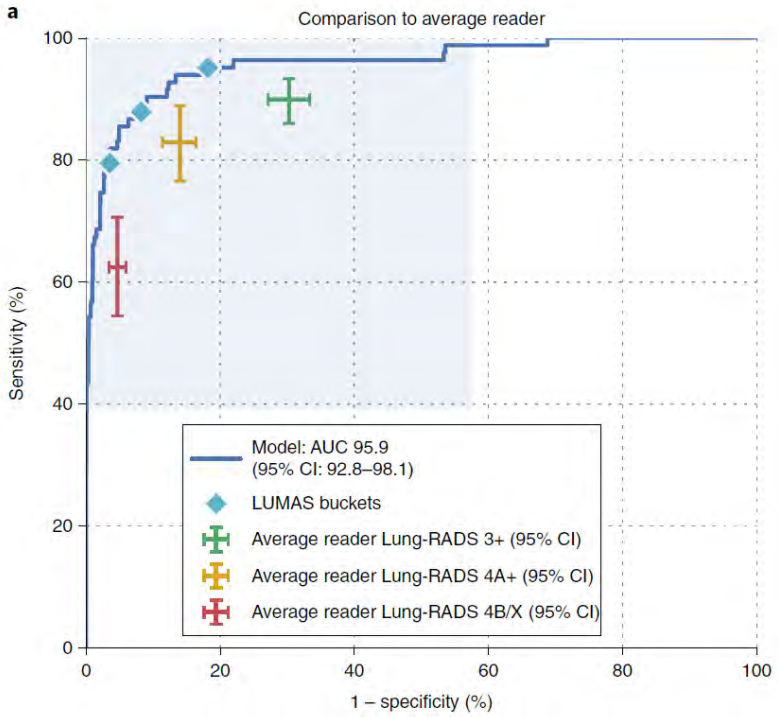


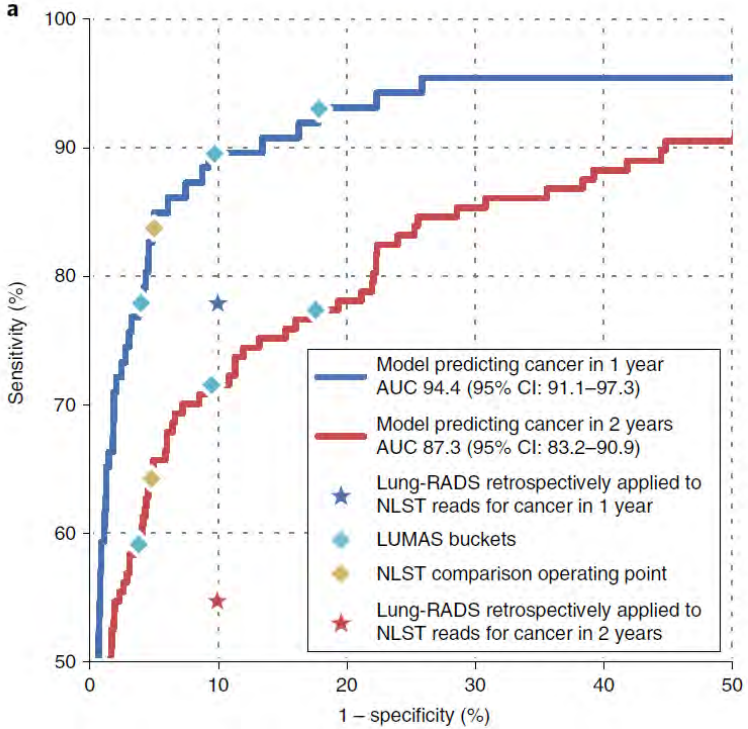




Massion et al. Am J Resp Crit Care Med. 2020;202(2):241-249

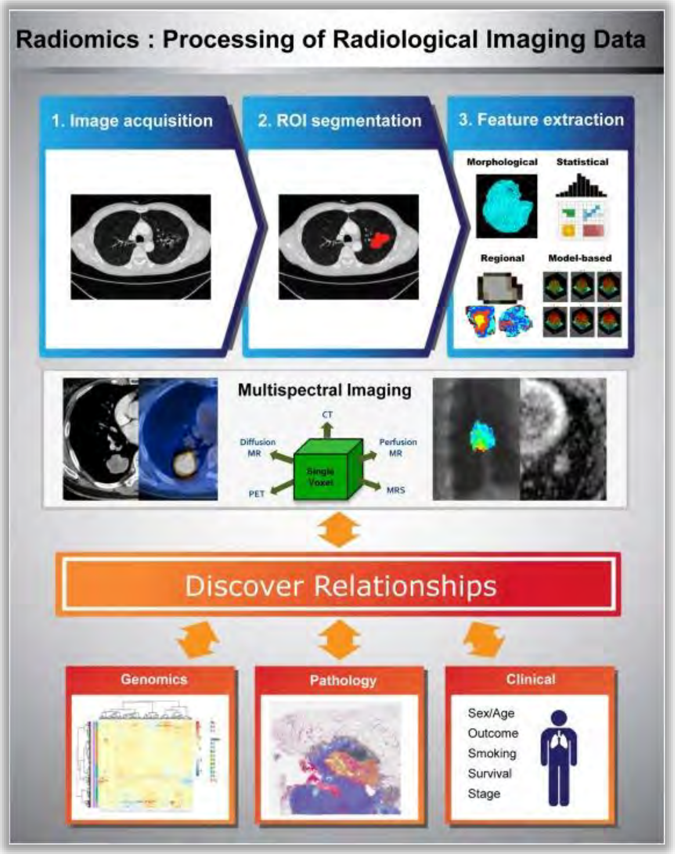
LCS: Radiomics vs Radiologists: LUMAS





Ardila et al. Nature Medicine. 2019

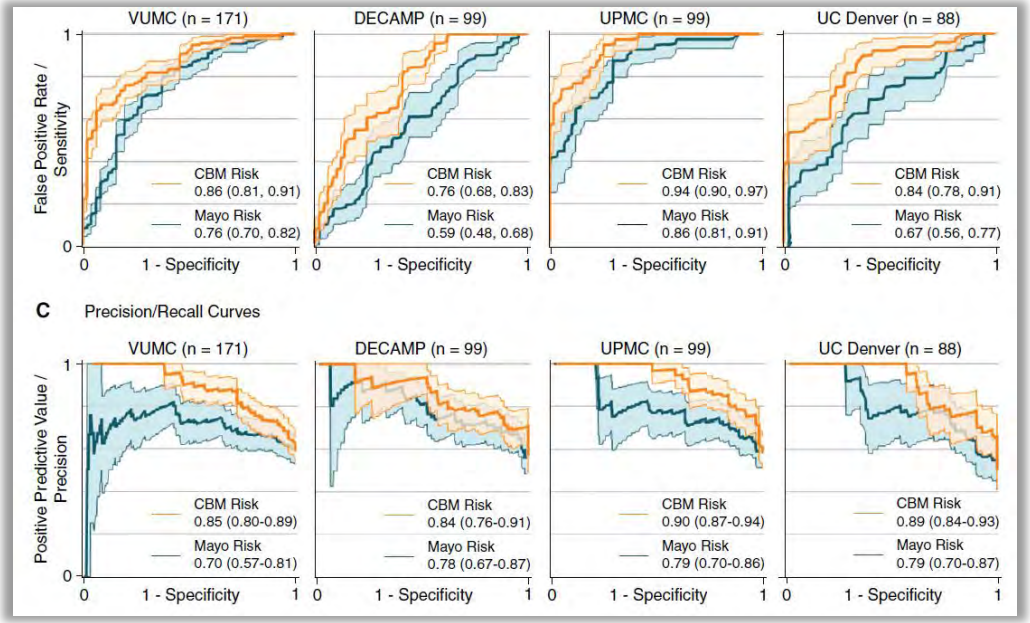
Trend: Combining radiomics and biomarkers



Lee et al. European J Radiol. 2017;86:297-307

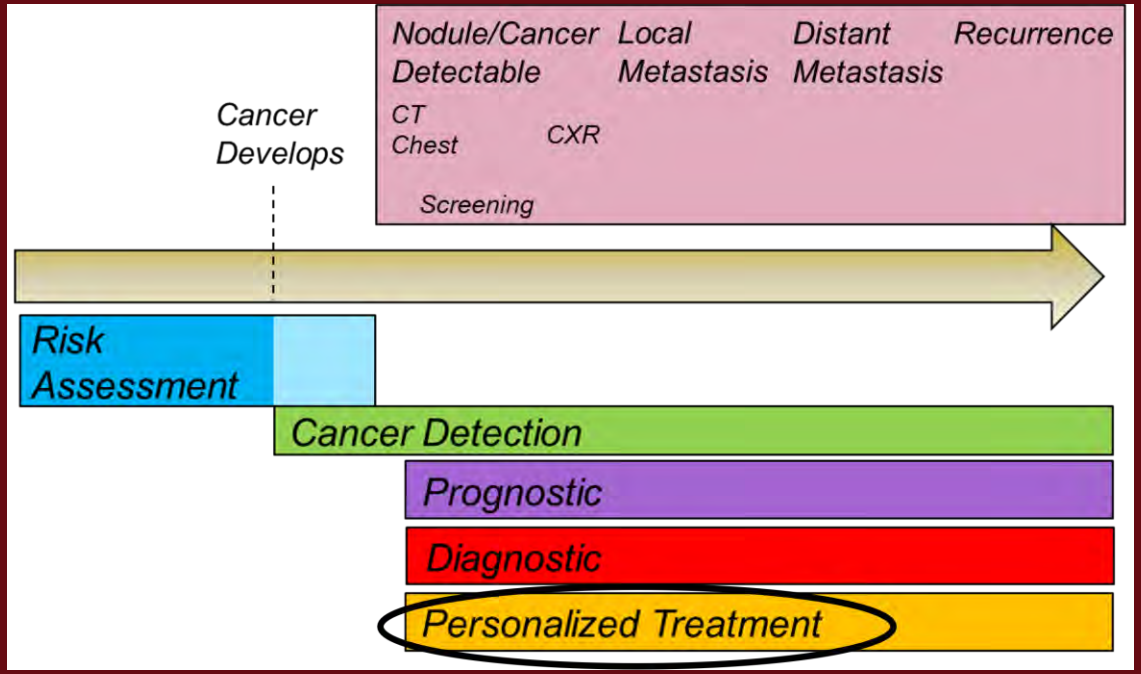
Trend: Combining Modalities for Nodule Diagnosis Combined Blood, Imaging, Clinical Biomarkers (CBM)

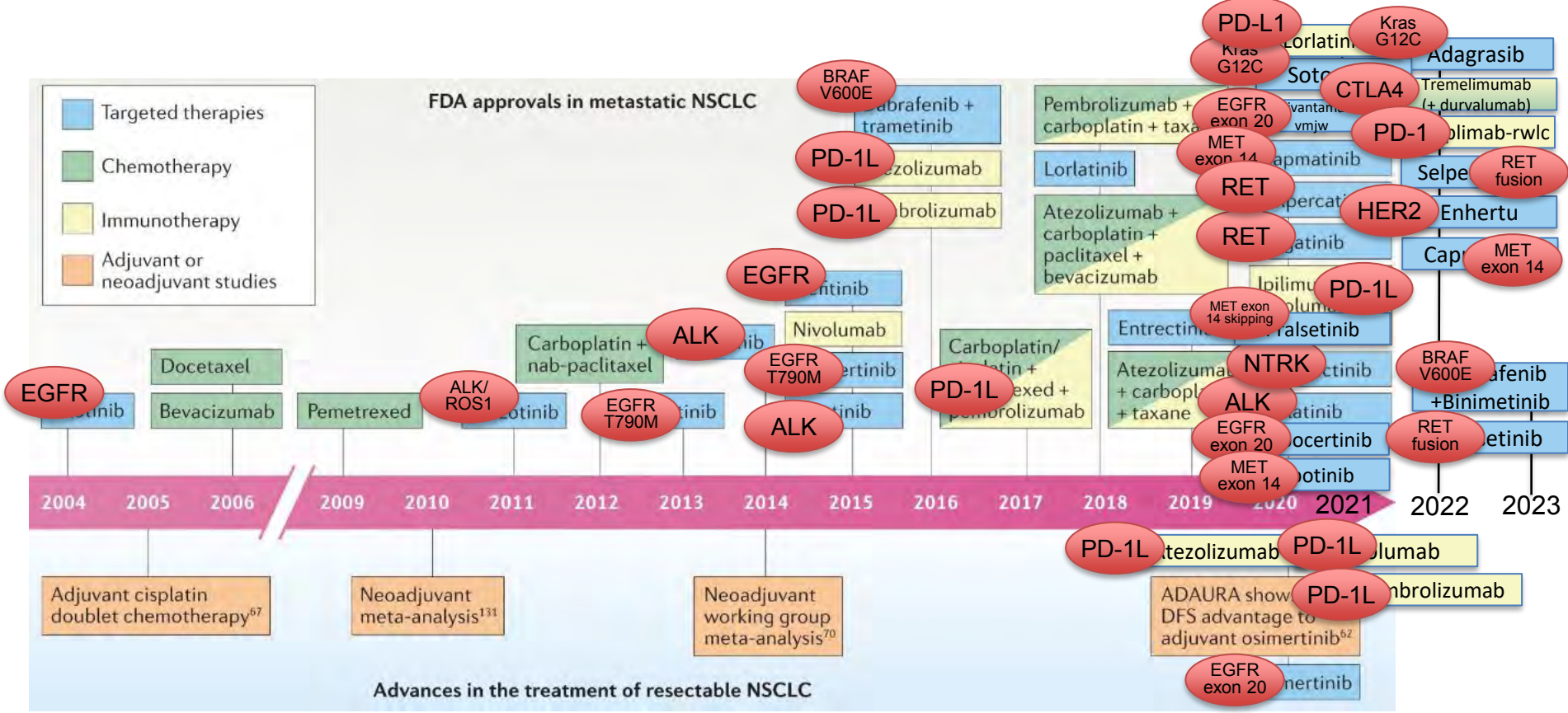
Blood (CYFRA 21-1) + Risk Module (Mayo) + Radiomics (nodule machine learning)



Kammer et al. Am J Respir Crit Care Med. 2021.

Biomarkers for Personalized Treatment





Chaft et al. Nature Rev Clinical Onc. 2021

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Table 3: Recent representative studies using deep learning to predict gene status in lung cancer patients on CT images.

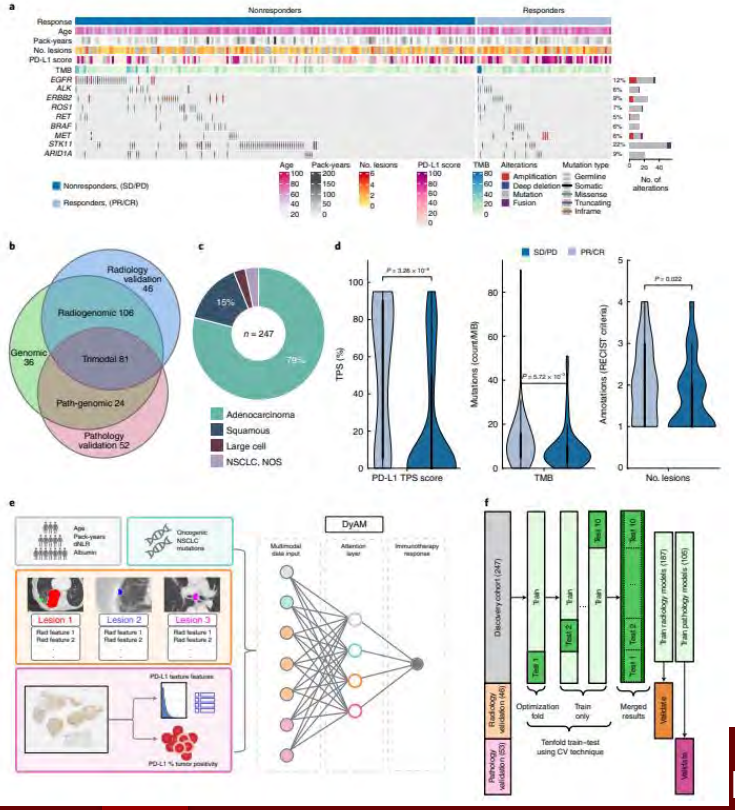
Author	Year	Design	Dataset	Training cohort	Validation cohort	Test cohort	Model	Outcome	Performance reported
Baihua Zhang	2021	Retrospective multicenter on CT	914 LUAD	638	NA	71 internal; 205 external	SE-CNN + radiomics mapping	EGFR mutation	AUC 0.910 and 0.841 in internal and external test cohorts, respectively
Wei Mu	2020	Retrospective multicenter on PET/CT	681 NSCLCs	429	187	65 external	CNN	EGFR mutation treatment response	AUC 0.86, 0.83, and 0.81 in the training, internal validation, and external test cohorts, respectively
Shuo Wang	2019	Retrospective multicenter on CT	844 LUAD	603	Five-fold cross validation; 241 independent	NA	CNN	EGFR mutation	AUC 0.85 in the primary cohort; AUC 0.81 in the independent validation cohort
Wei Zhao	2019	Retrospective multicenter on CT	616 LUAD	348	116	115 internal; 37 public	CNN 3D DenseNets	EGFR mutation	AUC 0.758 and 0.750 in the internal test set and public test set
Junfeng Xiong	2018	Retrospective single-center on CT	503 LUAD	345	158	NA	CNN	EGFR mutation	An AUC (CNN) of 0.776 and an AUC (a fusion model of CNNs and clinical features) of 0.838 in the validation set
Panwen Tian	2021	Retrospective multicenter on CT	939 NSCLCs	750	93	96	KNN	PD-L1 expression treatment response	AUC 0.78, 0.71, and 0.76 in the training, validation, and test cohorts
Ying Zhu	2020	Retrospective single-center on CT	127 LUAD	NA	Five-fold cross validation	NA	CNN 3D DenseNets	PD-L1 expression	AUC more than 0.750
Zhengbo Song	2020	Retrospective multicenter on CT	1028 NSCLCs	651	286	91	CNN 3D ResNet10	ALK fusion status Treatment response	AUC(CNN) 0.8046 and 0.7754 in the primary and validation cohorts, AUC (trained by both CT images and clinicopathological information) 0.8540 and 0.8481 in the primary and validation cohorts

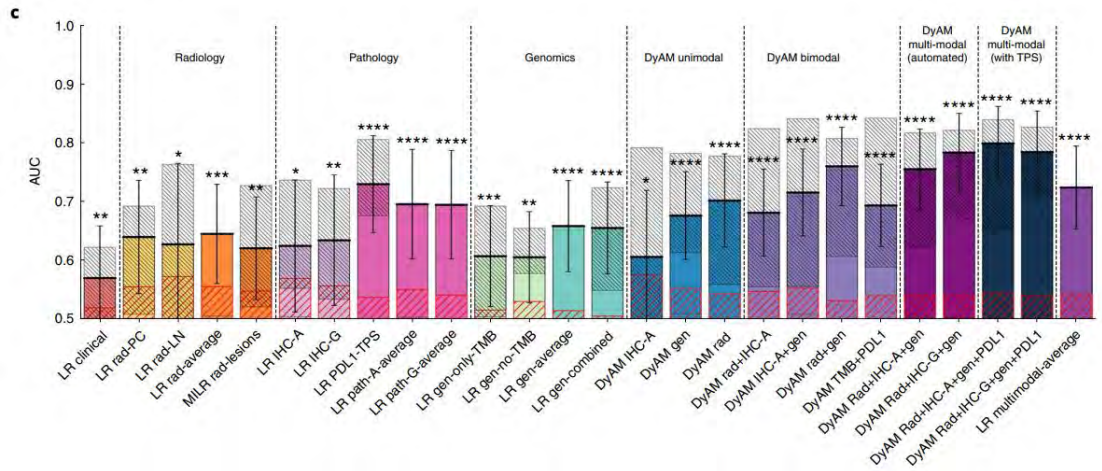
LUAD: lung adenocarcinoma; NSCLC: non-small-cell lung cancer; CNN: convolutional neural network; KNN: k-nearest neighbor; NA: not applicable.

Wang C et al. J Oncol. 2024

Kim, S et al. Sci Rep. 2024

Trend – Combining Tumor Characteristics with Radiomics - Response to ICI (DyAM)





- Training cohort (247), Radiology validation (46), Pathology validation (53)

Vanguri RS et al. *Nat cancer.* 2024

Conclusions

- Rapid expansion of biomarkers in conjunction with imaging
  - Lung cancer screening/Earlier lung cancer diagnosis
  - Nodule risk assessment
- Increasing biomarker availability*
- Trends in early lung cancer diagnosis
  - Less invasive
  - Multi-omics (not cancer specific)
  - Prediction of utility
  - Clinical utility studies to determine if estimates predict usefulness in practice
  - Radiomics
  - Combining clinical, radiologic and biomarker characteristics to improve performance and predict response to therapy