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Predicting Invasiveness in Early Stage Lung Cancer

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Predicting Invasiveness in Early Stage Lung Cancer

Julie (Linek) Barta, MD

November 19, 2014

Division of Pulmonary, Critical Care, and Sleep Medicine
Icahn School of Medicine at Mount Sinai, New York

Outline

- Clinical case
- Invasion and metastasis
- Biomarkers of invasion
  - Biomarker validation
  - High-throughput approaches to biomarker discovery
- Clinical approaches
  - Role of FNA in the diagnosis of lung cancer
- Future directions
  - Important tumor biology and clinical questions

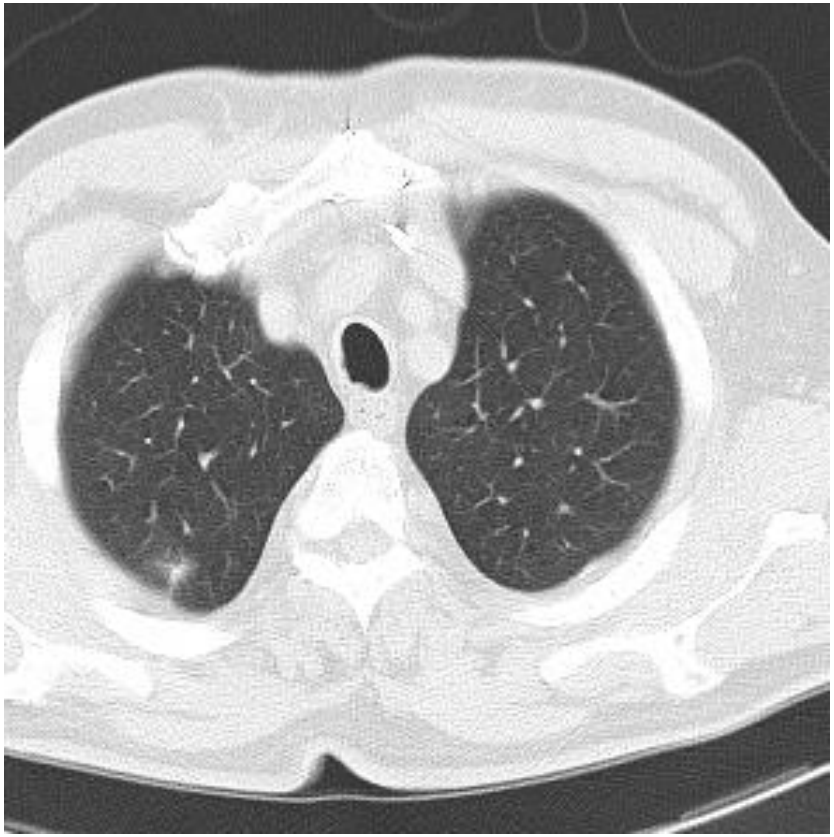
Clinical case

- A 73 year-old man presents with a RUL nodule since 2010, when he first presented with a cough.
- PMHx: COPD, peripheral vascular disease, obesity (BMI=30.4)
- Smoking history: Former smoker, 1 pack per day for 40 years, quit 13 years ago
- PFTs: Mild obstruction (FEV1= 1.9 L, 66% predicted)

CT chest

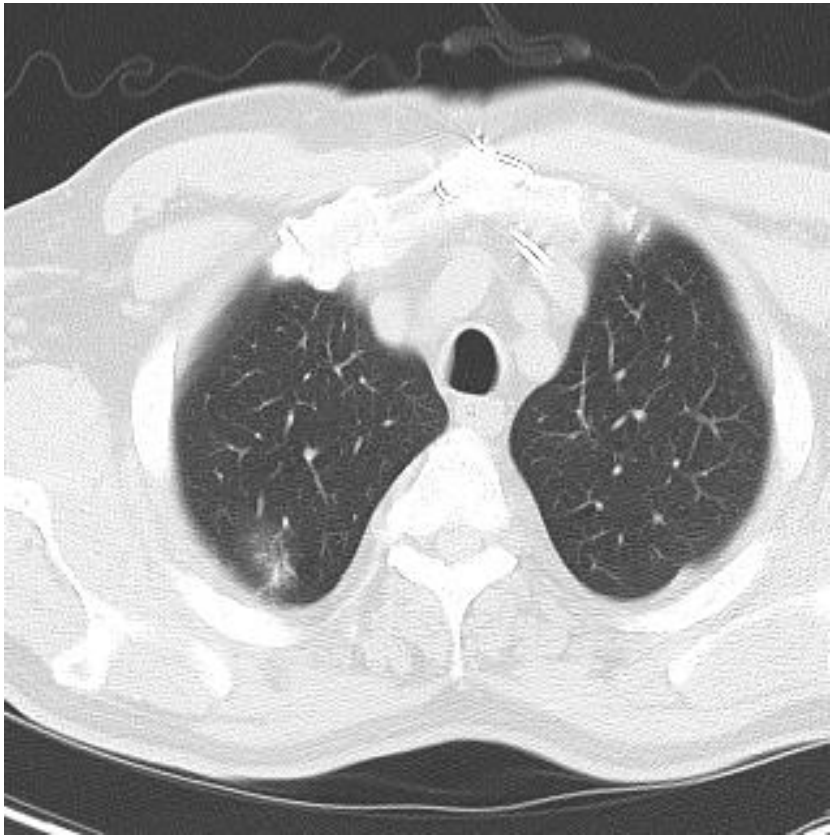
September 2010

8mm mixed partly ground glass/ partly solid nodule



July 2014

Increase in solid component to 7mm, with peripheral ground glass component now measuring 1.3cm



FNA

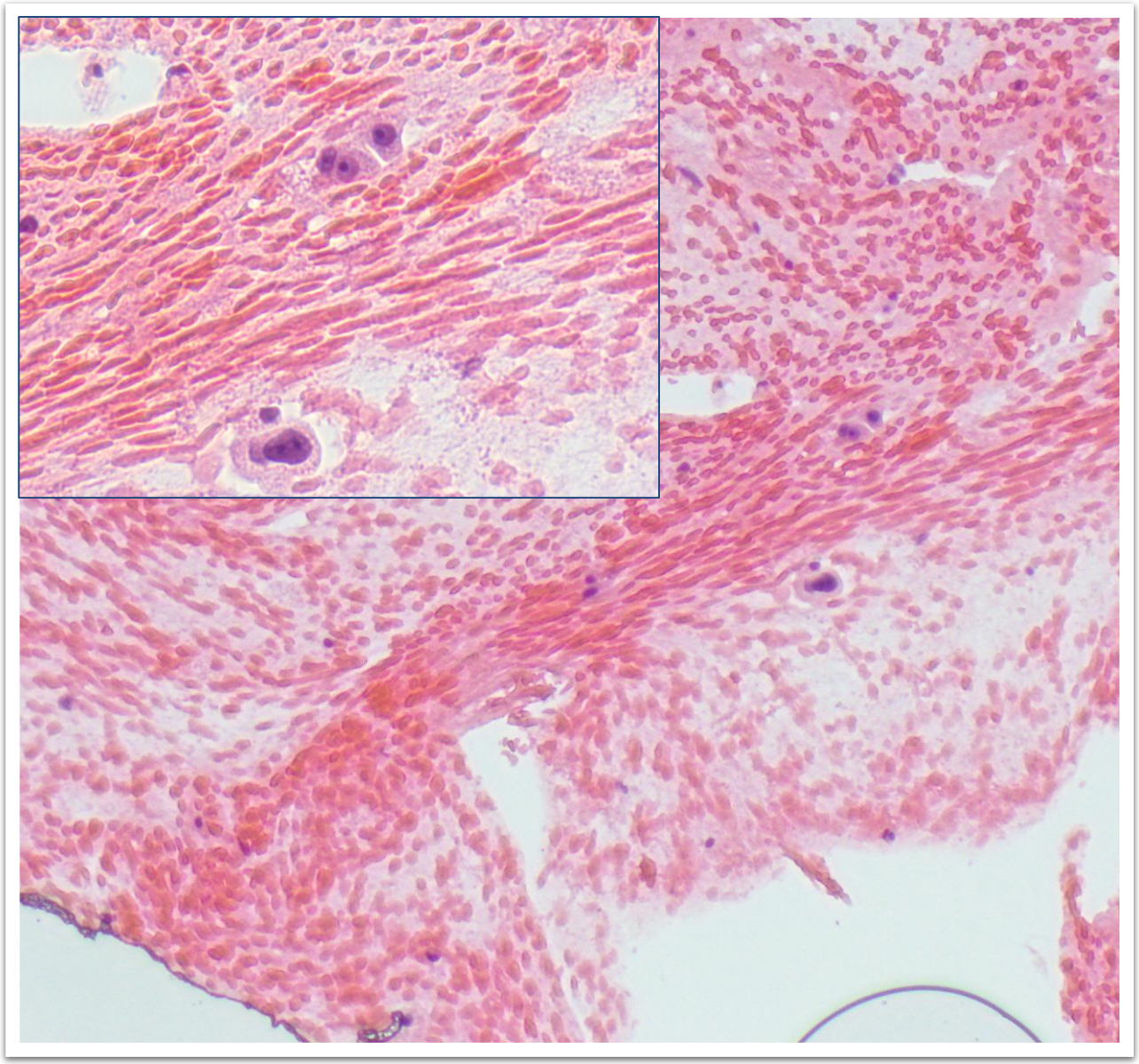
July 2014

Non-small cell carcinoma, favor adenocarcinoma

CK7 positive

CK20 negative

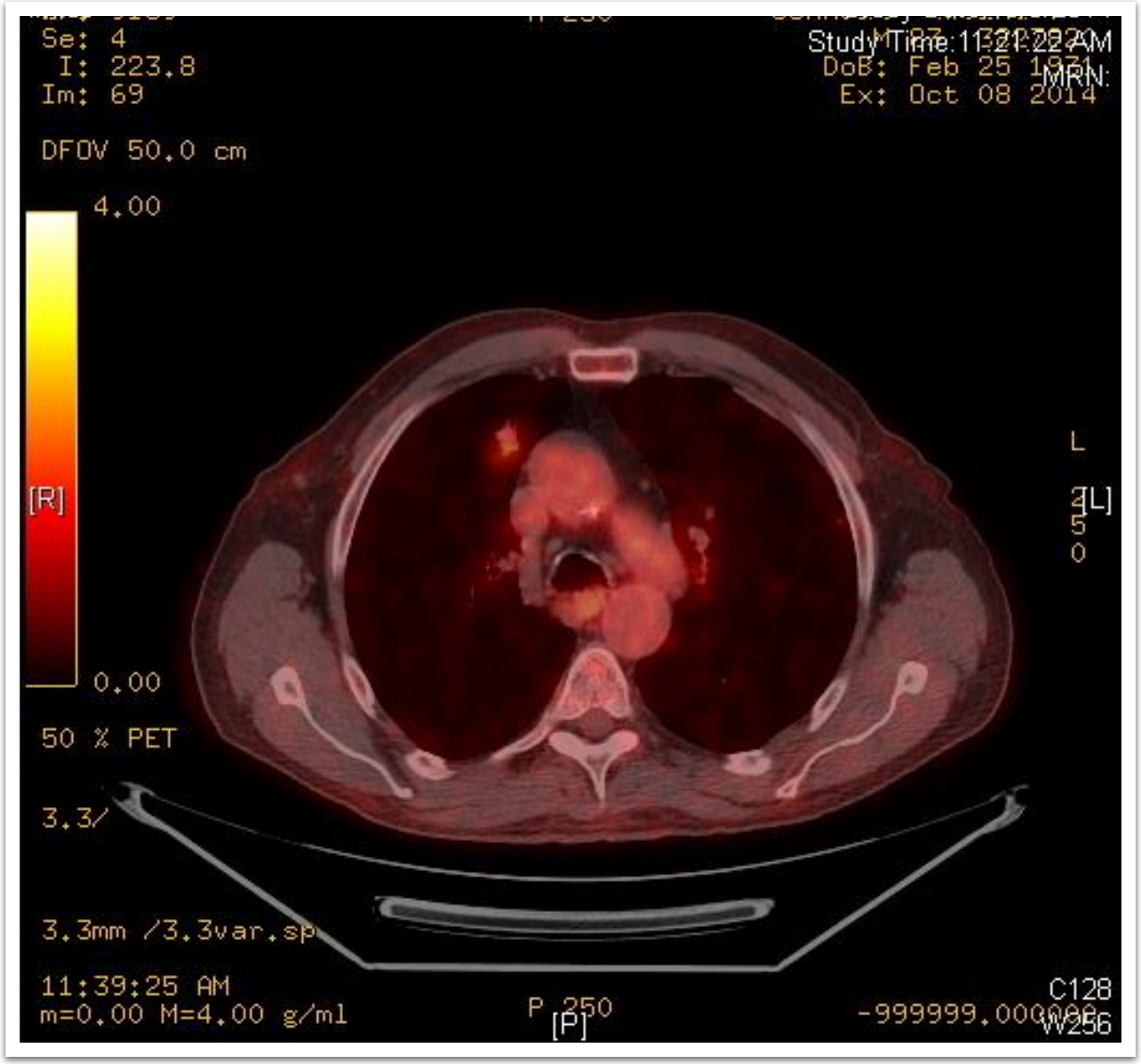
TTF-1 positive



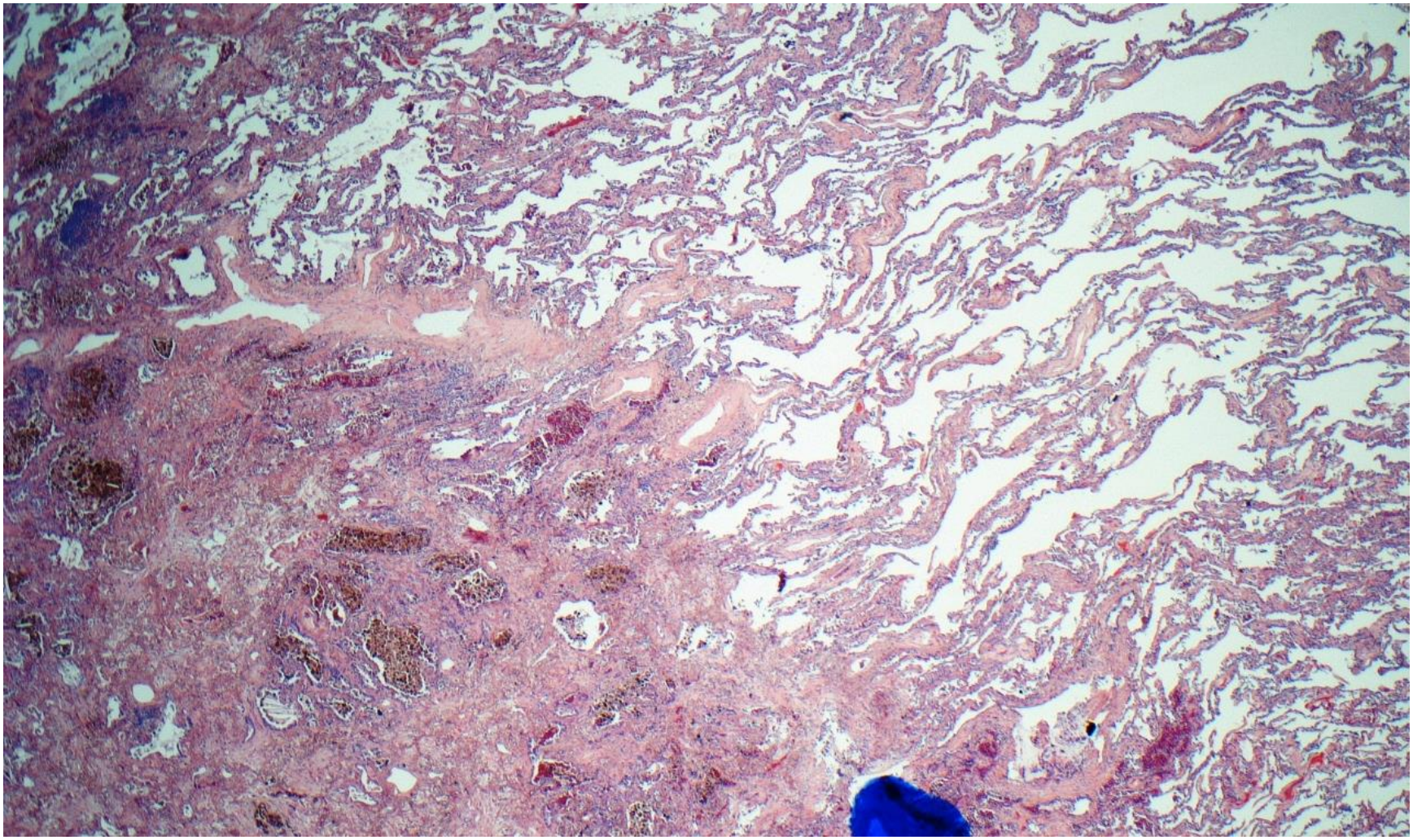
PET-CT

July 2014

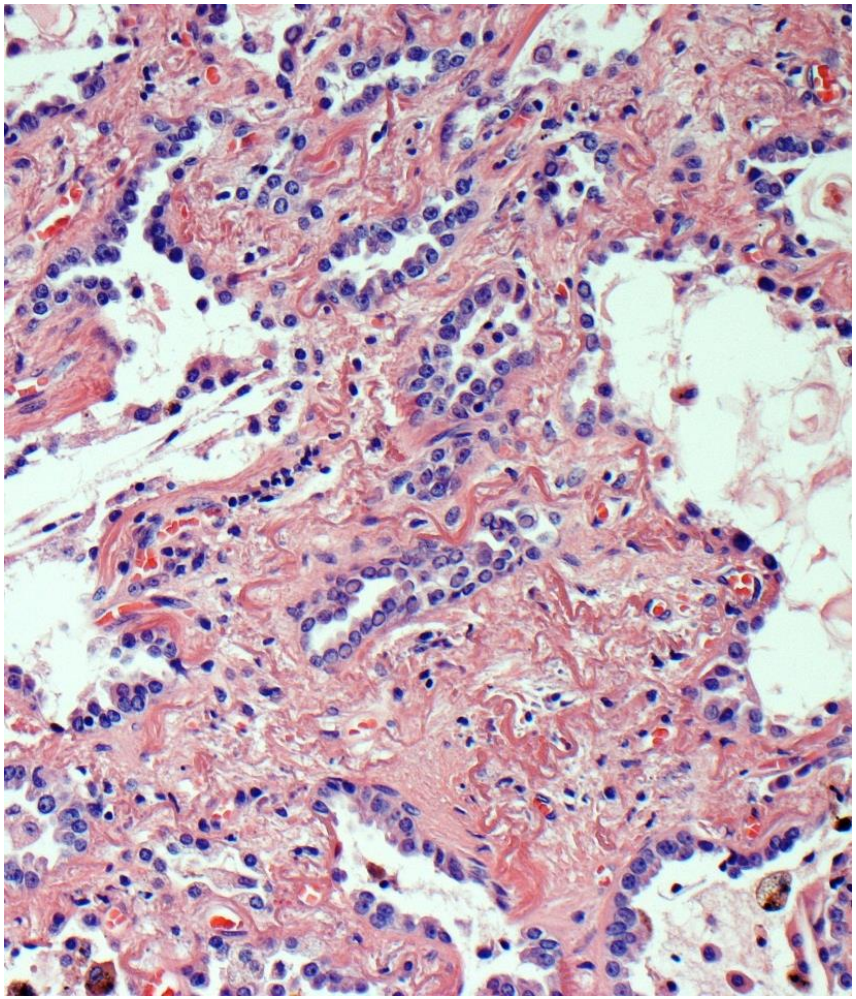
Minimally FDG-avid (SUVmax 1.5) 1.8cm RUL opacity

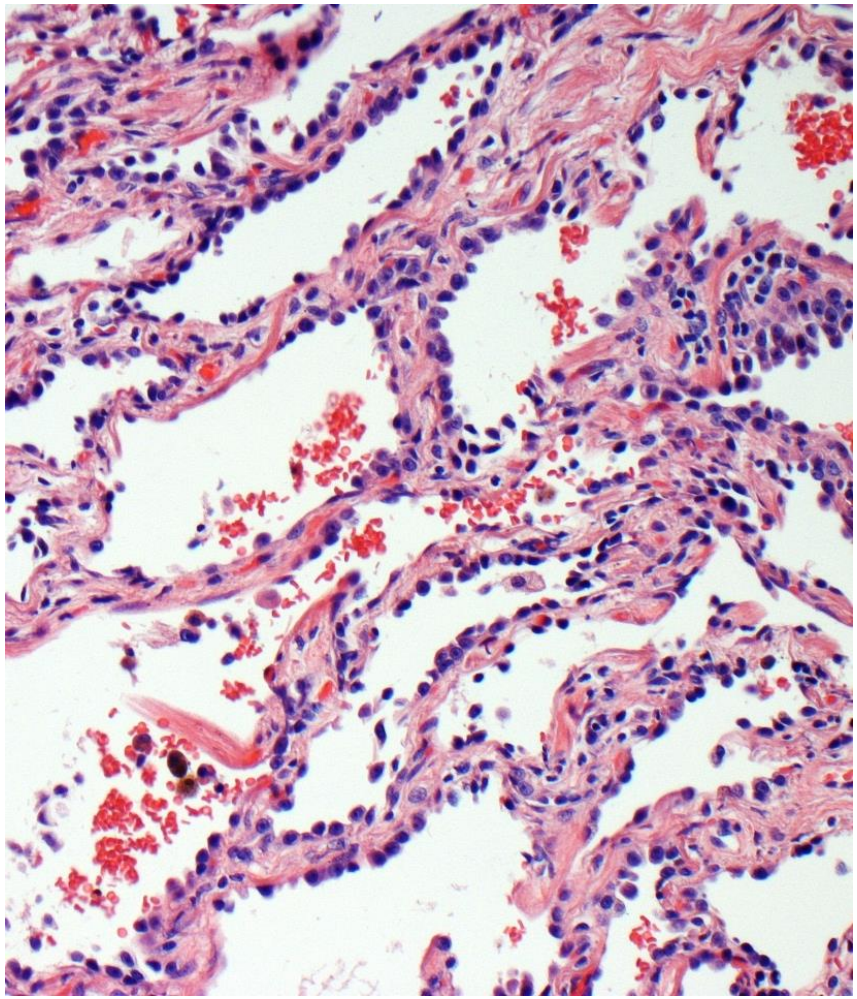


VATS RUL wedge resection and mediastinal lymph node dissection



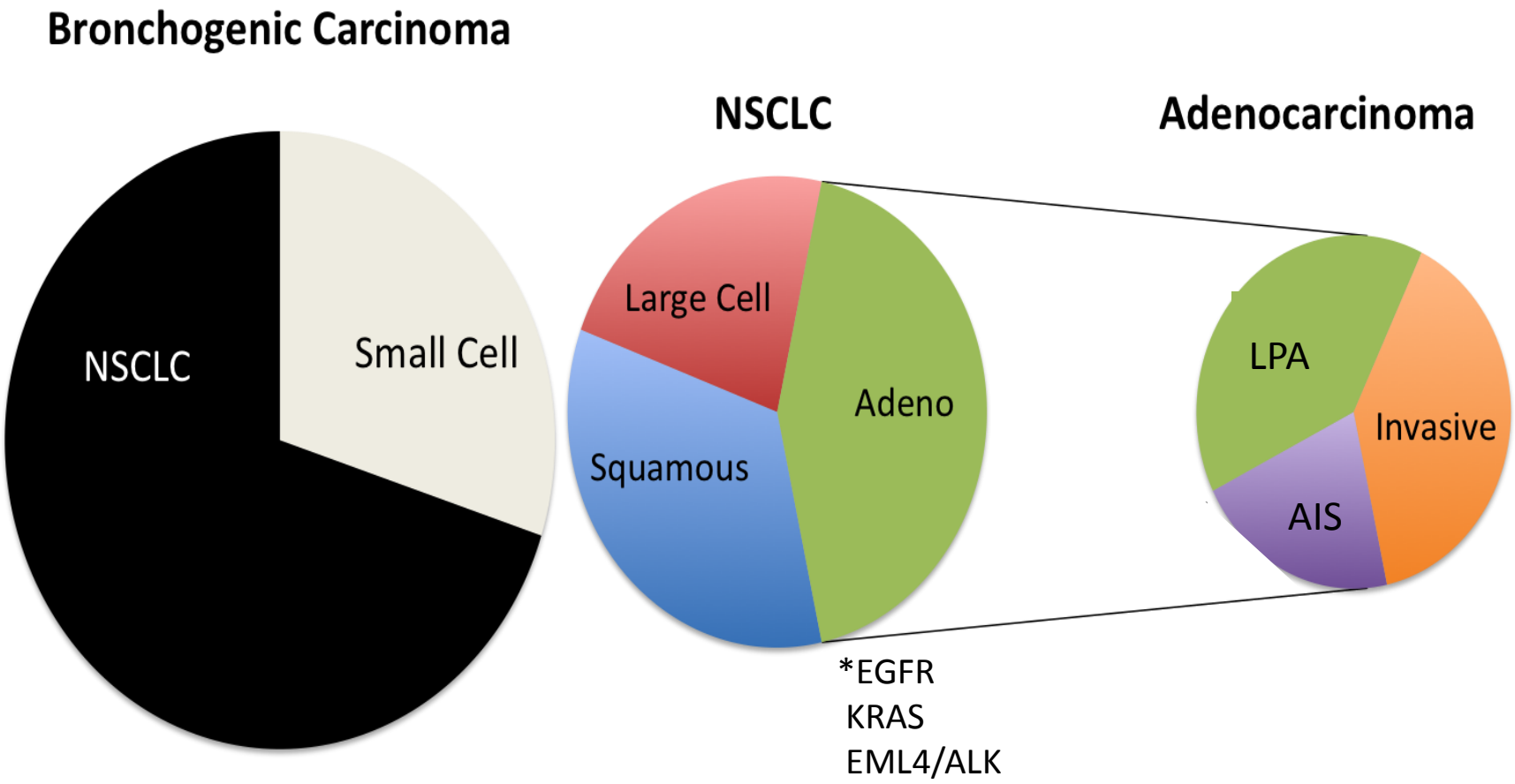
Lepidic predominant adenocarcinoma (LPA)



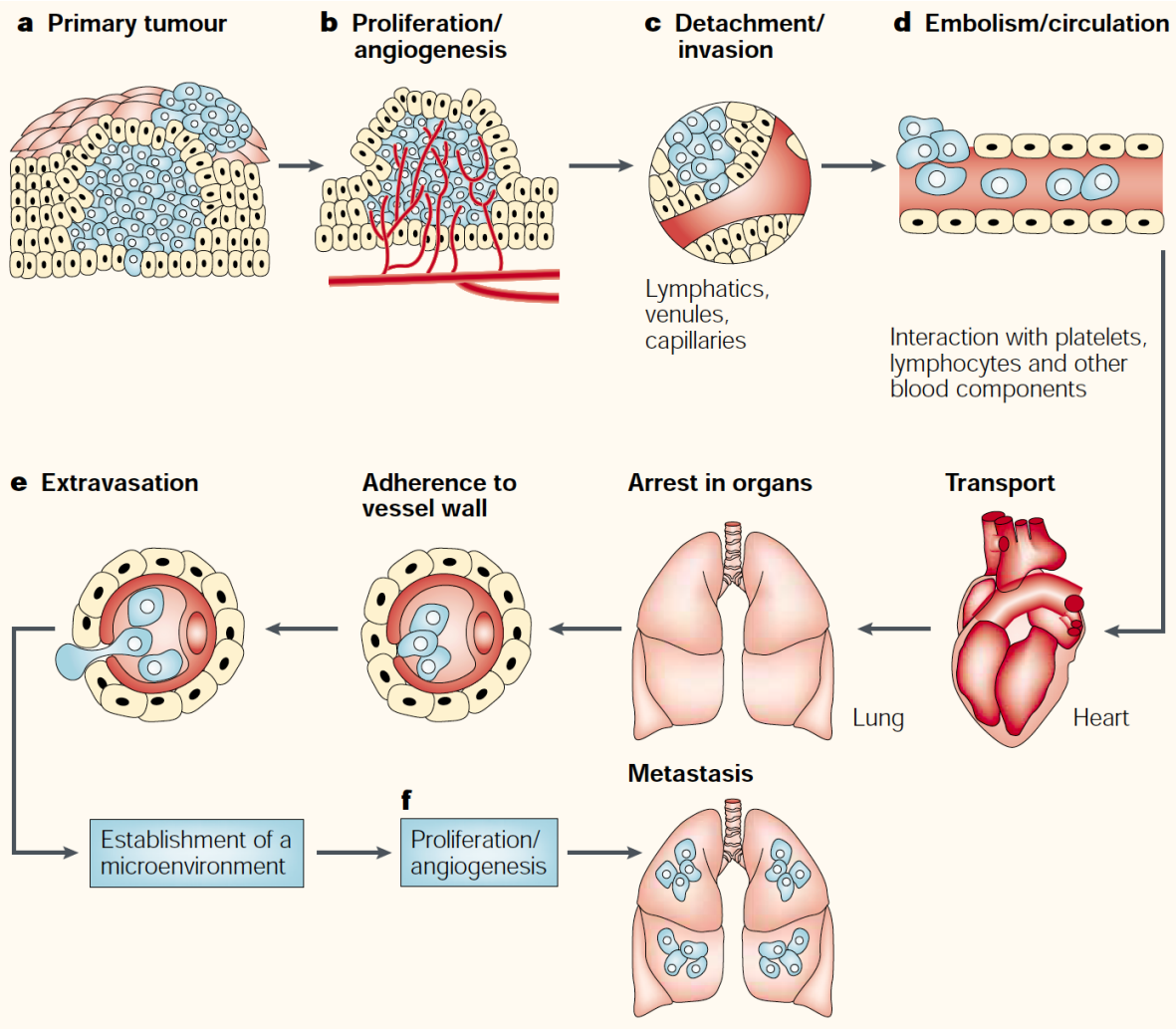


Lung adenocarcinoma: Invasion and metastasis

Lung cancer is a heterogeneous disease



Tumor invasion and metastasis



Initial step for metastasis:

- Loss of adhesion, mobility, basement membrane degradation

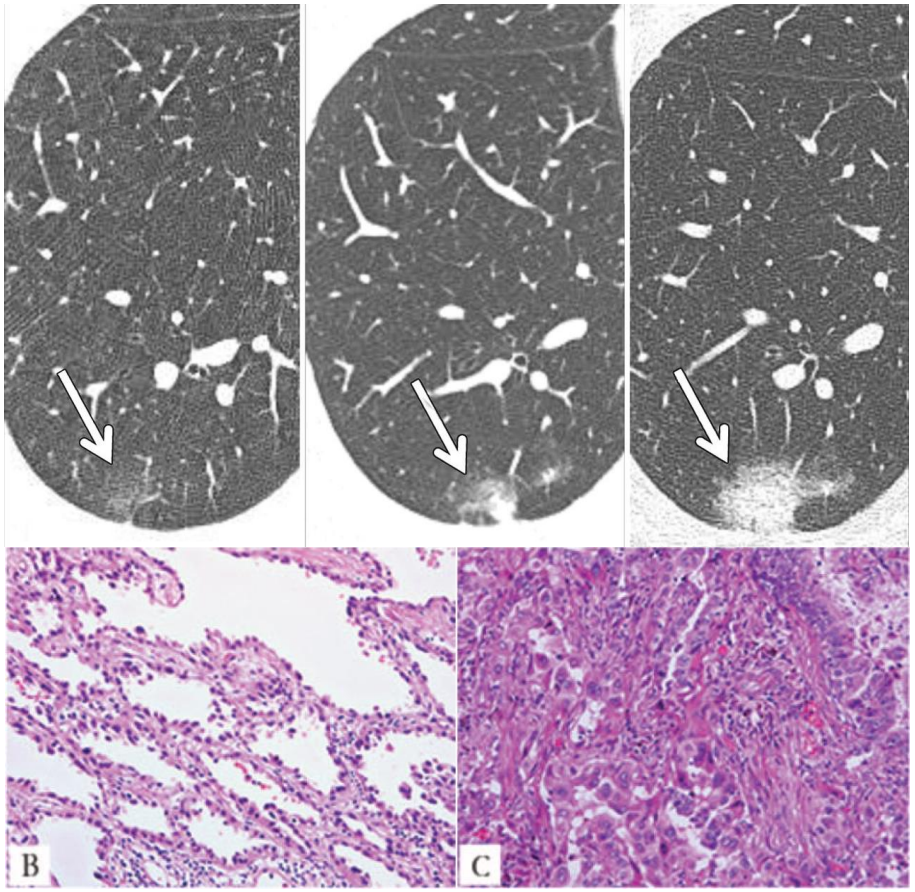
Subsequent steps:

- Intravasation into circulation, extravasation, and angiogenesis at target site

Fidler, *Nature Rev Cancer,* 2003

Adenocarcinoma subtype is defined by degree of tumor invasion

- Lung adenocarcinoma histologic subtypes are associated with tumor invasion and have characteristic radiologic correlates.
- The degree of tumor invasion on histologic examination predicts clinical outcomes.

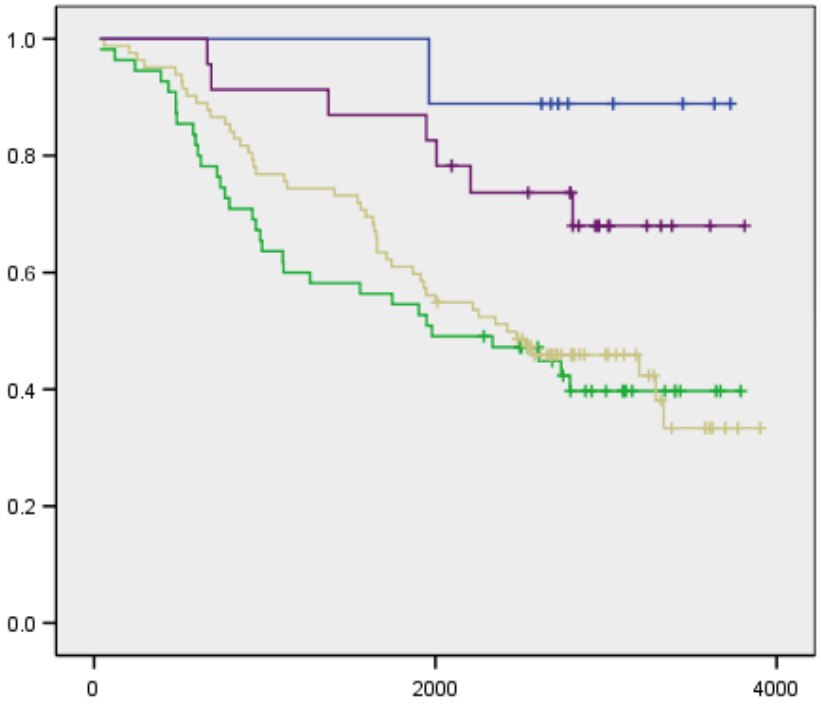


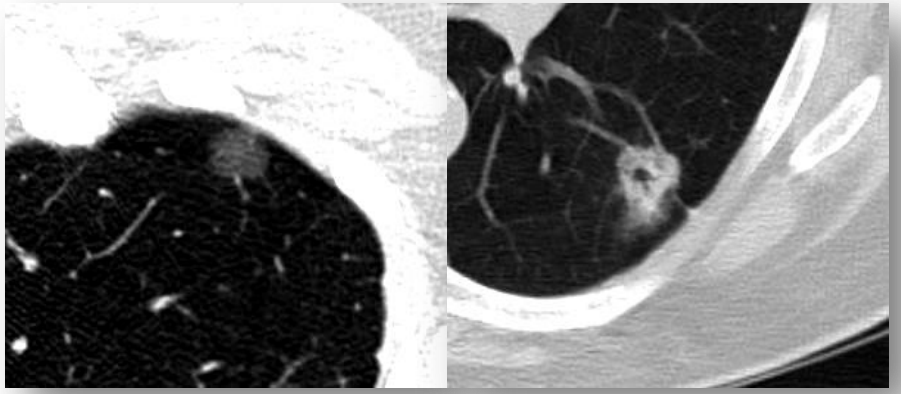
Adenocarcinoma in situ

Invasive adenocarcinoma

Travis, *J Thoracic Onc*, 2011; Naidich, *Radiology*, 2013

Extent of invasion is independently associated with survival in stage I, II, IIIA adenocarcinoma (n=183)

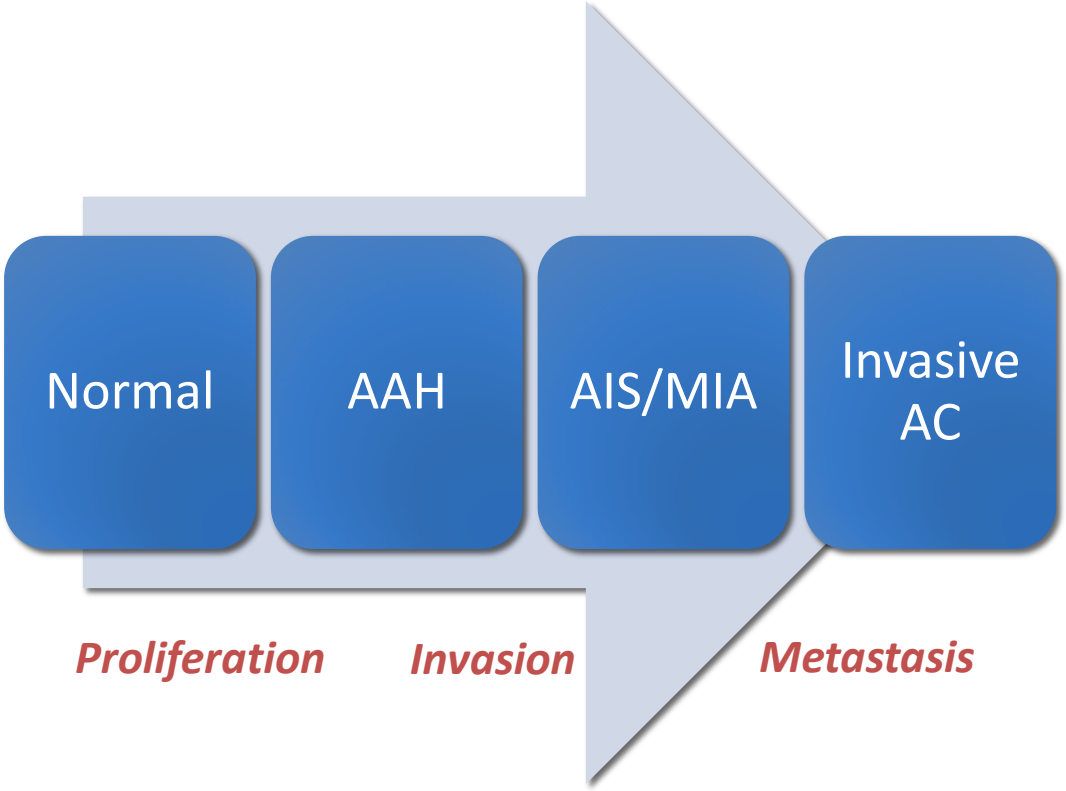


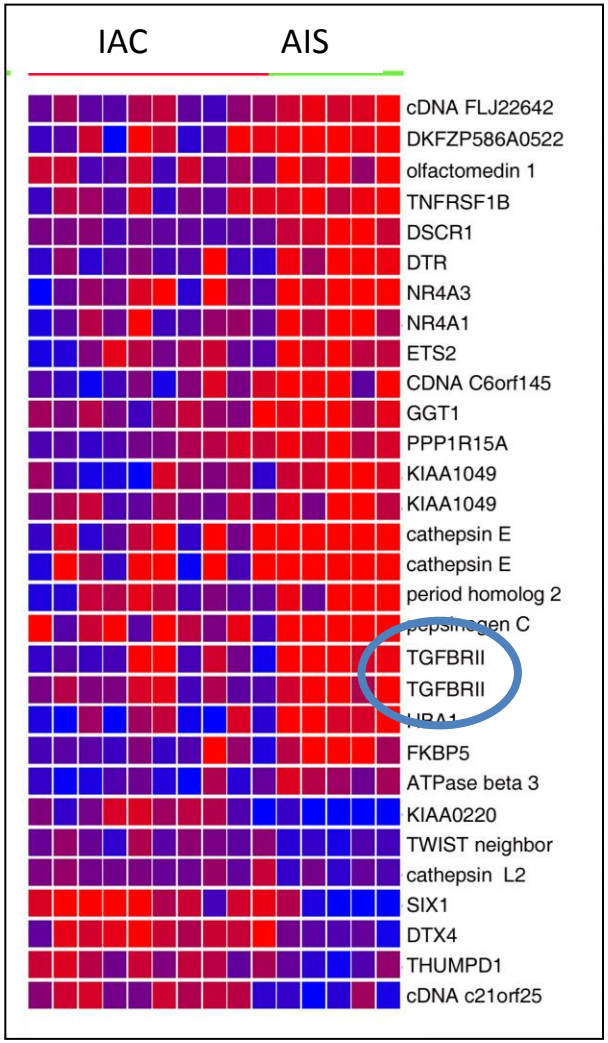


Category	Median survival
BAC	Not reached
Microinvasive (≤ 5mm)	Not reached
Mixed Subtype	2421 days
Pure Invasive	1980 days

Borczuk, Powell, *Am J Surg Pathol,* 2009

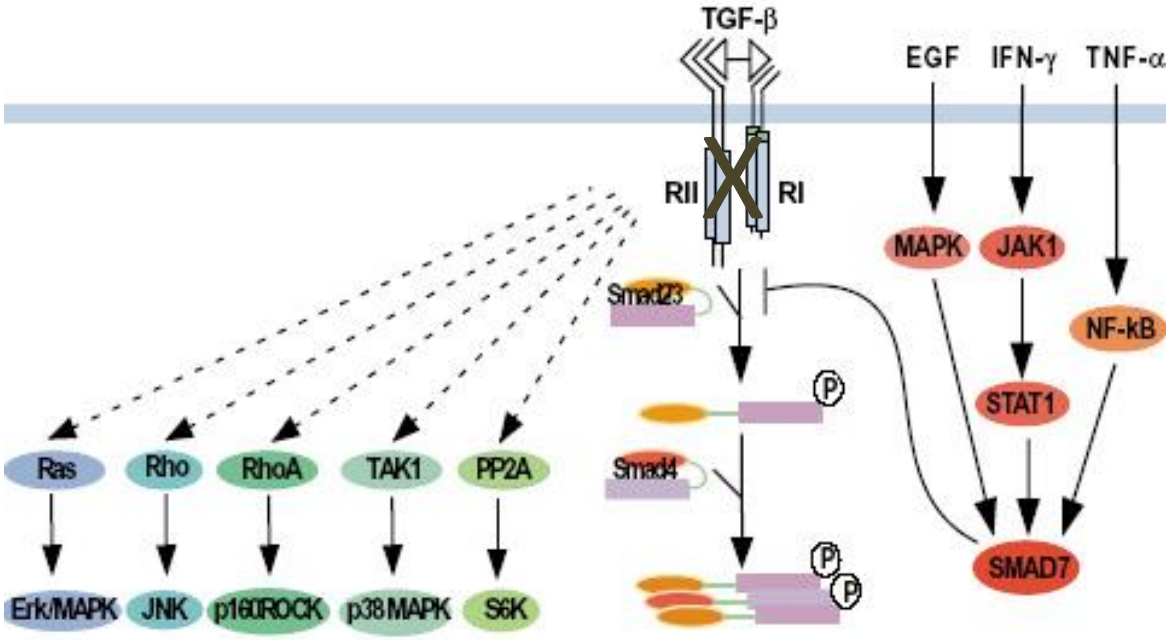
Lung adenocarcinoma progression is associated with differentially expressed genes

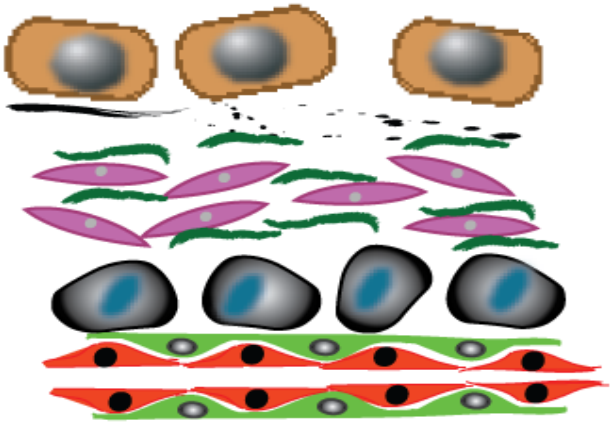




Borczuk, Powell, *AJRCCM,* 2005; Borczuk, Powell, *Cancer Res,* 2011

Mediators of lung adenocarcinoma invasion in TGFβRII–deficient tumors





Migration
MEMBRANE DEGRADATION
Fibroblast Proliferation
Fibronectin Deposition
Macrophage accumulation/ activation

Angiogenesis

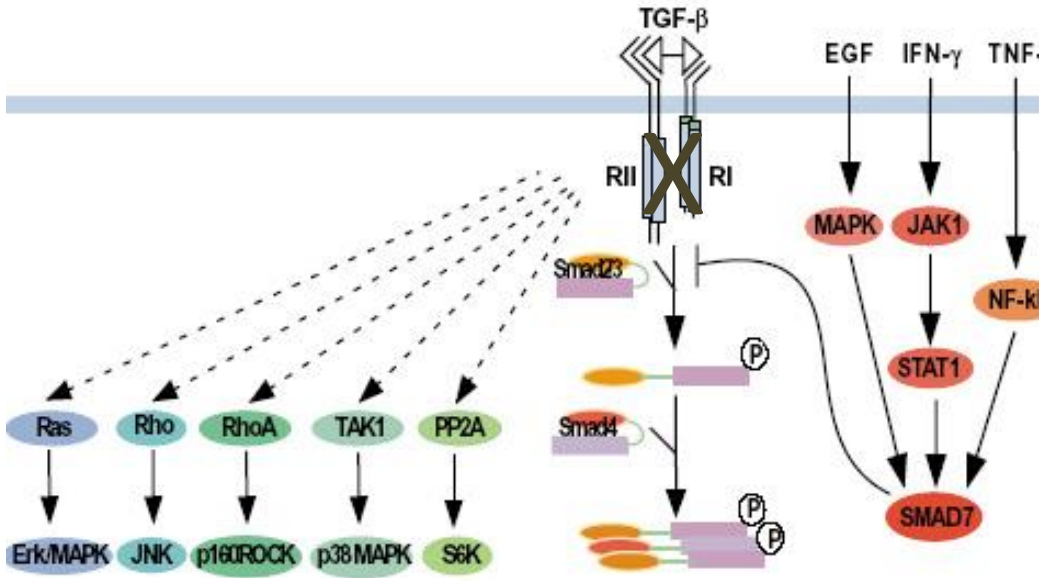
Borczuk, Powell, *PATS,* 2009

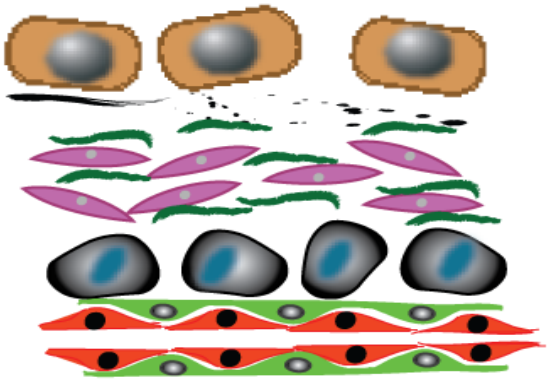
LOX family genes and CCL5 are differentially expressed in invasive lung adenocarcinoma

Affymetrix Probe Set ID	GO Function/Gene Name	Gene Symbol	Fold Change*
	Adhesion		
201130_s_at	Cadherin 1, type 1, E-cadherin (epithelial)	CDH1	0.23
205328_at	Claudin 10	CLDN10	2.94
204750_s_at	Desmocollin 2	DSC2	0.42
204751_x_at	Desmocollin 2	DSC2	0.42
202267_at	Laminin, γ2	LAMC2	0.38
203726_s_at	Laminin, α3	LAMA3	0.29
208083_s_at	Integrin, β6	ITGB6	0.13
208084_at	Integrin, β6	ITGB6	0.24
226535_at	Integrin, β6	ITGB6	0.42
215446_s_at	Lysyl oxidase	LOX	4.00
202998_s_at	Lysyl oxidase-like 2	LOXL2	2.70
214154_s_at	Plakophilin 2	PKP2	0.17
207717_s_at	Plakophilin 2	PKP2	0.42
	Cell–Cell Signaling		
205290_s_at	Bone morphogenetic protein 2	BMP2	0.18
205289_at	Bone morphogenetic protein 2	BMP2	0.22
1405_i_at	Chemokine (C-C motif) ligand 5	CCL5	3.70
1555759_a_at	Chemokine (C-C motif) ligand 5	CCL5	2.94

Borczuk, Powell, *AJRCCM,* 2005

CCL5/RANTES and LOX and LOXL2 are important mediators of lung adenocarcinoma invasion in TGFβRII–deficient tumors





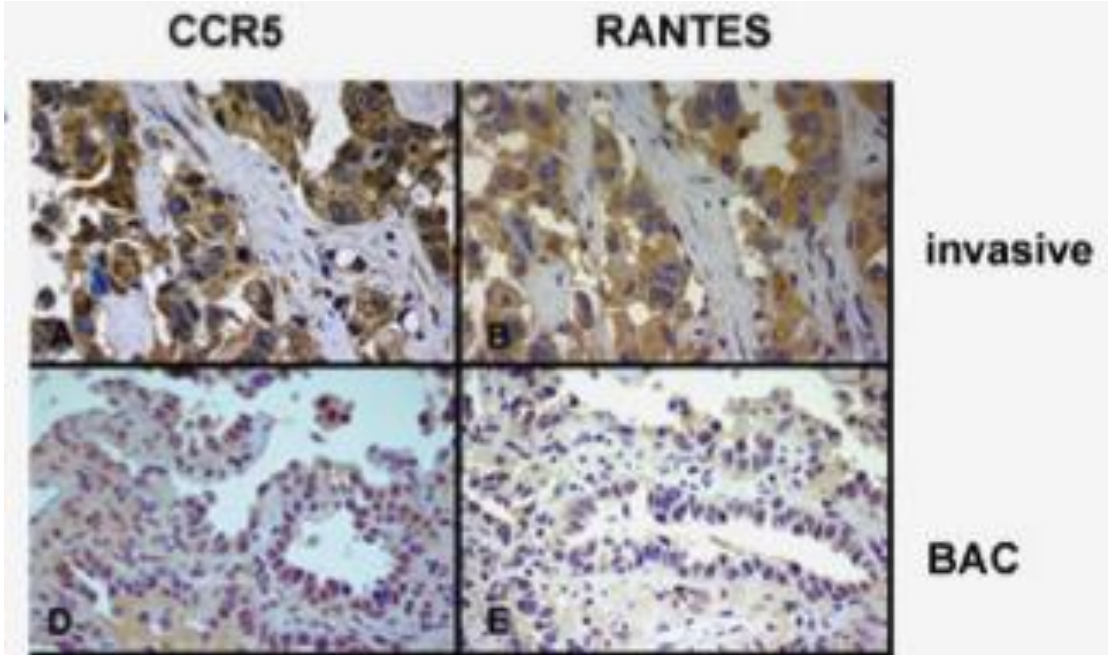
Migration
MEMBRANE DEGRADATION
Fibroblast Proliferation
Fibronectin Deposition
Macrophage accumulation/ activation

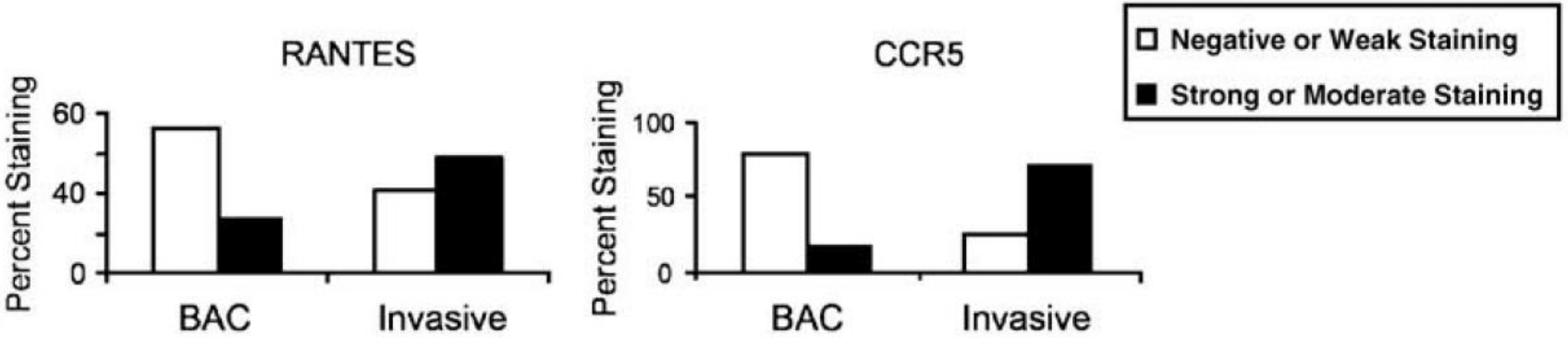
Angiogenesis

CCL5/RANTES; LOX and LOXL2

Borczuk, Powell, *PATS,* 2009

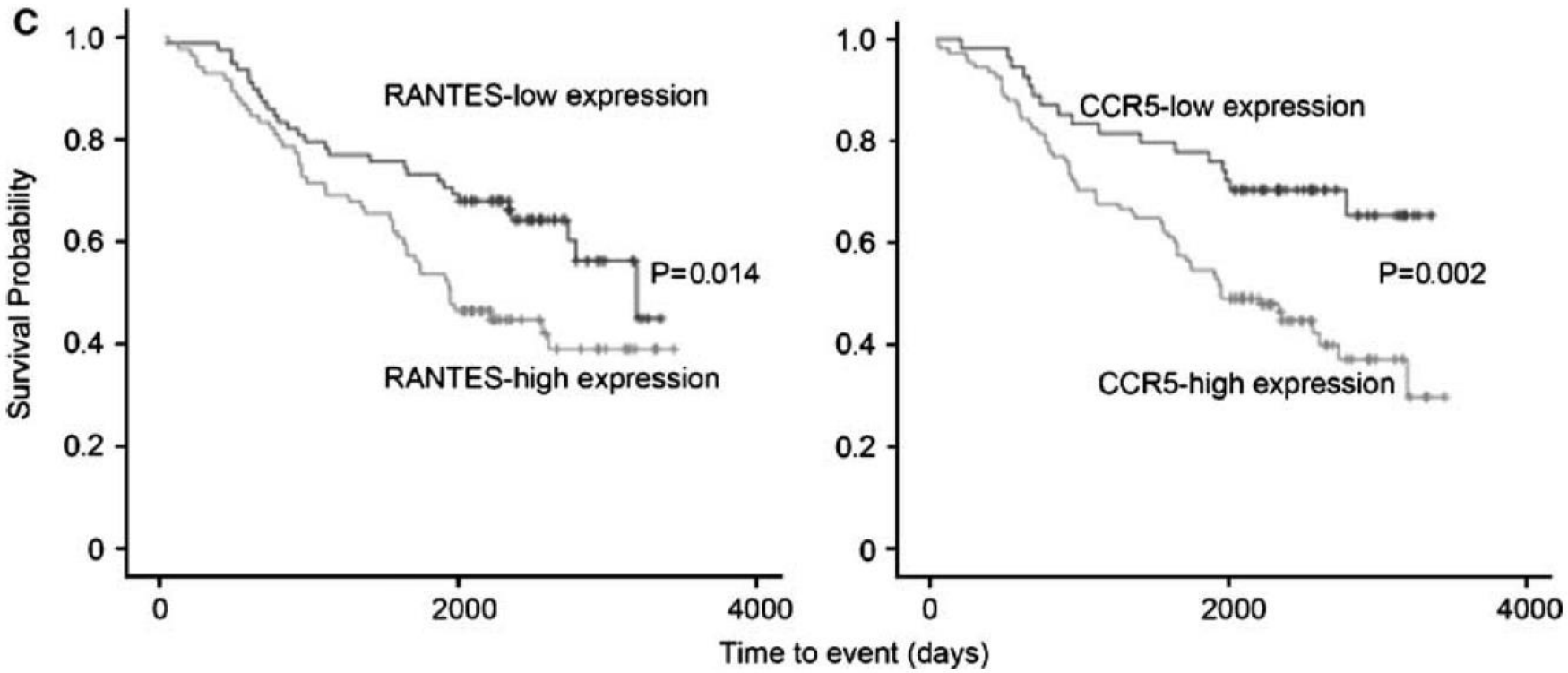
CCR5/RANTES expression in lung adenocarcinoma is associated with invasion and clinical outcome





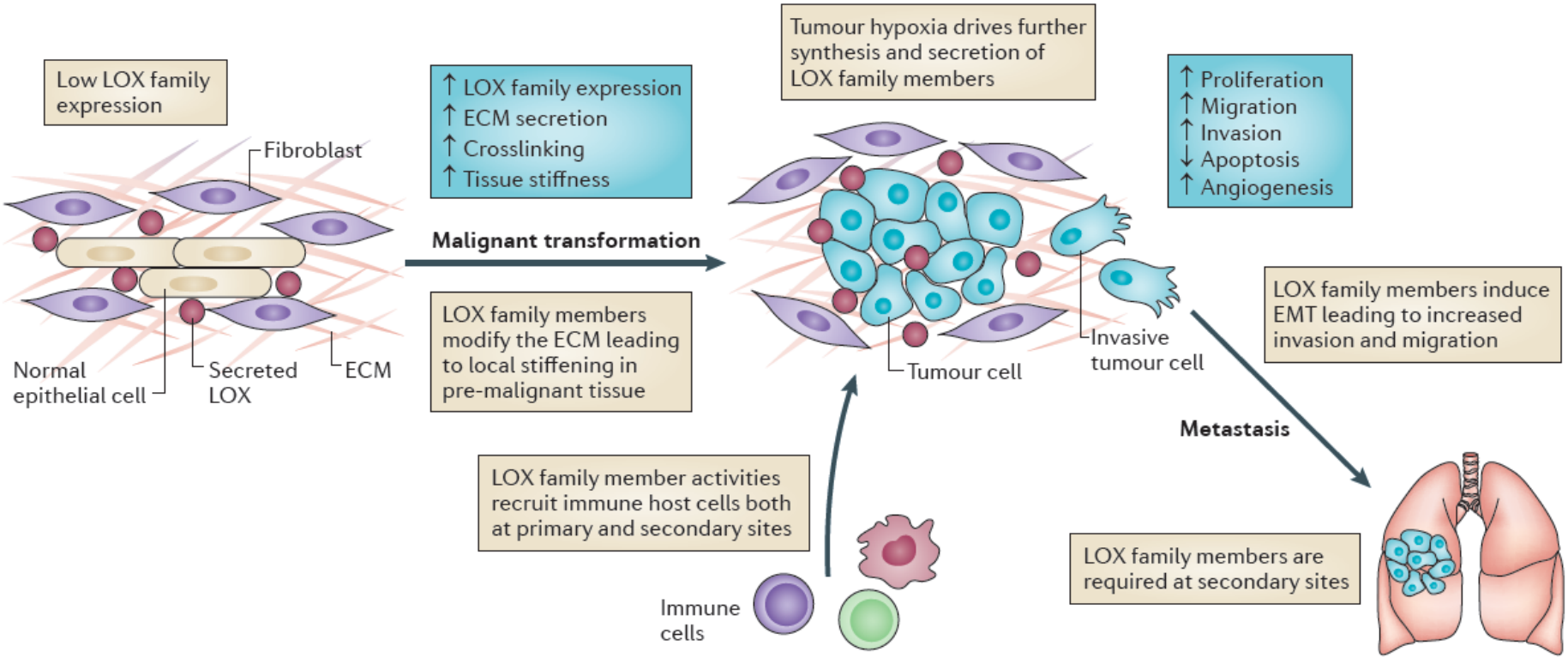
Borczuk, Powell, *Oncogene*, 2008

CCR5/RANTES expression in lung adenocarcinoma is associated with invasion and clinical outcome



Borczuk, Powell, *Oncogene*, 2008

Lysyl oxidase (LOX) acts on the ECM to facilitate fibrosis, tumor cell invasion, and metastasis



Barker, *Nature Rev Cancer*, 2012

Lysyl Oxidase: A Lung Adenocarcinoma Biomarker of Invasion and Survival

May-Lin Wilgus, MD[1]; Alain C. Borczuk, MD[2]; Mark Stoopler, MD[3]; Mark Ginsburg, MD[4]; Lyall Gorenstein, MD[4]; Joshua R. Sonett, MD[4]; and Charles A. Powell, MD[1]

Table 2. Tumor LOX Expression Is Associated With Invasive Subclass

Tumor	Low LOX Staining, No. of Cases (% Each Subclass)	High LOX Staining, No. of Cases (% Each Subclass)
Pure BAC	10 (100%)	0 (0%)
Microinvasive	19 (86%)	3 (14%)
Mixed-type	65 (82%)	14 (18%)
Invasive	34 (62%)	21 (38%)

LOX indicates lysyl oxidase; BAC, bronchioloalveolar cancer.

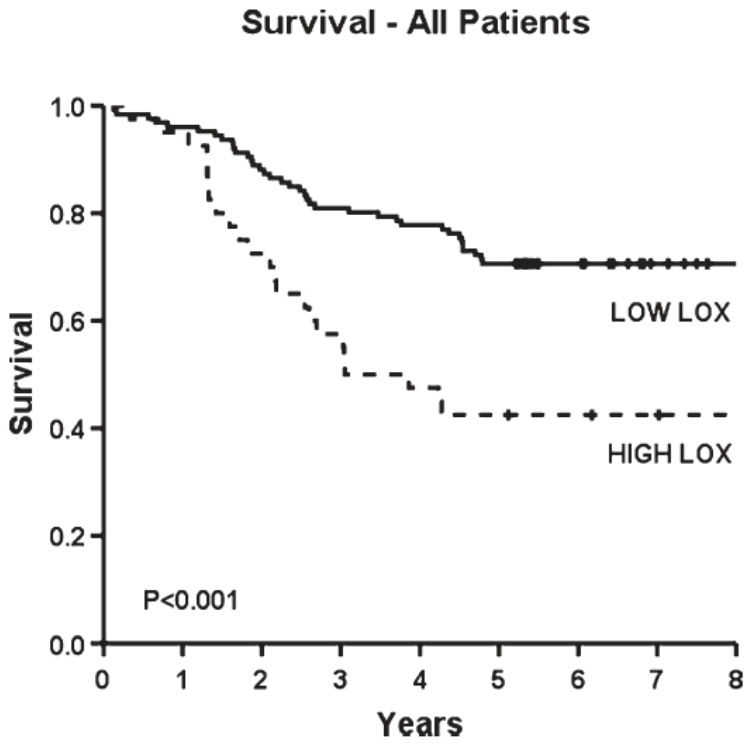
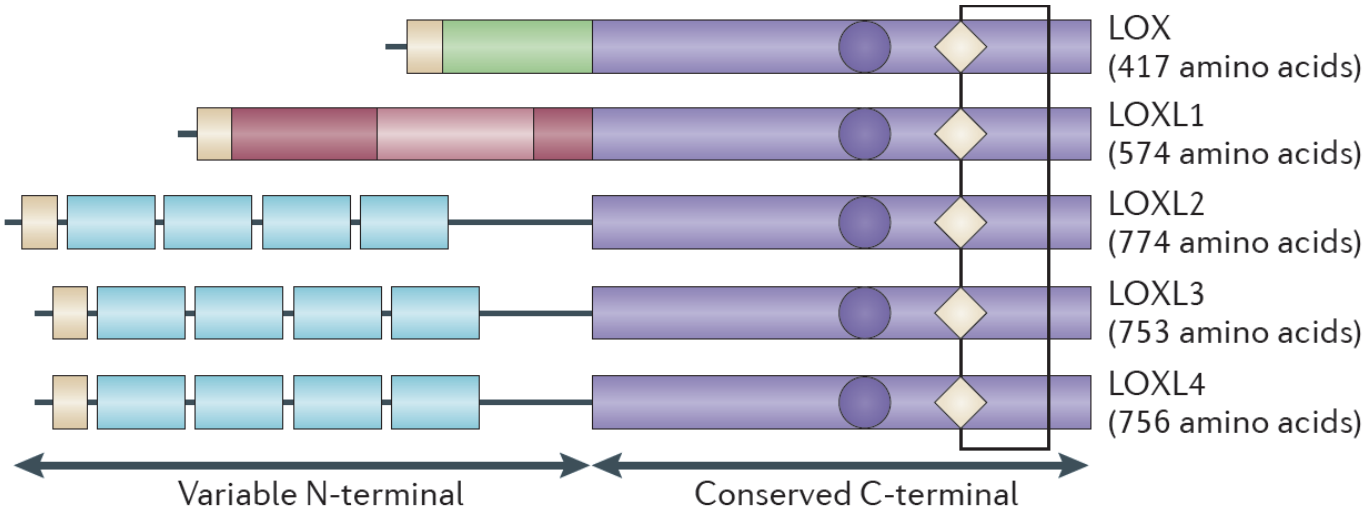


Figure 2. Survival for all patients is shown. High lysyl oxidase (LOX) expression is associated with increased 5-year mortality (log-rank $P < .001$).

Wilgus, Powell, *Cancer*, 2011

The LOX family of proteins has been implicated as both tumor suppressors and metastasis promoters

- Lysyl oxidase (LOX) and LOXL2 are important for lung and breast tumor invasion. Expression distinguishes pre-invasive from invasive histology and is associated with poor survival in lung adenocarcinoma.



Wilgus, Powell, *Cancer*, 2011; Barker, *Nature Rev Cancer,* 2012

Table 2 | **The LOX family in cancer**

Cancer	Member	Role
Colorectal	LOX	Increased expression associated with increased invasion, metastatic potential and SRC activation
	LOXL2	Co-expression with RAMP3 and TIMP1 in tumour versus normal tissue
		Increased expression in tumour-associated stroma versus normal stroma
Bladder	LOXL1 and LOXL4	Epigenetic silencing of *LOXL1* and *LOXL4* detected
Pancreatic	LOXL2	Silencing renders cells sensitive to chemotherapy
		Increased expression in adenocarcinoma-associated stroma versus normal stroma
Breast	LOX	Increased expression associated with increased metastasis and decreased survival
	LOXL2	Aberrant and decreased expression significantly correlated with distant metastatic incidence and poor survival
		Co-expression with RAMP3 in tumour versus normal tissue
		Increased expression in tumour-associated stroma versus normal stroma
HNSCC	LOX	Increased expression associated with decreased patient disease-free and overall survival
Laryngeal	LOXL2	Increased expression in stroma and tumour cells is associated with decreased survival
Lung	LOXL2	Increased expression is associated with decreased survival
		Decreased mRNA in tumour versus non-tumour tissue is associated with disease progression
Gastric	LOXL2	Co-expression with RAMP3 in tumour versus normal tissue
		Increased expression in tumours promotes progression and can be abrogated by immunological targeting

Barker, *Nature Reviews Cancer*, 2012

Early Stage Lung Cancer: Biomarker discovery, validation, and new applications

Hypothesis

- Gap: There is a need for validated biomarkers in pre-resection specimens that predict tumor invasion and metastasis.

- **Cellular expression of LOX and LOXL2 in pre-resection cytologic specimens predicts lung adenocarcinoma invasiveness.**

- Objective: To examine the association of LOX and LOXL2 expression in fine needle aspirate (FNA) cytology specimens with extent of invasion in paired, surgically resected lung adenocarcinoma specimens.

Methodology

- LOX and LOXL2 gene expression was performed by **quantitative polymerase chain reaction (qPCR)** of RNA extracted from surgically resected formalin fixed and paraffin embedded (FFPE) tumors and using Taqman probes.

- **Immunohistochemistry (IHC) /Immunocytochemistry (ICC)** for LOX and LOXL2 expression was performed on FFPE cytologic cell blocks from FNAs and paired, surgically resected FFPE tumors.

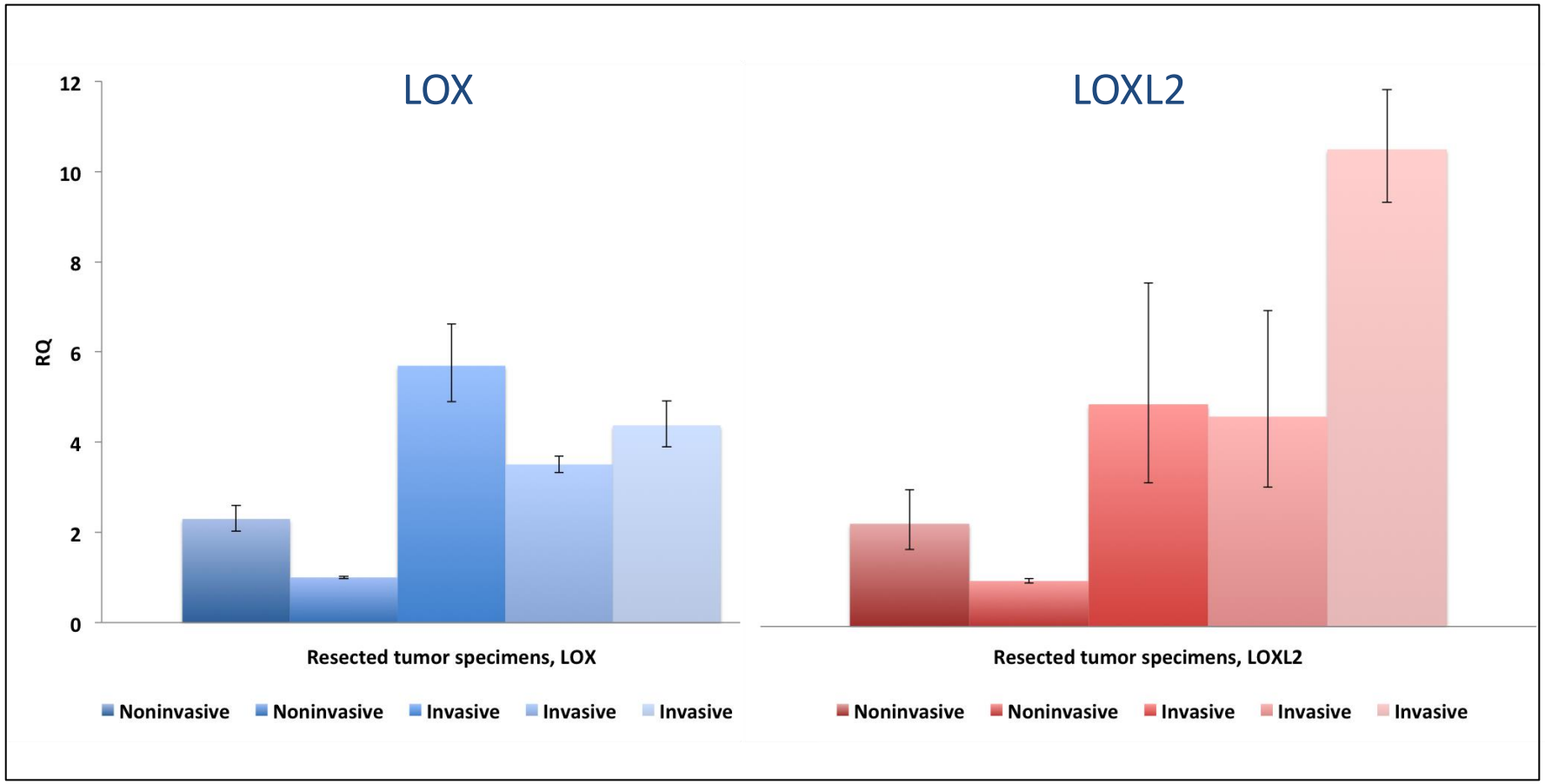
- Immunostaining in tumor cell cytoplasm was scored as negative (0) or positive on a scale of 1 (faint) to 3 (strong). In positive cases, an assessment of percent staining within tumors was performed.

1° antibody	Tissue	Cytology cell block	Source
Anti-LOX	1:3000	1:3000	Abcam
Anti-LOXL2	1:1000	1:1000	Santa Cruz Biotechnology

Patient and tumor demographics

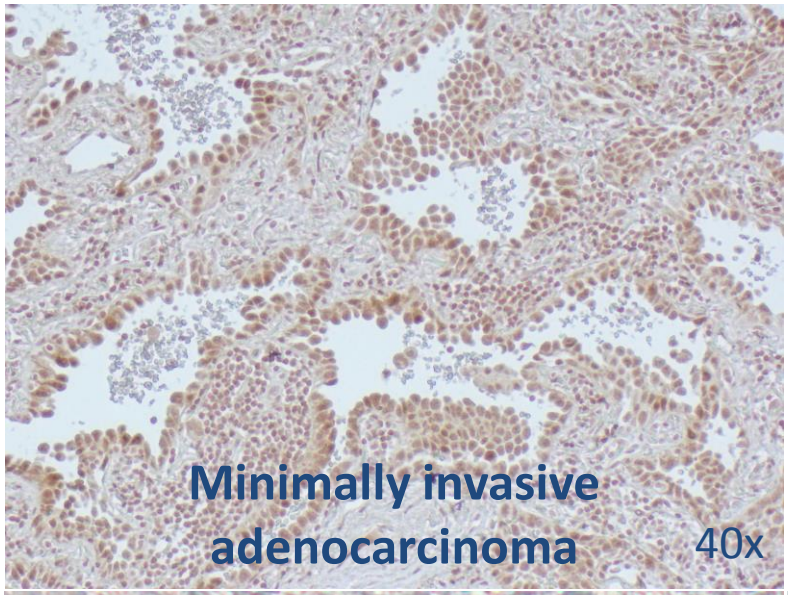
Demographics	Tumors (31)
Age, mean ± SD, y	70 ± 9
Male, n	12 (38%)
Tumor size, mean ± SD, cm	1.7 ± 1.1
Pathologic stage, n	
IA	28 (91%)
IB	2 (6%)
2A	1 (3%)
Adenocarcinoma subtype, n	
Adenocarcinoma in situ (AIS)	3 (10%)
Minimally invasive adenocarcinoma (MIA)	9 (29%)
Lepidic predominant adenocarcinoma (LPA)	4 (13%)
Invasive, acinar predominant	9 (29%)
Invasive, papillary predominant	4 (13%)
Invasive, solid predominant	2 (6%)
Molecular markers, n	
EGFR	4 (13%)
KRAS	4 (13%)
ALK gene rearrangement	1 (3%)

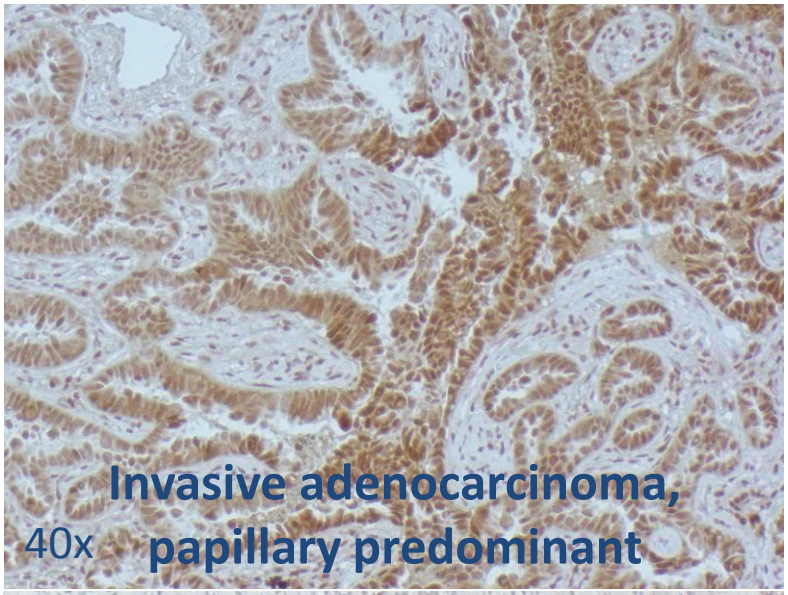
LOX and LOXL2 expression is higher in resected invasive lung adenocarcinoma compared with noninvasive tumors



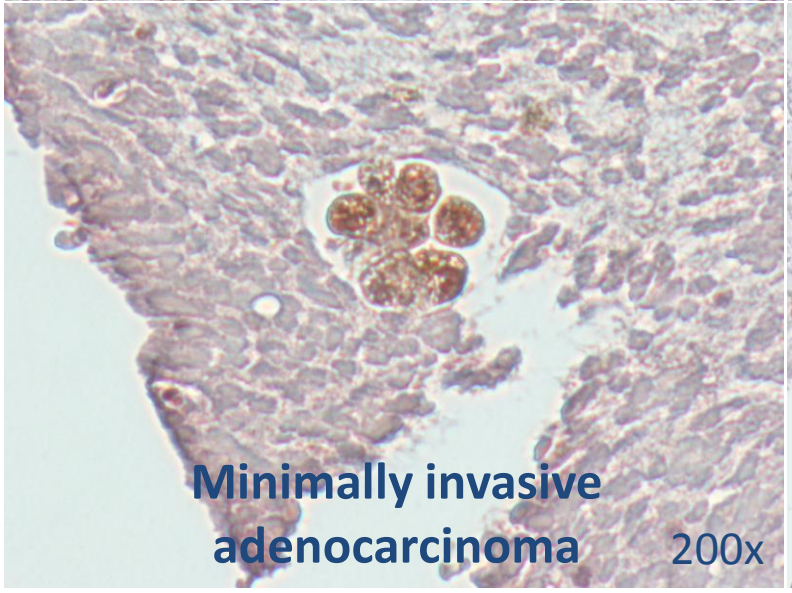
LOX expression in lung adenocarcinoma

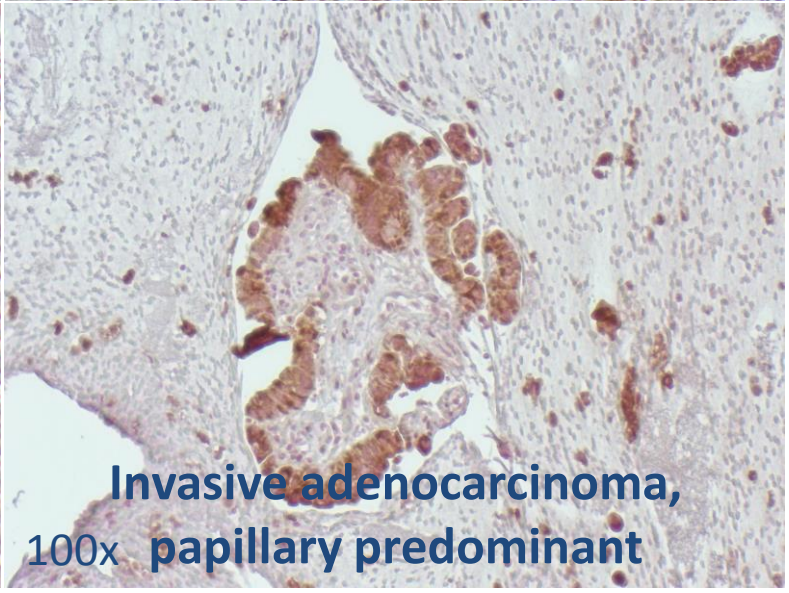
Surgically resected tumor





Cytologic cell block from FNA



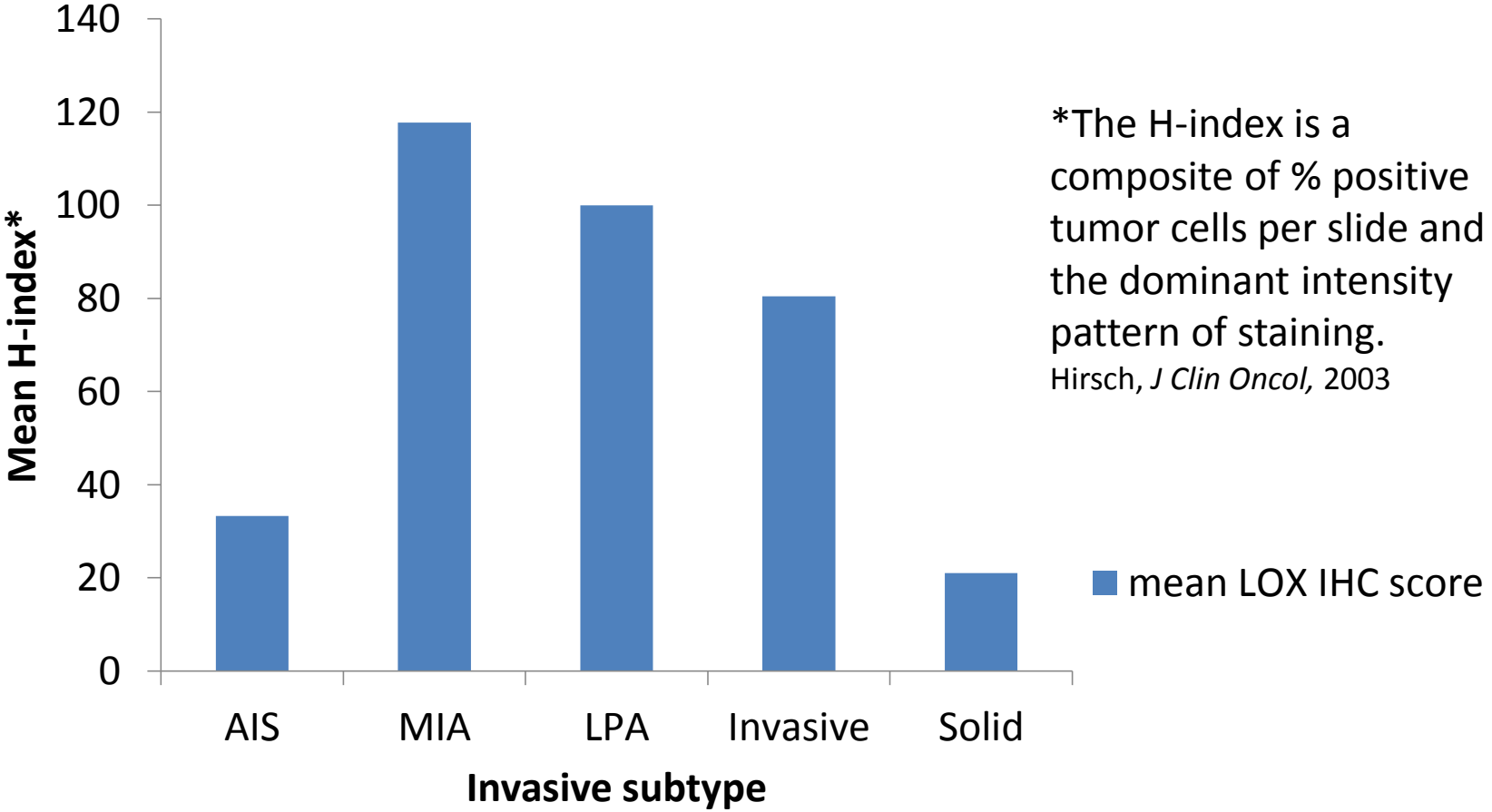


The frequency of LOXL2 expression is reduced in pre-invasive resected adenocarcinoma

Cytoplasmic staining positive*	AIS	MIA	LPA	Invasive non-solid	Invasive solid
LOX					
Surgical specimens, n= 19/31	1/3 (33%)	7/9 (78%)	2/4 (50%)	7/10 (70%)	2/5 (40%)
Cytologic specimens, n= 17/18	No specimens	6/6 (100%)	0/1 (0%)	6/6 (100%)	5/5 (100%)
LOXL2					
Surgical specimens, n= 25/31	2/3 (67%)	7/9 (78%)	4/4 (100%)	10/10 (100%)	2/5 (40%)
Cytologic specimens, n= 20/20	No specimens	7/7 (100%)	1/1 (100%)	7/7 (100%)	5/5 (100%)

*LOX and LOXL2 expression is defined by IHC/ICC intensity score 1+, 2+, 3+

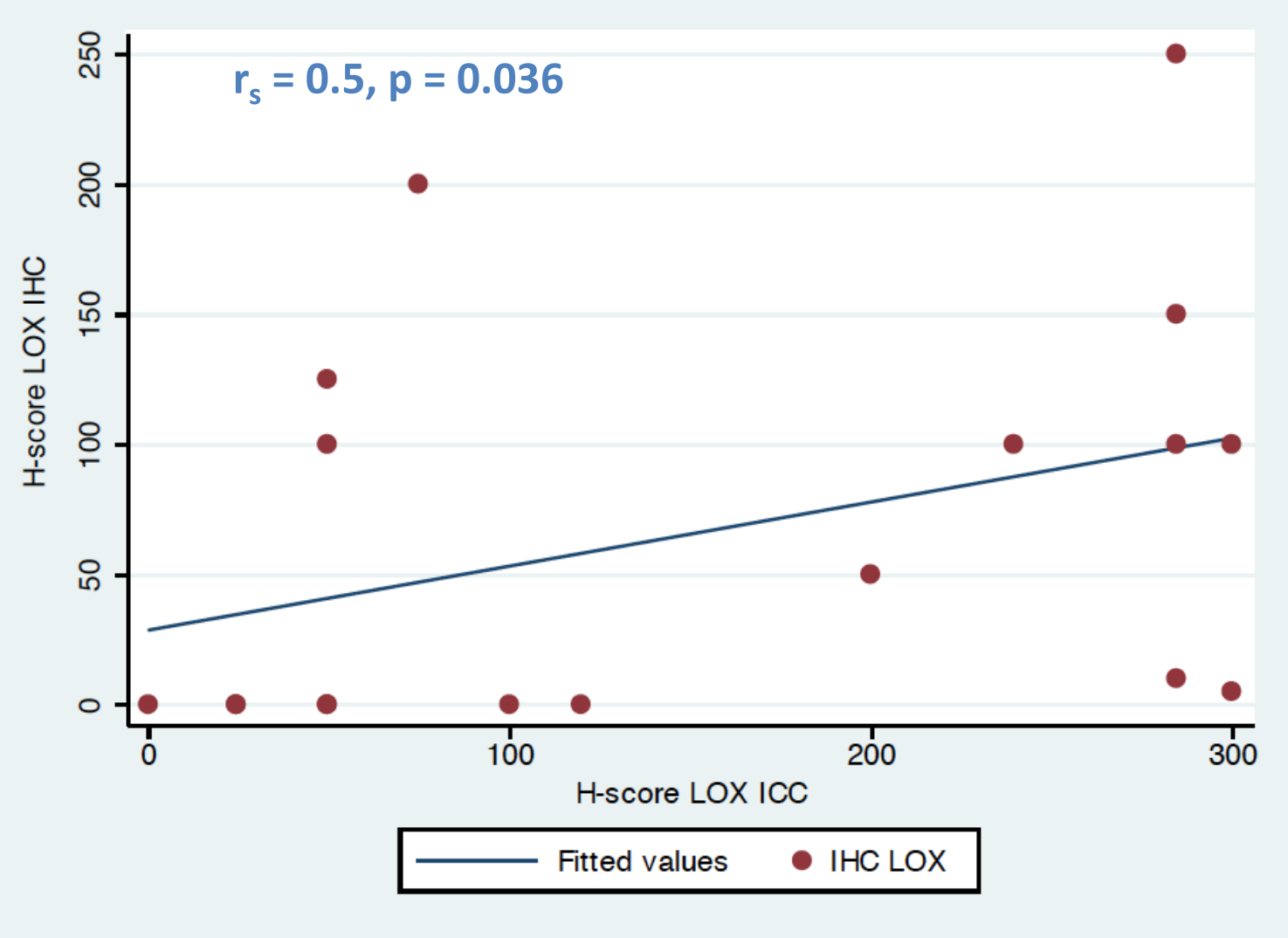
LOX expression in biopsy and resection specimens is associated with extent of invasion



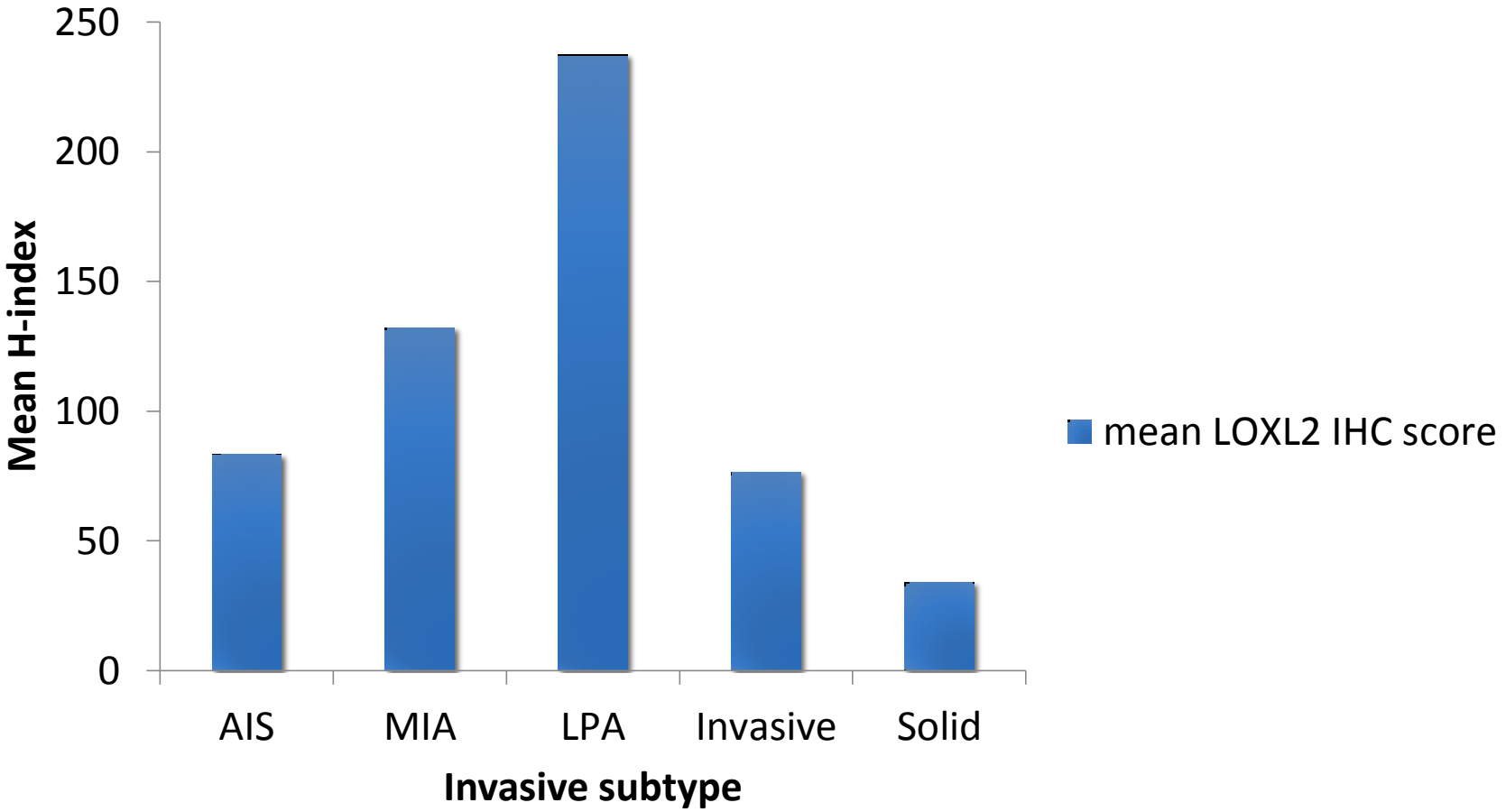
*The H-index is a composite of % positive tumor cells per slide and the dominant intensity pattern of staining.
Hirsch, *J Clin Oncol,* 2003

Needle biopsy immunostaining correlates with tissue immunostaining. Spearman's correlation for LOX ICC and LOX IHC, r_s= 0.50, p= 0.036.

Spearman's rank correlation of LOX expression in biopsy and resection specimens

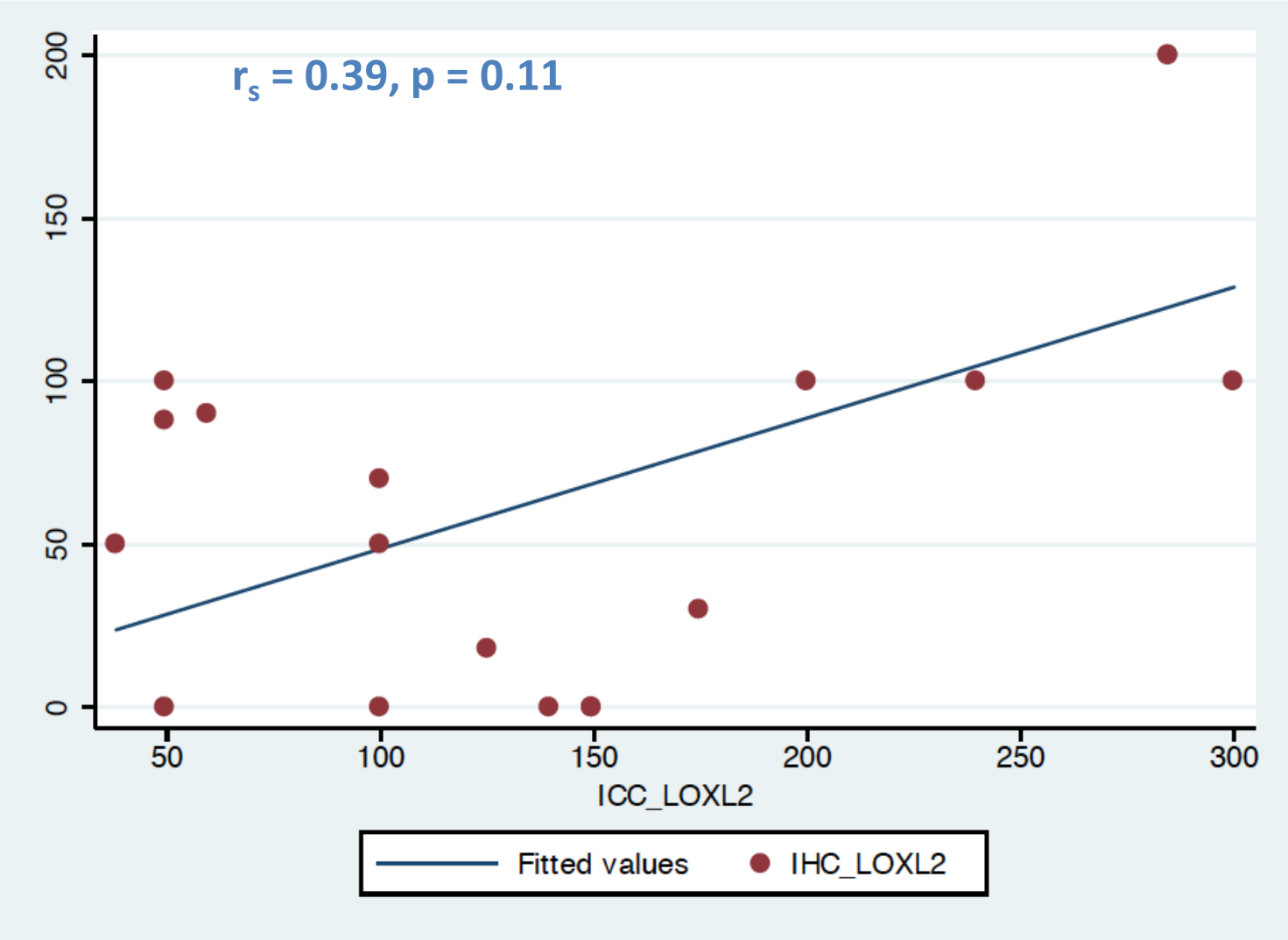


LOXL2 expression in resected adenocarcinoma is increased in the progression from AIS to LPA



There is no correlation between LOXL2 ICC and LOXL2 IHC.

Spearman's rank correlation of LOXL2 expression in biopsy and resection specimens



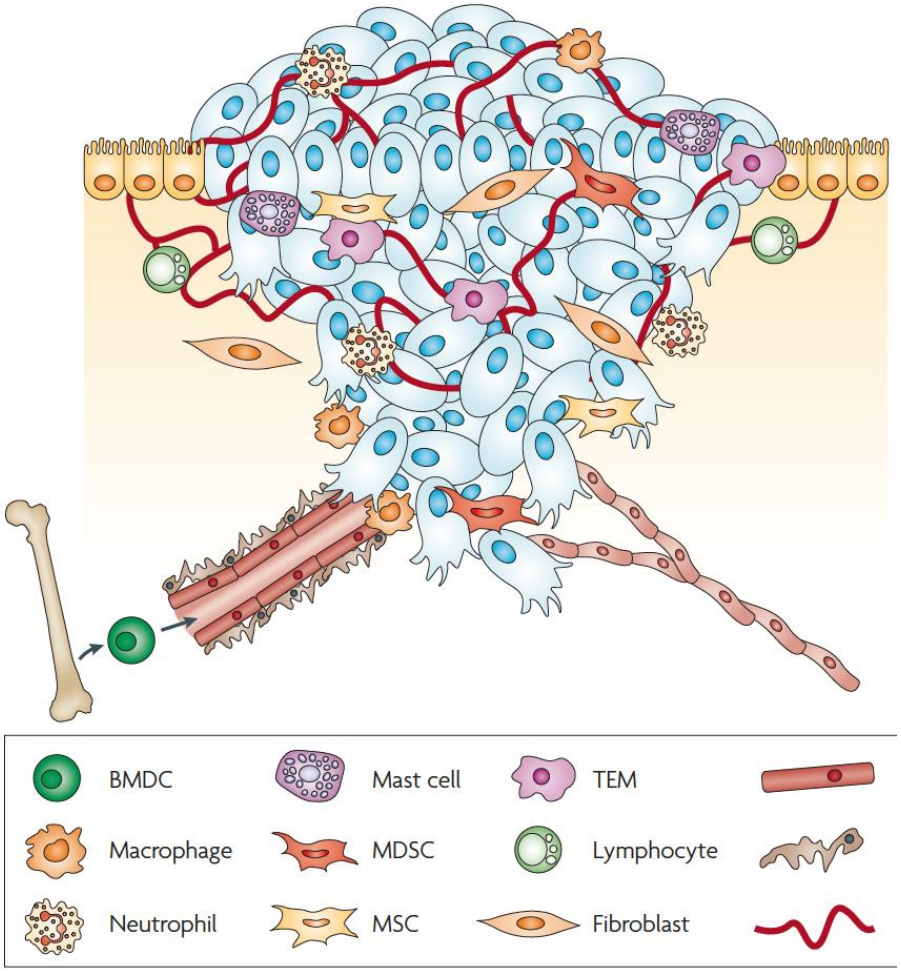
LOX and LOXL2 conclusions

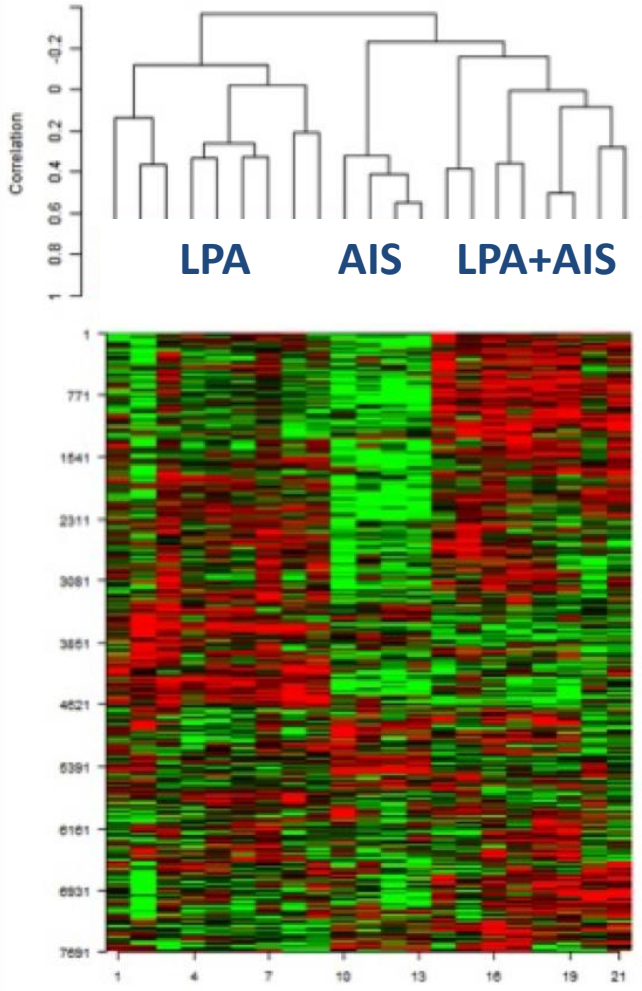
- These data confirm the association between adenocarcinoma invasive subtype and LOX and LOXL2 expression in lung adenocarcinoma.

- **Cellular expression of LOX in pre-resection biopsy specimens is associated with lung adenocarcinoma invasiveness.**

- Prospective validation of tumor biopsy LOX expression patterns in larger sample sets could lead to development of new lung cancer biomarkers. When confirmed, these data suggest a role for LOX immunostaining as a biomarker to predict tumor aggressiveness.

The tumor microenvironment modulates invasion and metastasis



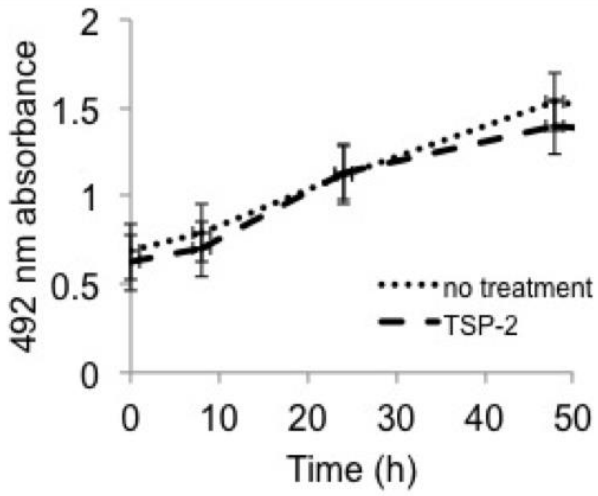


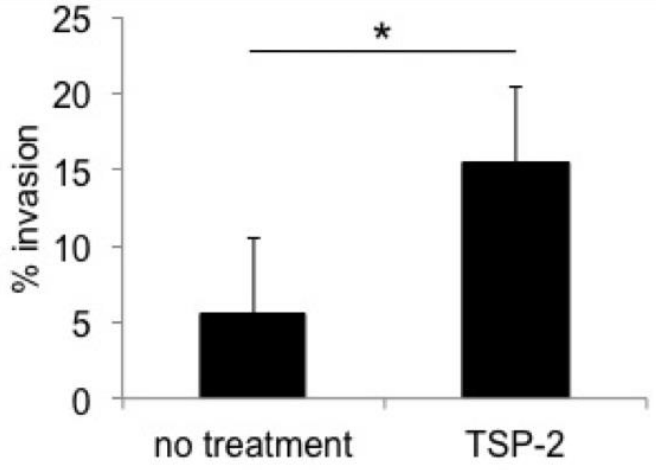
Joyce, *Nature Rev* Cancer, 2009; Powell, unpublished data

Thrombospondin-2 (TSP-2) is a stromal marker of invasion and poor prognosis

- TSP-2 is overexpressed in the stroma of human and murine invasive lung adenocarcinoma.

- Treatment of SKLU-1 cells with exogenous recombinant TSP-2 had no effect on cell proliferation but increased invasion through Matrigel membrane by 2.5-fold.

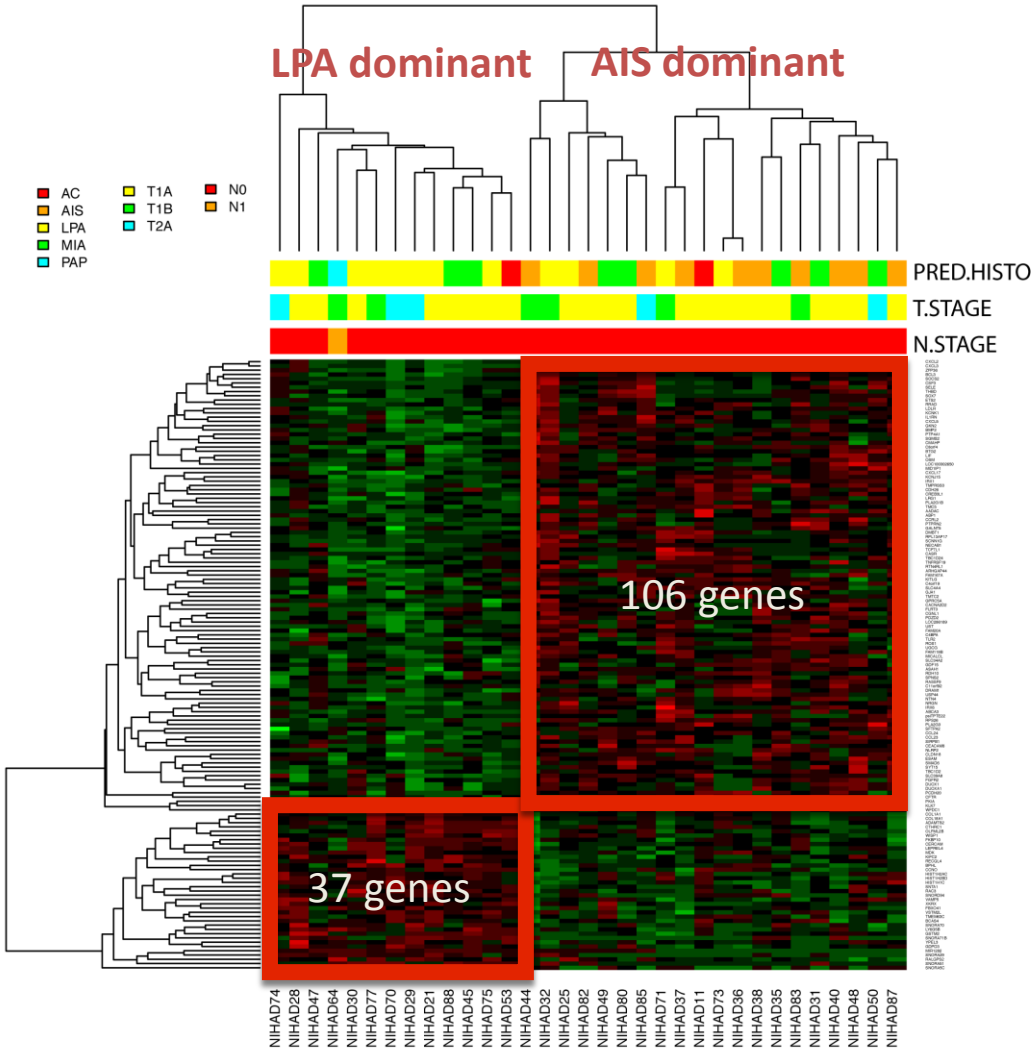




Powell, unpublished data

RNA Sequencing of invasive and noninvasive lung adenocarcinoma specimens reveals 143 differentially expressed genes

- Supervised clustering of differentially expressed genes for 12 LPA and 21 AIS lung adenocarcinoma specimens.
- Class comparison between invasive (LPA) and noninvasive (AIS) genes was restricted to a significance threshold p <0.01 and those genes with >1.5-fold difference in expression.



Yoo, Zhu, unpublished data

Clinical implications

- Biomarkers of invasion in lung adenocarcinoma, such as LOX and LOXL2, MDM2 and CDK4, and TSP-2 may be important mediators of tumor invasion and metastasis.

- High-throughput approaches such as RNA Sequencing will bring forth new targets for prospective validation in larger sample sets.

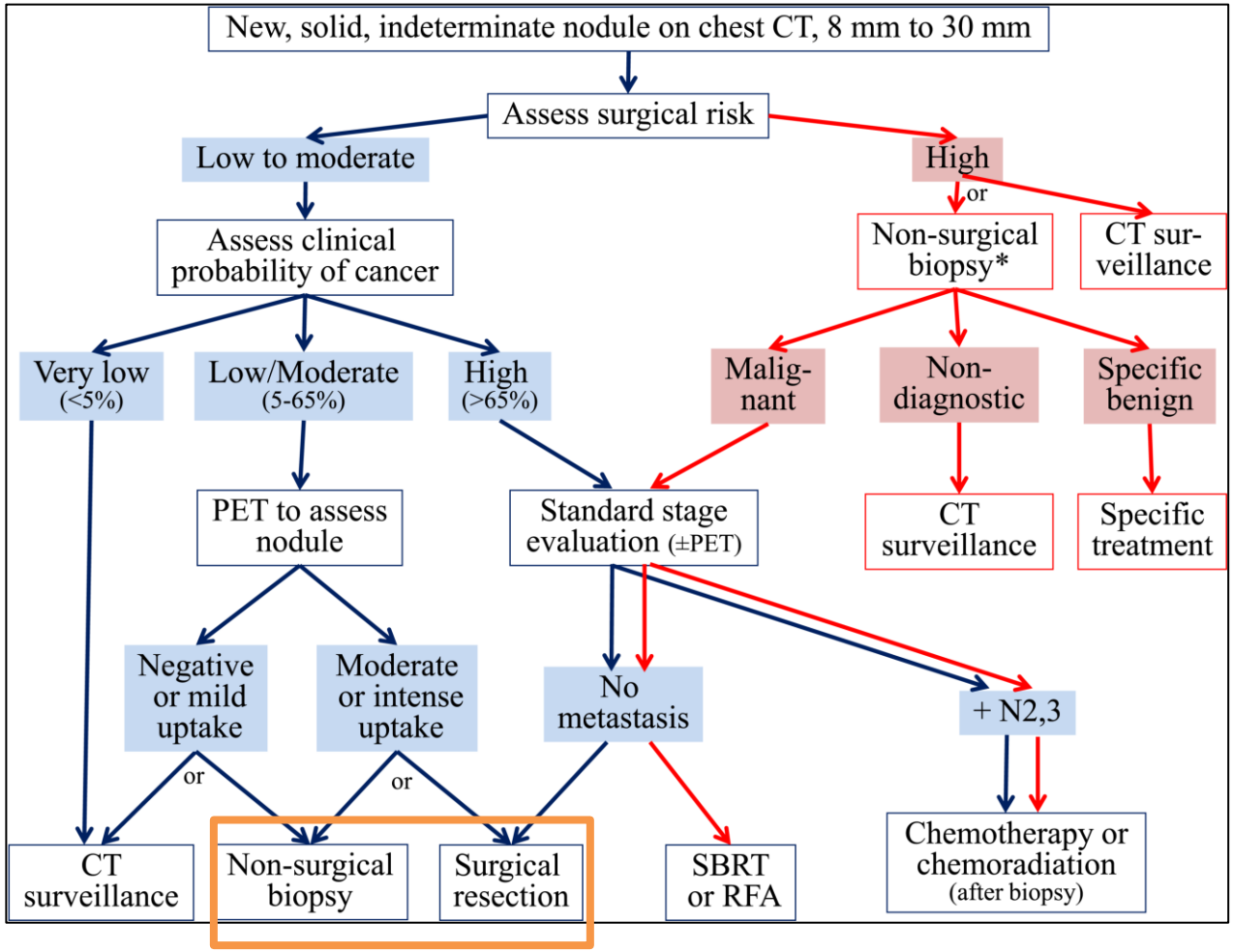
- **Early prediction of tumor invasion and aggressiveness can stratify risk and guide clinical decision-making regarding optimal therapy for small, early-stage lung cancers.**

Early Stage Lung Cancer: FNA as a diagnostic tool

What is an acceptable rate of non-malignant diagnoses in lung cancer resection surgery?

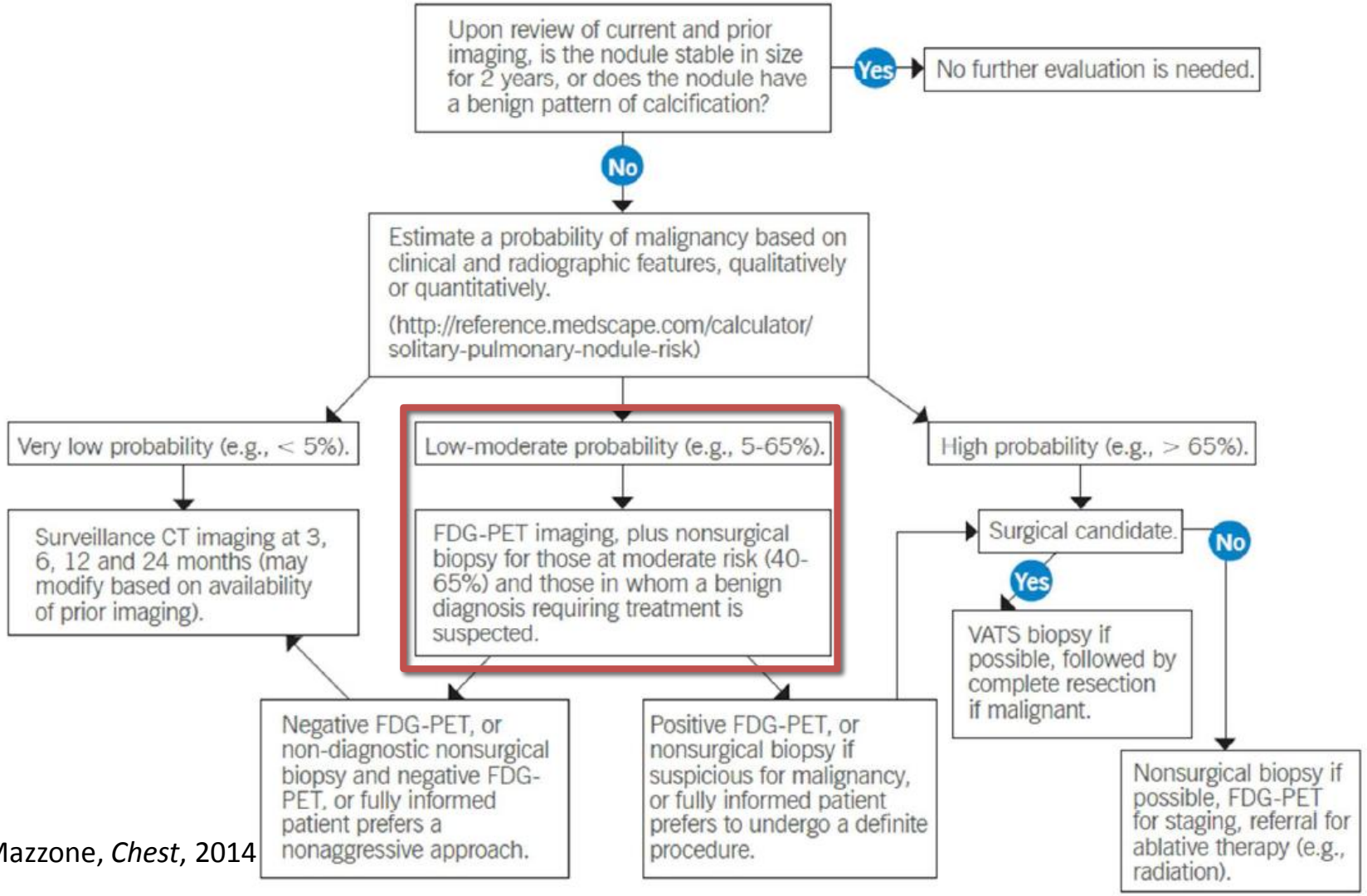
- Principle:
  - Minimize risks in resecting non-malignant lesions.
  - Maximize resection of all lung cancers that require resection.

What is the role of FNA in the diagnosis of early stage (screen-detected) suspected lung cancer?



Gould, *Chest,* 2013

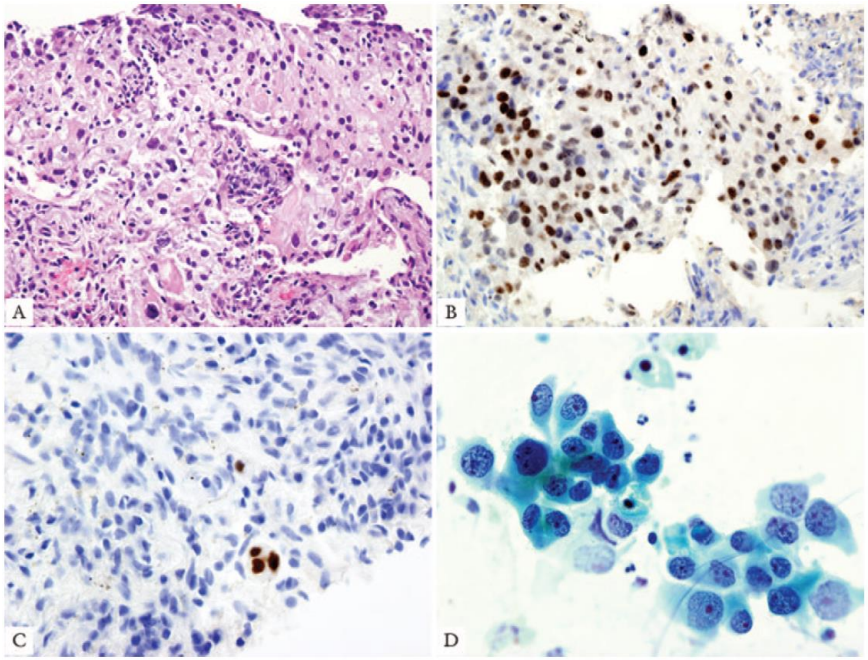
What is the role of FNA in the diagnosis of early stage (screen-detected) suspected lung cancer?



Mazzone, *Chest*, 2014

FNA utility and accuracy

- CT-guided FNA obtains a cytologic aspirate for:
  - H&E staining for cellular morphology
  - Immunohistochemistry for histologic subtyping
  - Cell block preservation for molecular analysis



- A pooled analysis of 24 studies of CT-guided transthoracic needle aspiration revealed:
  - Sensitivity of 0.92 (95% CI, 0.9-0.94)
  - Specificity of 0.97 (95% CI, 0.96-0.98)

Schreiber, *Chest*, 2003; Travis, *J Thoracic Onc,* 2011

Hypothesis

- **Pre-operative FNA performed in a high-volume lung cancer center will decrease the rate of surgical resection for non-malignant disease.**

- Objective: To retrospectively examine the non-malignant resection rate (NMRR) and determine concordance of FNA results and surgical resection pathology.

Methodology

- Consecutive thoracic operations performed by the Mount Sinai Department of Thoracic Surgery during the 6-month period between February 1, 2014 and August 1, 2014 for known or suspected first primary lung cancer presenting with a lung nodule or mass.

- Not included:
  - Patients with metastatic disease
  - Patients with prior lung cancer
  - Patients who received neoadjuvant chemotherapy prior to surgical resection

- Data extracted from EMR:
  - Patient demographics
  - Smoking history
  - Nodule characteristics
  - Thoracic operation data

Characteristics of entire cohort

Patient demographics (n=119)	
Age, mean ± SD, y	66.1 ± 11.2
Female, n	81 (64%)
BMI, mean ± SD	26.6 ± 5
Smoking history	
Never smoker, n	37 (29%)
Current smoker, n	20 (16%)
Former smoker, n	70 (55%)
Years smoked*, mean ± SD, y	34 ± 16.3
COPD or emphysema, n	54 (43%)
Personal history of cancer, n	39 (31%)

Nodule and operative characteristics (n=127)	
Nodule size, mean ± SD, mm	20.9 ± 11.7
PET scan performed, n	104 (82%)
PET SUVmax#, mean ± SD	4.3 ± 3.4
Thoracic operation	
VATS, n	94 (74%)
Thoracotomy, n	33 (26%)
Extent of resection	
Sublobar, n	74 (58%)
Lobectomy, n	53 (42%)

*Smoking history available for 120 of 127 current or formerly smoking subjects, with range 3-70 years

#SUVmax unavailable for 11 of 105 subjects who underwent PET

Characteristics of entire cohort

Cytology and pathology results	N (%)
Pre-operative core biopsy	9 (7%)
FNA or core biopsy categorization (n=78, 61%)	
No pre-operative FNA performed	49 (39%)
Malignant	62 (49%)
Suspicious	8 (6%)
Atypical	3 (2%)
Nondiagnostic	2 (1%)
Benign	3 (2%)
Resection pathology	
Primary lung neoplasm	107 (84%)
Non-malignant disease	20 (16%)

*PET avidity defined as SUVmax >2

Preliminary results

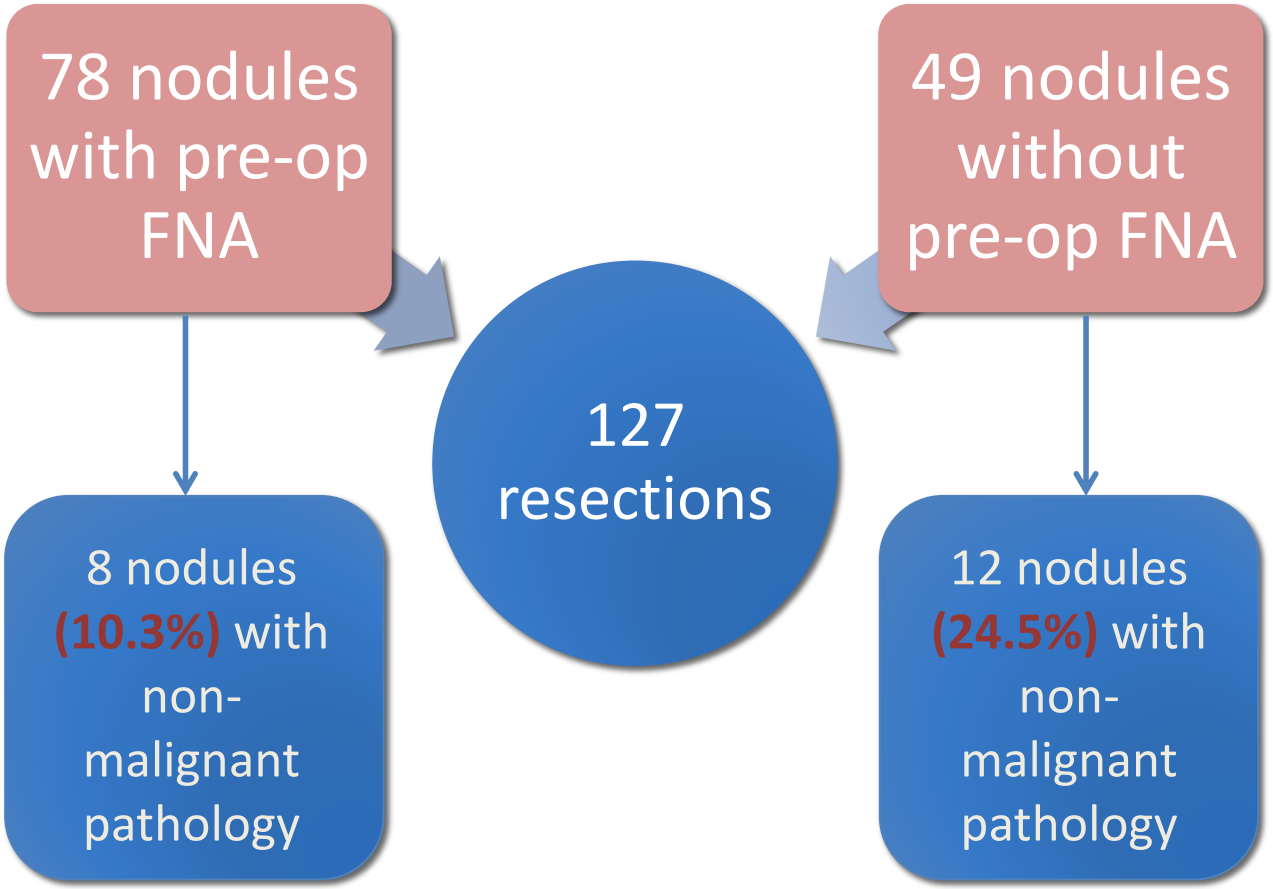
- 127 surgical resections from 119 patients among 10 cardiothoracic surgeons

- Overall non-malignant resection rate

  (NMRR) = 20/127 **(15.7%)**

  - Among 10 surgeons, the non-malignant resection rate varied from 6.5% to 36%.

NMRR is decreased in patients with a pre-operative diagnostic FNA

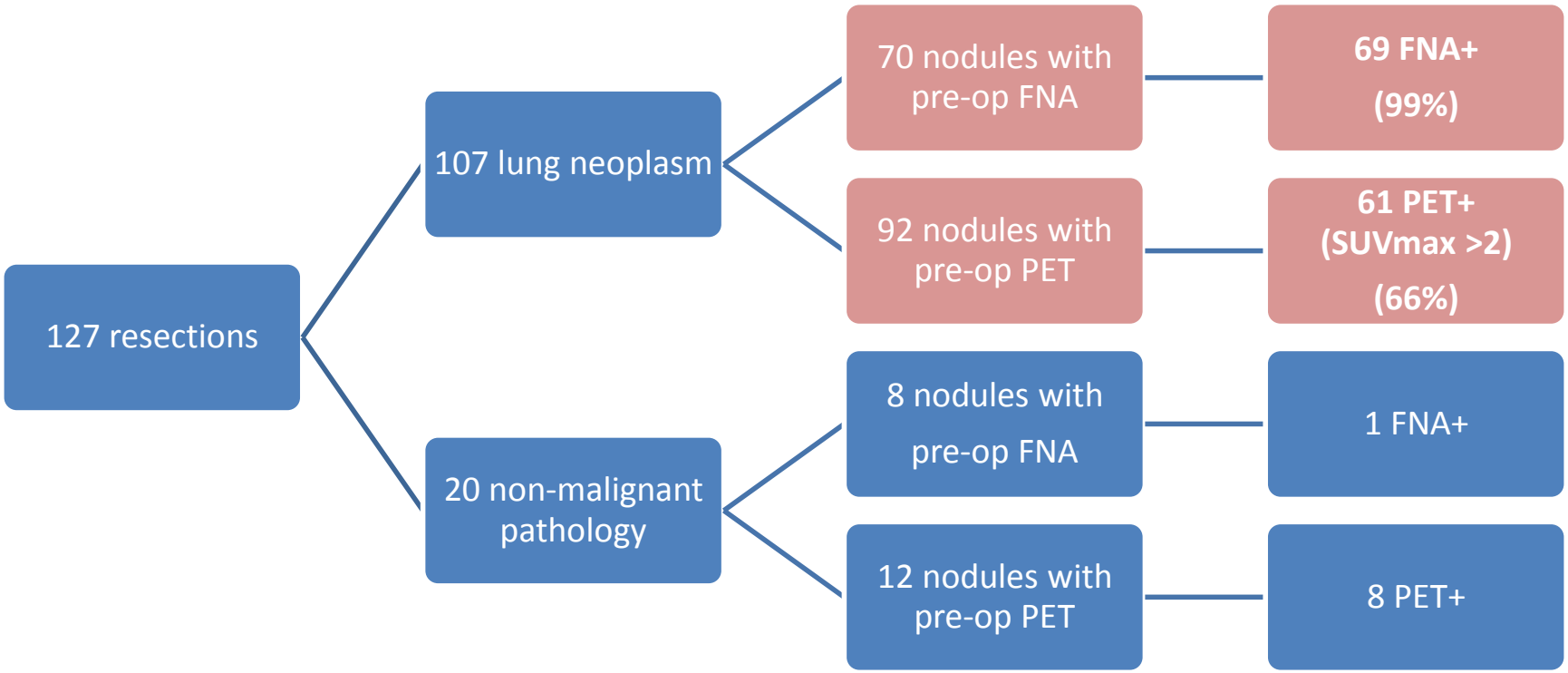


		Resection pathology				
		Malignant (107)	Non-malignant (20)		Malignant (107)	Non-malignant (20)
Pre-operative diagnostic testing	**FNA/ Core Positive* (70)**	**69**	**1**	**PET+#**	**41**	**0**
				PET-	**23**	**1**
				No PET	**5**	**0**
	FNA Atypical (3)	1	2	**PET+**	0	1
				PET-	0	1
				No PET	1	0
	FNA/ Core Benign (3)	0	3	**PET+**	0	3
				PET-	0	0
	FNA/ Core Nondiagnostic (2)	0	2	**PET+**	0	0
				PET-	0	1
				No PET	0	1
	FNA Not done (49)	**37**	**12**	**PET+**	**20**	**4**
				PET-	**8**	**1**
				No PET	**9**	**7**

*FNA positive includes Malignant and Suspicious categorizations.

#PET+ defined by SUVmax >2; PET- by SUVmax ≤2.

A positive FNA result more accurately predicts resection pathology compared with PET



Characteristics of patients with non-malignant pathology on resection

Patient demographics (n=20)	
Age, mean ± SD, y	58.2 ± 11.6
Female, n	10 (50%)
BMI, mean ± SD	27.8 ± 5.6
Smoking history	
Never smoker, n	13 (65%)
Current smoker, n	1 (5%)
Former smoker, n	6 (30%)
Years smoked*, mean ± SD, y	21.5 ± 7.4
COPD or Emphysema, n	5 (25%)
Personal history of cancer, n	7 (35%)

Nodule and operative characteristics (n=20)	
Nodule size, mean ± SD, mm	15.7 ± 8
PET scan performed, n	12 (60%)
PET SUVmax#, mean ± SD	3.1 ± 1.5
Thoracic operation	
VATS, n	17 (85%)
Thoracotomy, n	3 (15%)
Extent of resection	
Wedge resection, n	18 (90%)
Lobectomy, n	2 (10%)

*Smoking history available for 4 of 7 current or formerly smoking subjects, with range 5-43 years
#SUVmax available for 11 of 12 subjects who underwent PET, with range 1.4-6.

Cytology and pathology results, n	N (%)
Pre-operative core biopsy (nondx or benign)	2 (10%)
FNA categorization	
No pre-operative FNA performed	12 (60%)
Malignant	1 (5%)
Suspicious	0 (0%)
Atypical	2 (10%)
Nondiagnostic	1 (5%)
Benign	2 (10%)
Resection pathology	
Granulomatous disease	12 (60%)
Other	6 (25%)
Organizing pneumonia	2 (10%)
Intra-parenchymal lymph node	1 (5%)
Interstitial/ peribronchial inflammation	1 (5%)
Lymphoplasmacytic infiltrates	1 (5%)
Apical cap	1 (5%)
Benign tumors (hamartoma, inflammatory myofibroblastic tumor)	2 (10%)

*PET avidity defined as SUVmax >2; PET status unknown for 3 patients.

FNA Conclusions

- Practice patterns vary widely among cardiothoracic surgeons within a single lung cancer center.
- Non-malignant resection rate is lower in patients who have a pre-operative FNA, compared with those who do not.
- FNA more accurately predicts resection pathology compared with PET among patients who undergo resection for a lung neoplasm.
  - FNA can provide a definitive diagnosis and allows for further molecular studies to be performed on biopsy specimens.

Future directions

Additional tumor biology questions

- What are additional pathways important in acquisition of tumor invasiveness and metastasis, and can targeted therapies effectively halt tumor growth and invasion?

- Targets for immune activation and checkpoint inhibition (anti-PD1 and anti-PDL1 antibodies).

- These and other biomarkers can be investigated in neoadjuvant window of opportunity clinical trials of targeted therapeutics.

Additional clinical questions

- How should screen-detected non-solid lung nodules be managed?
  - How quickly do they grow? Can they be observed?
  - Should sub-solid nodules be resected once a solid component develops?
  - Do biomarkers of invasiveness predict outcome and guide clinical decision making?

- What is the role of limited surgical resection?
  - Do wedge resection and segmentectomy have similar outcomes when compared with lobectomy?
  - Which nodules (size, location, density) are most amenable to sub-lobar resection?

Summary points

- Pathways for lung cancer metastasis and invasion provide potential targets for therapy and biomarkers by which to guide prognostication and clinical decision-making.

- Clinical workup of nodules suspicious for lung cancer requires further investigation to determine the most accurate diagnostic modalities based upon a priori risk.

- Lung cancer screening for high-risk current and former smokers will further highlight these controversies through a sharp increase in the number of early stage lung cancers. Tools that allow us to distinguish indolent from aggressive tumors will maximize benefit and reduce harms from lung cancer screening.

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