新しい肺癌WHO分類（第4版）の要点と問題点

筑波大学医学医療系診断病理学
野口雅之

第28回細胞診従事者講習会　2016.3.6.

2015 WHO CLASSIFICATION OF THE PATHOLOGY AND GENETICS OF TUMORS OF THE LUNG

LUNG CANCER: IASLC GLOBAL INITIATIVES

September 8, 2015

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IASLC 40 YEARS

WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart

Edited by
William D. Travis, Elisabeth Brambilla, Allen P. Burke, Alexander Marx, Andrew G. Nicholson

WHO

http://whobluebooks.iarc.fr/

WHO Booth #403– Exhibition Hall

INCREASING COMPLEXITY

- 1967 WHO
- 1981 WHO
- 1999 WHO
- 2004 WHO

- 2015 WHO

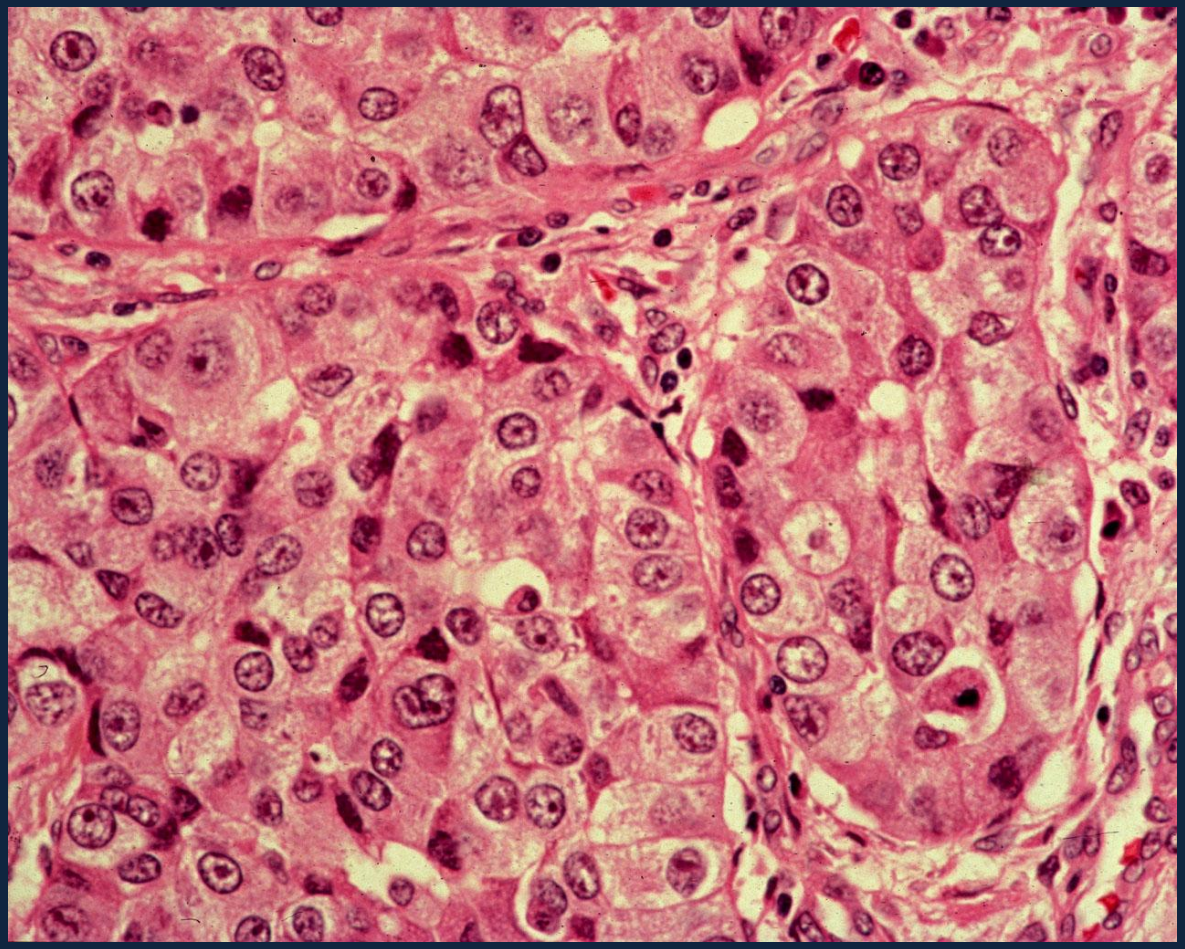
- H&E
- H&E & Mucin
- H&E, EM & IHC
- H&E, EM, IHC & Genetics
- H&E, Cytology, IHC, Genetics, Mucin, Radiology

INCREASING RELEVANCE FOR PERSONALIZED MEDICINE

Major Changes in Classification that Impact Diagnosis of Surgically Resected Patients

- Main advances in lung adenocarcinoma: adopted the 2011 IASLC/ATS/ERS Lung Adenocarcinoma classification
- Restrict large cell carcinoma to tumors lacking clear differentiation by both IHC and morphology
- Reclassify squamous cell ca: keratinizing, nonkeratinizing and basaloid
- Group NE tumors together (TC,AC, LCNEC, SCLC)

LARGE CELL CARCINOMA



Diagnosis can only be made in a resection specimen

2015 WHO CLASSIFICATION: WHERE WILL THE LARGE CELL CARCINOMAS GO?

- Pneumocyte marker (TTF-1) positive LCC → solid adenoca
- Squamous marker positive (p40) LCC → nonkeratinizing squamous cell ca
- Large cell neuroendocrine carcinoma
  - Combined LCNEC → NE tumors
- Basaloid carcinoma → Squamous ca

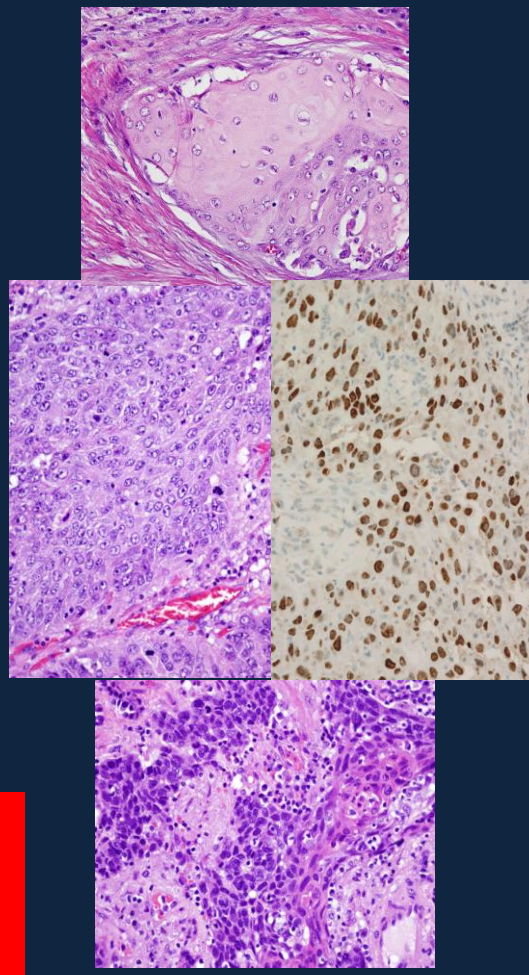
LARGE CELL CARCINOMA
2015 WHO Classification

- Large cell carcinoma with null immunohistochemical features and no mucin
- Large cell carcinoma with unclear immunohistochemical features
- Large cell carcinoma with no stains available

2015 WHO CLASSIFICATION SQUAMOUS CELL CARCINOMA

- Keratinizing
- Non-keratinizing
- Basaloid carcinoma

now need IHC –
P40 positive,
TTF-1 negative



now need IHC –
(+p40, -TTF1 & NE markers)
r/o LCNEC & SCLC

2015 WHO CLASSIFICATION NEUROENDOCRINE TUMORS

- Small cell carcinoma
  - Combined SCLC
- Large cell neuroendocrine carcinoma
  - Combined LCNEC
- Carcinoid tumor
  - Typical carcinoid
  - Atypical carcinoid

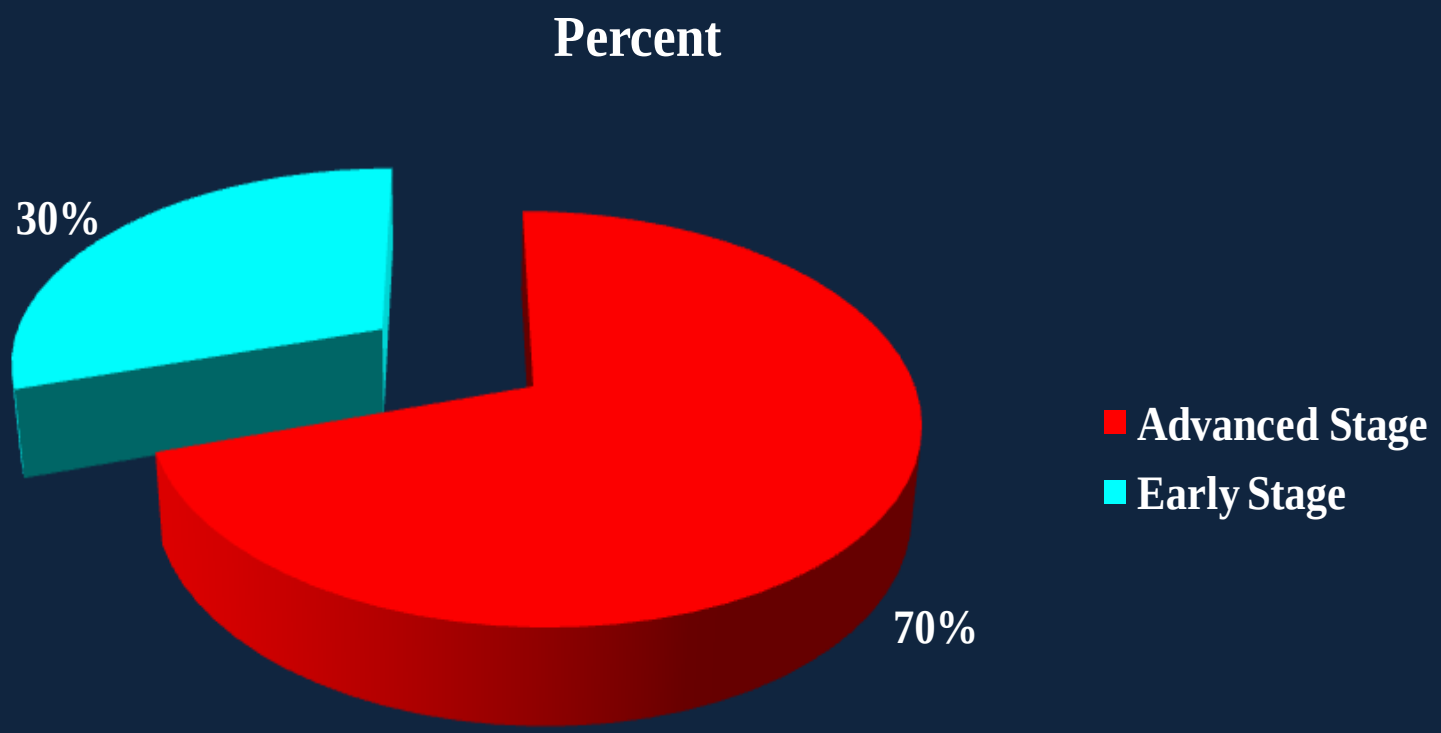
OTHER EVOLVING TOPICS RELATED TO COMPREHENSIVE HISTOLOGIC SUBTYPING OF LUNG ADENOCARCINOMA

- Cribriform pattern – poor prognosis
- Comparing multiple tumors
- Grading – architectural, second pattern, nuclear, mitoses, tumor budding
- Molecular – histologic correlations

2015 WHO Classification of Lung Cancer

- WHO is the team?
- WHERE was WHO developed?
- WHAT is a WHO classification?
- HOW will the 2015 WHO have an impact on management of
  - Advanced Lung Cancer?
  - Resected Lung Cancers?
- WHEN can WHO be updated (future work)?

NON-SMALL CELL LUNG CANCER: 70% PRESENT IN ADVANCED STAGE



2015 Classification: Impact on Management of Advanced Lung Cancer Patients

Criteria/terminology for small bx/cytology
More accurate histologic subtyping
Strategic management of small tissues
Streamlining workflow for molecular testing
Need for local multidisciplinary team

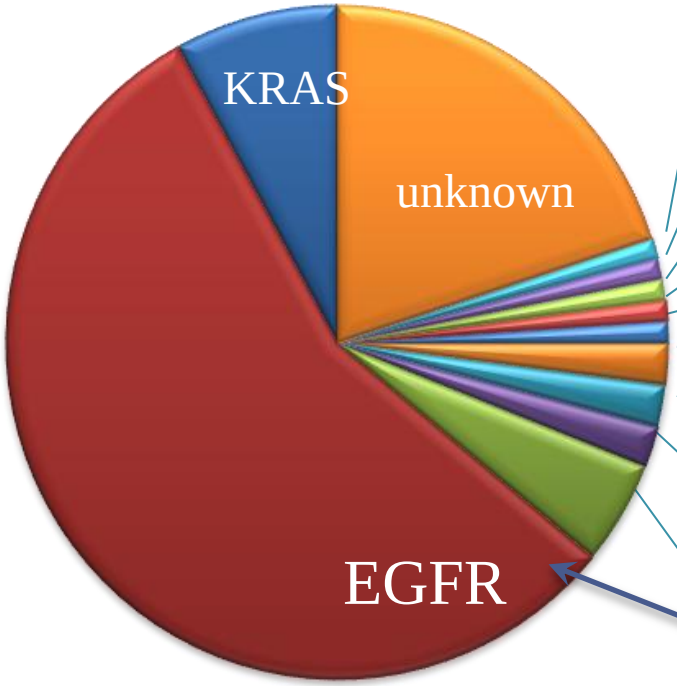
THERAPEUTIC ADVANCES IMPACTED NEED FOR MORE ACCURATE HISTOLOGIC DIAGNOSIS AND MOLECULAR TESTING

- Predictive of response
  - *EGFR* mutation (adenoca) – TKI's
  - Adenoca or NSCC-NOS – pemetrexed
  - *ALK* fusion (adenoca)- crizotinib
- Predictive of toxicity
  - Bevacizumab – contraindicated in life-threatening hemorrhage in squamous carcinoma

遺伝子診断の持つ意味

Approved and Potentially effective molecular targeting agents（肺腺癌）

日本人での遺伝子異常頻度



RET fusion*
ROS1 fusion
NRAS
MAP2K1
AKT1
PIK3CA
BRAF
HER2
ALK fusions

Vandetanib, Sorafenib, Cabozantinib
Crizotinib
Selumetinib
Temsirolimus
Vemurafenib
Lapatinib
Crizotinib
Gefitinib, Erlotinib, Afatinib

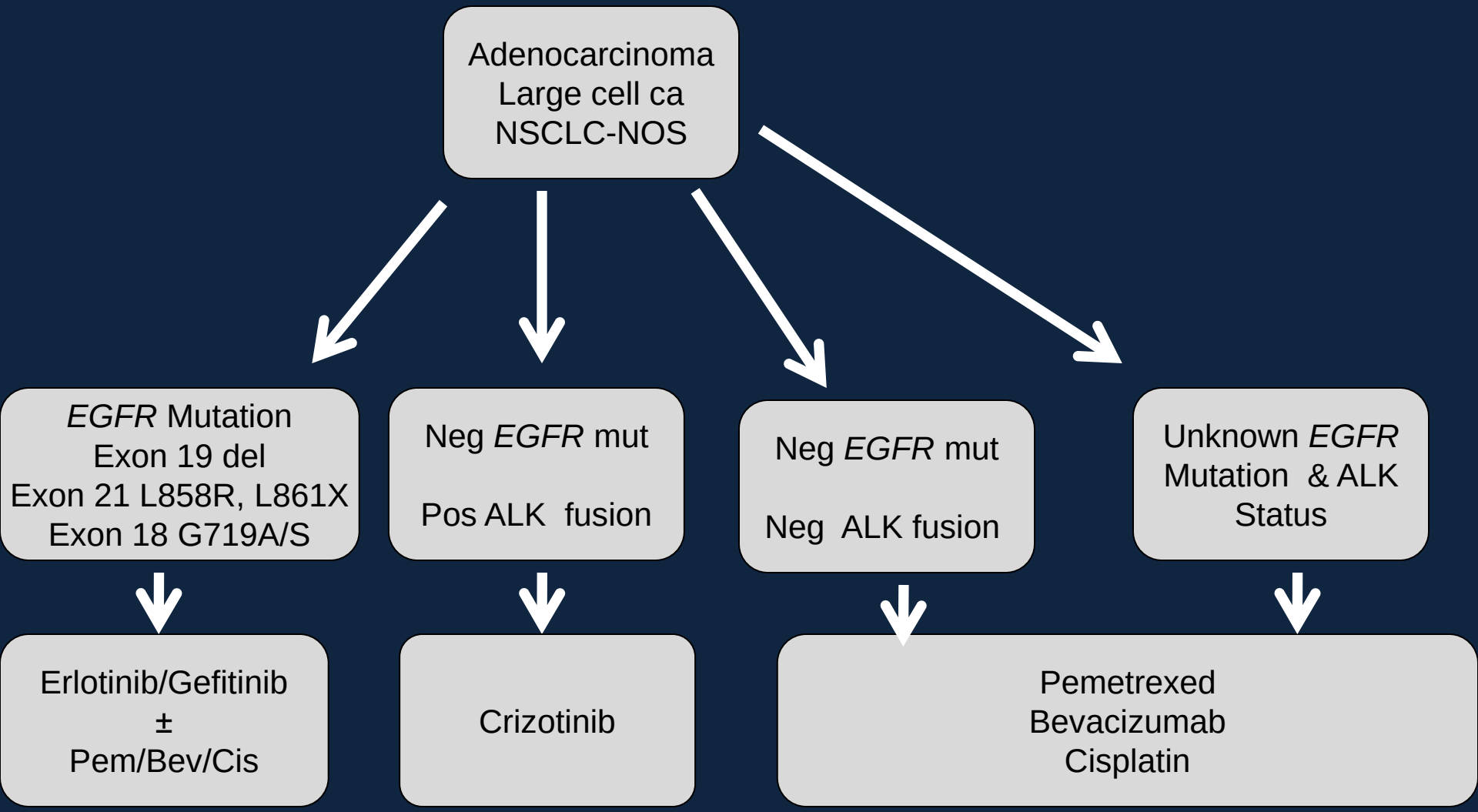
*Kohno T et al, Nature Medicine 18; 375-77, 2012

DRIVER MUTATIONS ARE TARGETS FOR MOLECULAR BASED THERAPY

Target	Drug
EGFR	Erlotinib Afatinib
ALK fusions	Crizotinib Ceritinib
BRAF V600E	Dabrafenib
ROS1 fusions	Crizotinib
RET fusions	Cabozantinib
MET splice site Exon 14 mutations	Cabozantinib (and crizotinib)

Courtesy of Greg Riely

Treatment of Advanced NSCLC is based on Histology and Genetics



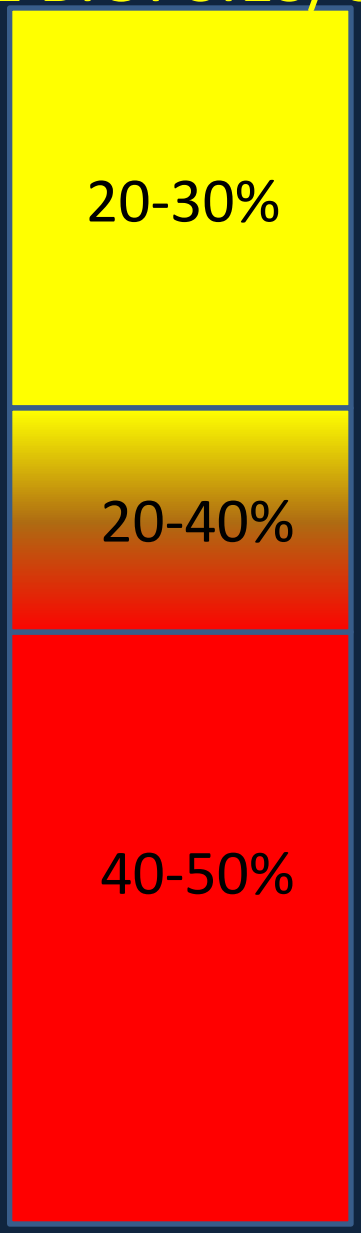
Modified from Mark Kris, Thoracic Oncology, MSKCC

NSCLC DIAGNOSED BY LIGHT MICROSCOPY IN SMALL BIOPSIES/CYTOLOGY

SQUAMOUS CELL CARCINOMA

NSCLC-NOS

ADENO-CARCINOMA



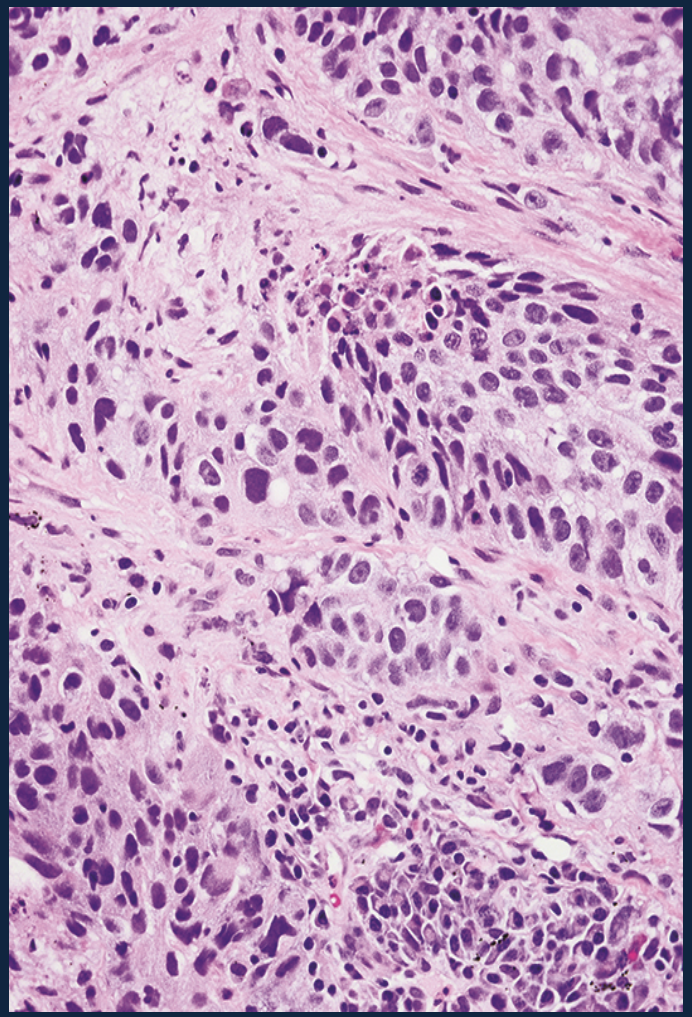
HISTORICALLY NSCLC-NOS HAS BEEN ENCOURAGED BECAUSE THERE WAS NO REASON TO CLASSIFY THESE TUMORS FURTHER

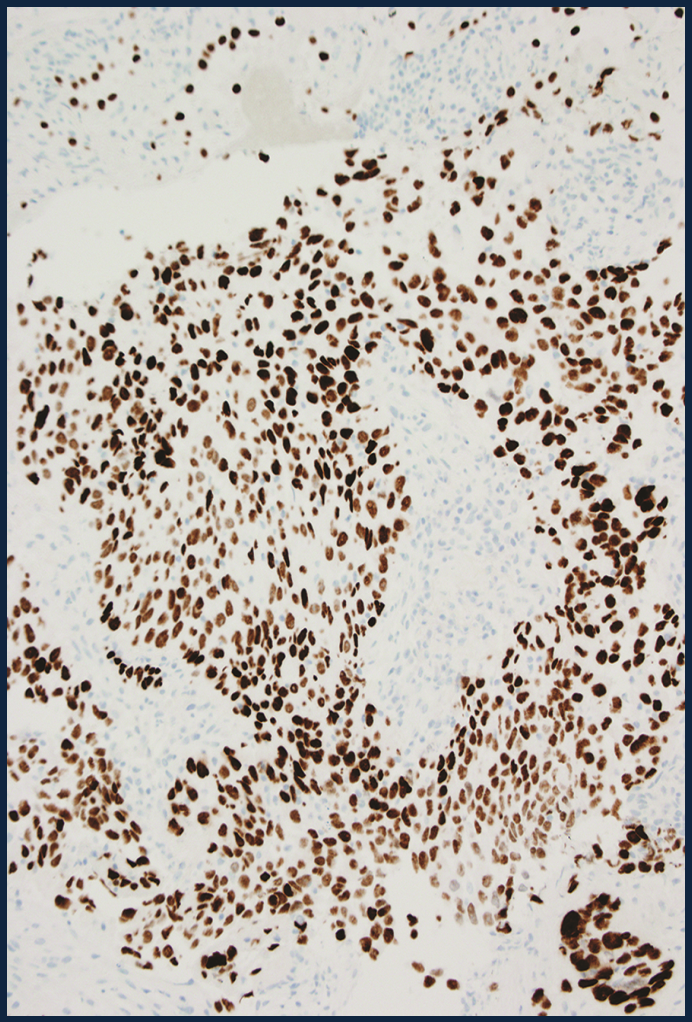
AS A RESULT 20-40% OF NSCLC IN SMALL BIOPSIES/CYTOLOGY ARE CURRENTLY BEING DIAGNOSED AS NSCLC-NOS

2015 WHO TERMINOLOGY FOR SMALL BIOPSIES AND CYTOLOGY

2015 WHO Resections	Small Biopsy/Cytology
ADENOCARCINOMA **Lepidic** **Acinar** **Papillary** **Micropapillary** **Solid**	***Morphologic adenocarcinoma patterns clearly present:*** **Adenocarcinoma, describe identifiable patterns present**
No 2004 WHO counterpart – most will be solid adenocarcinomas	***Morphologic adenocarcinoma patterns not present (supported by special stains; i.e TTF-1 +; p40 -):*** **Non-small cell carcinoma, favor adenocarcinoma**
SQUAMOUS CELL CARCINOMA **Keratinizing** **Nonkeratinizing** **Basaloid**	***Morphologic squamous cell patterns clearly present:*** **Squamous cell carcinoma**
No 2004 WHO counterpart	***Morphologic squamous cell patterns not present (supported by stains; i.e. p40+, TTF-1 -):*** **Non-small cell carcinoma, favor squamous cell carcinoma**
LARGE CELL CARCINOMA	**Non-small cell carcinoma, not otherwise specified (NOS)**

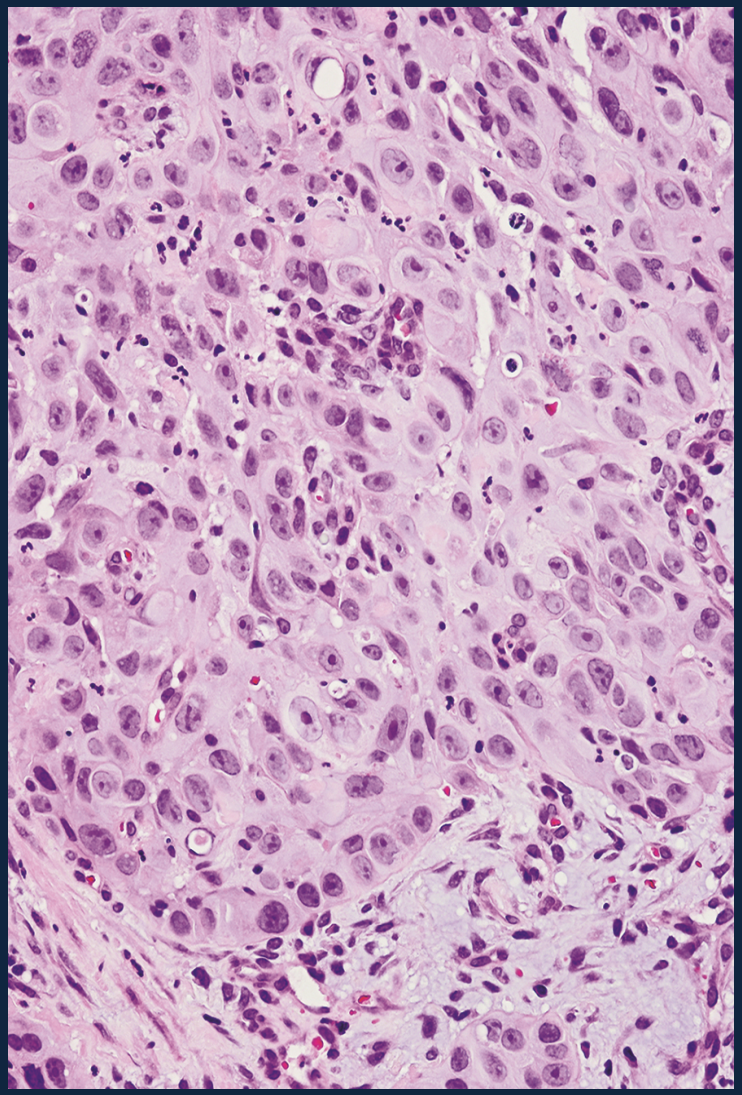
Nonsmall cell carcinoma, favor squamous cell carcinoma

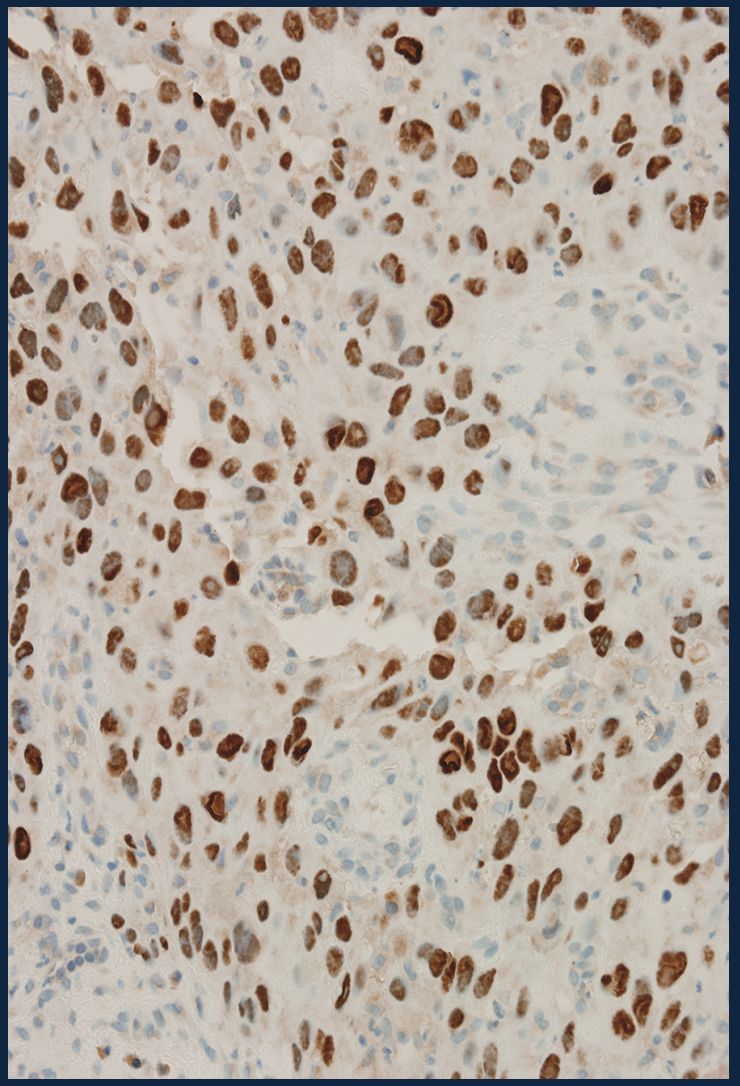




P40 (TTF-1 was negative)

NONSMALL CELL CARCINOMA, FAVOR ADENOCARCINOMA





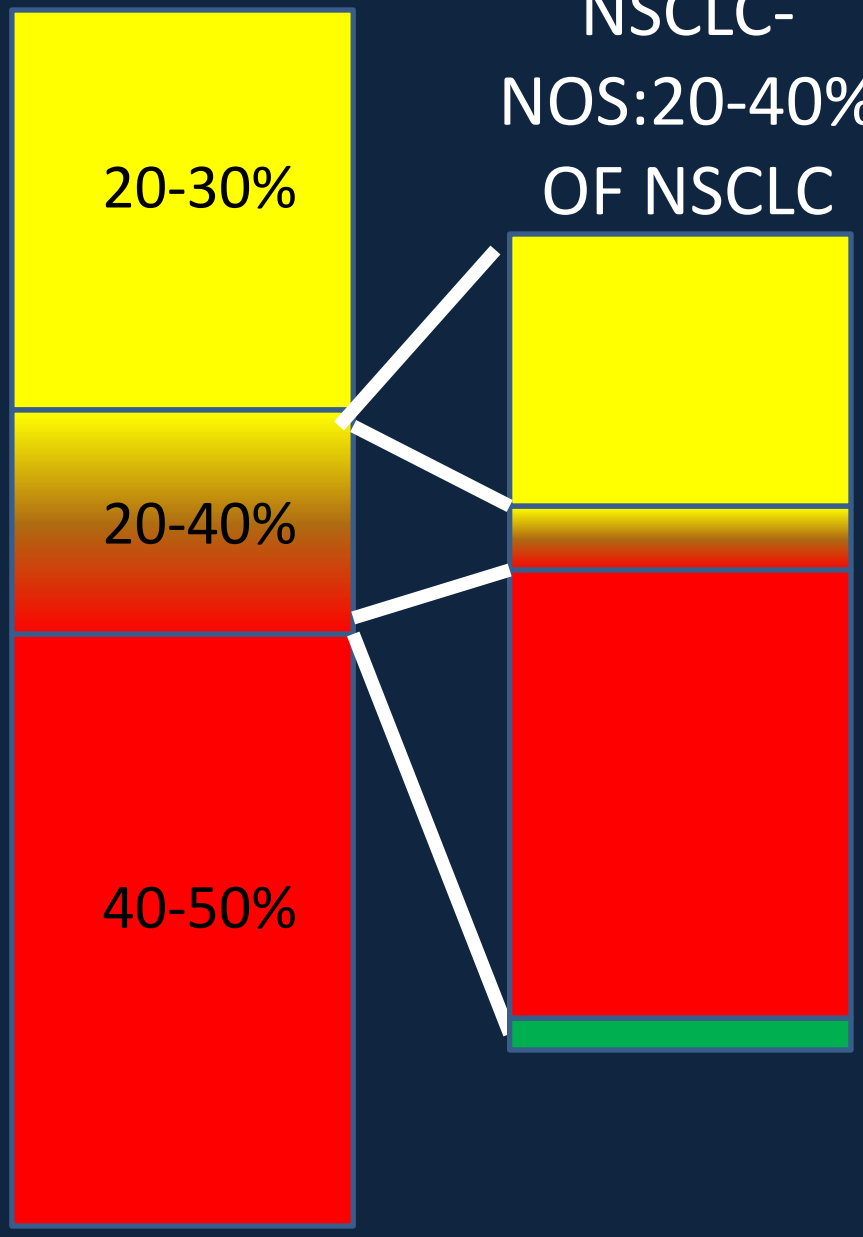
TTF-1 (p40 was negative)

LIGHT MICROSCOPY

SQUAMOUS CELL CARCINOMA

NSCLC-NOS

ADENO-CARCINOMA



FORMER NSCLC-NOS:20-40% OF NSCLC

NEW CLASSIFI-CATION

NSCLC, FAVOR SQUAMOUS CELL CARCINOMA

NSCLC-NOS <5%

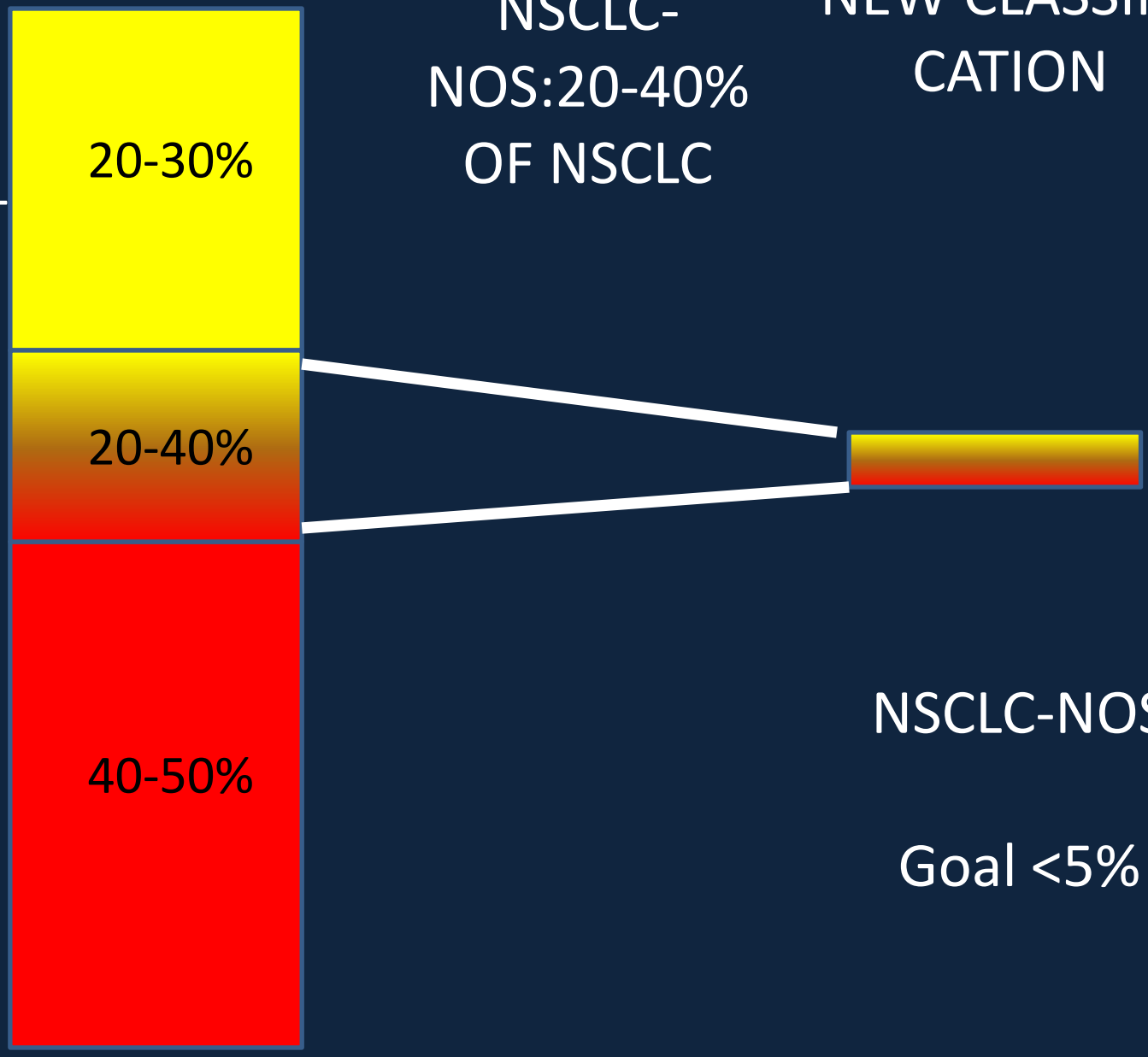
NSCLC, FAVOR ADENO-CARCINOMA

METASTASIS OR OTHER TUMOR

LIGHT MICROSCOPY

FORMER NSCLC-NOS:20-40% OF NSCLC

NEW CLASSIFI-CATION



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IASLC PATHOLOGY COMMITTEE

March 1, 2013; Baltimore, MD

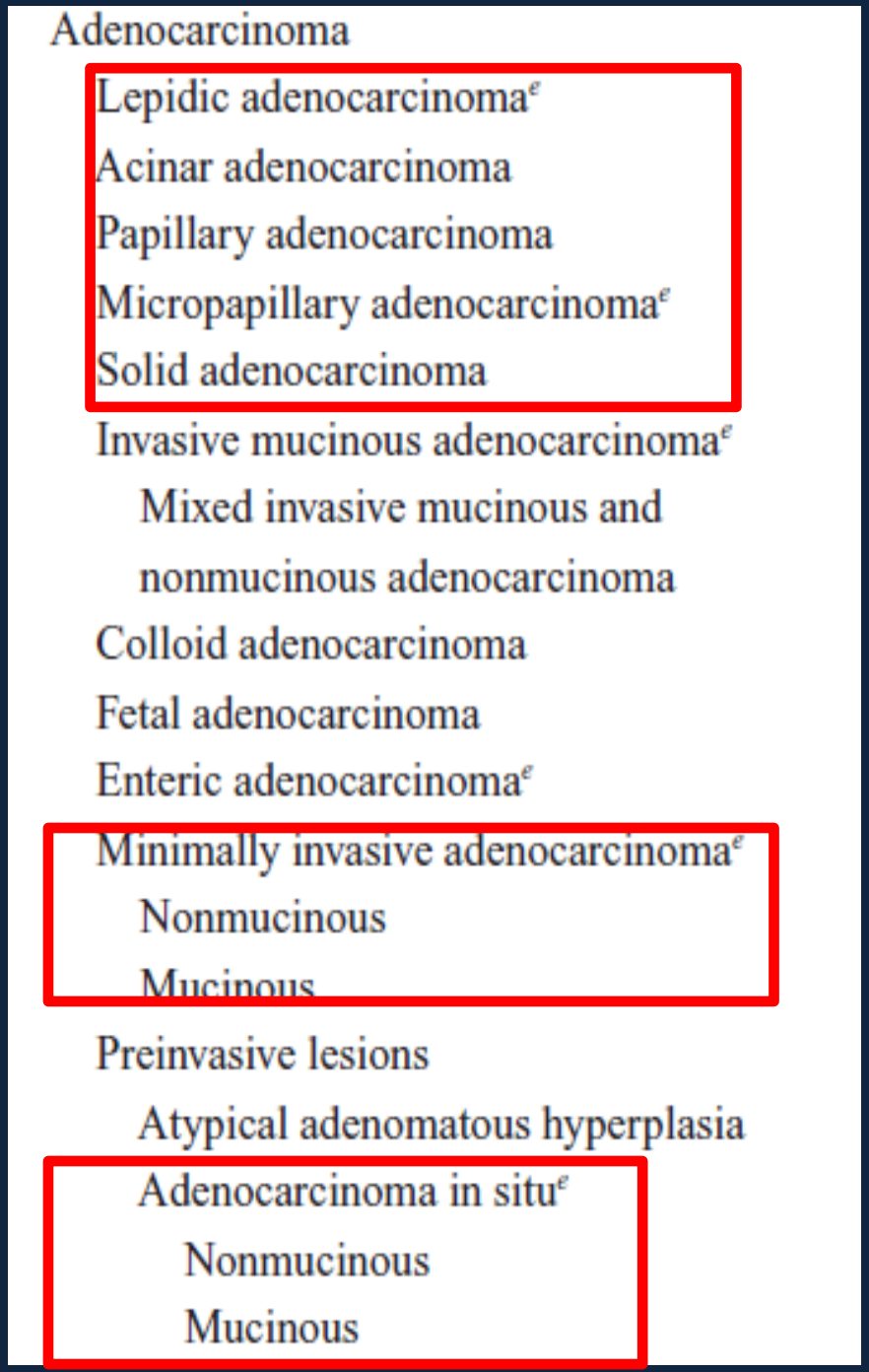
WHERE WAS WHO DEVELOPED?

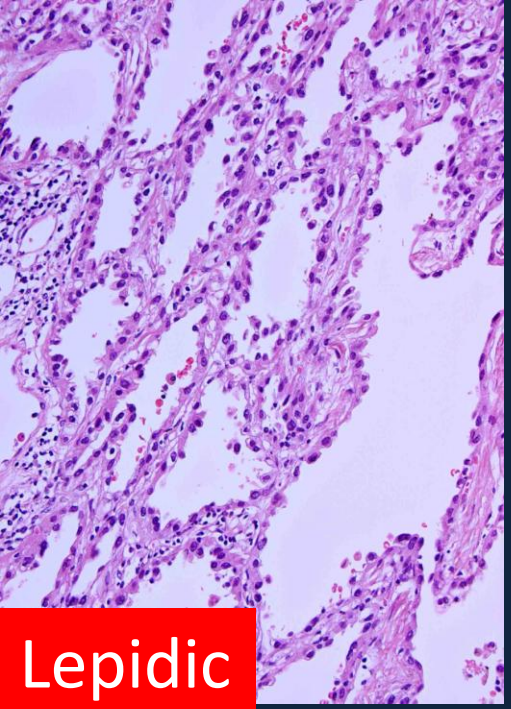
157 Authors from 29 countries

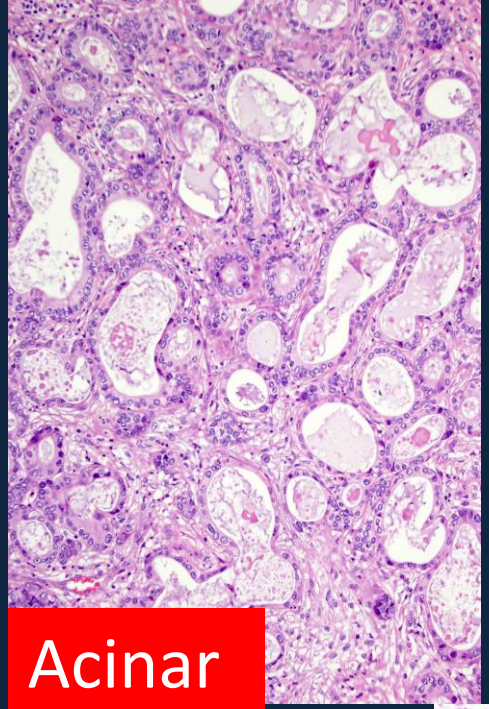
How does classification help management of surgically resected ADC patients?

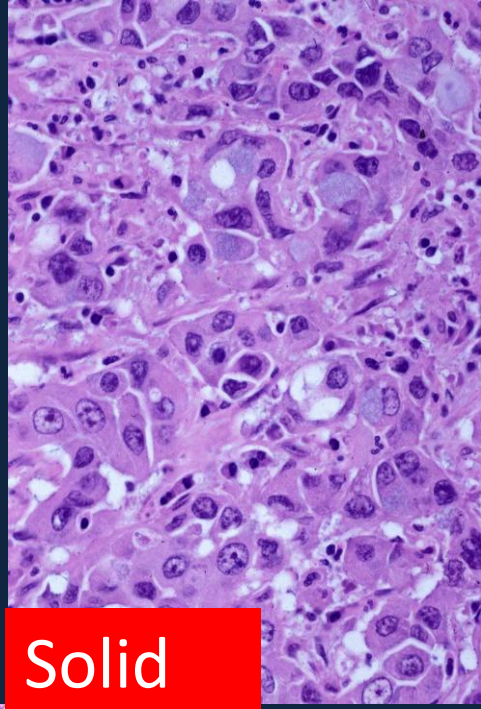
- Predicts survival and recurrence
- Predicts survival benefit with adjuvant cisplatin based chemotherapy
- Defines AIS & MIA: 100% & near 100% survival if completely resected
- Allows radiologic pathologic correlations
- Impacts TNM Staging
  - Invasive size
  - Comparing multiple tumors

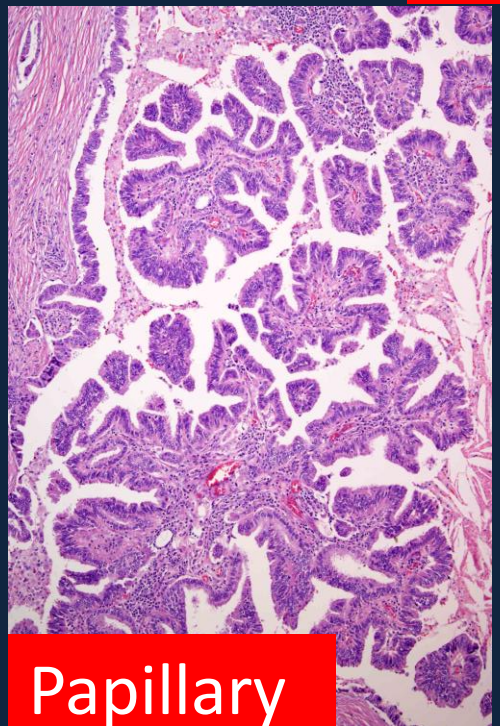
2015 WHO CLASSIFICATION OF LUNG ADENOCARCINOMA

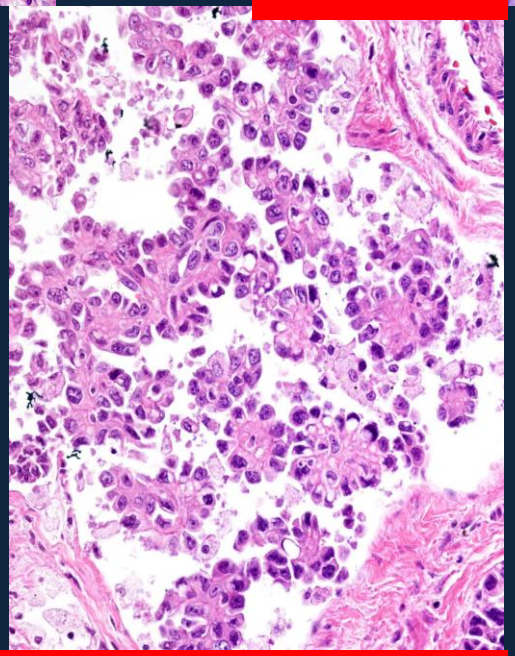



Lepidic

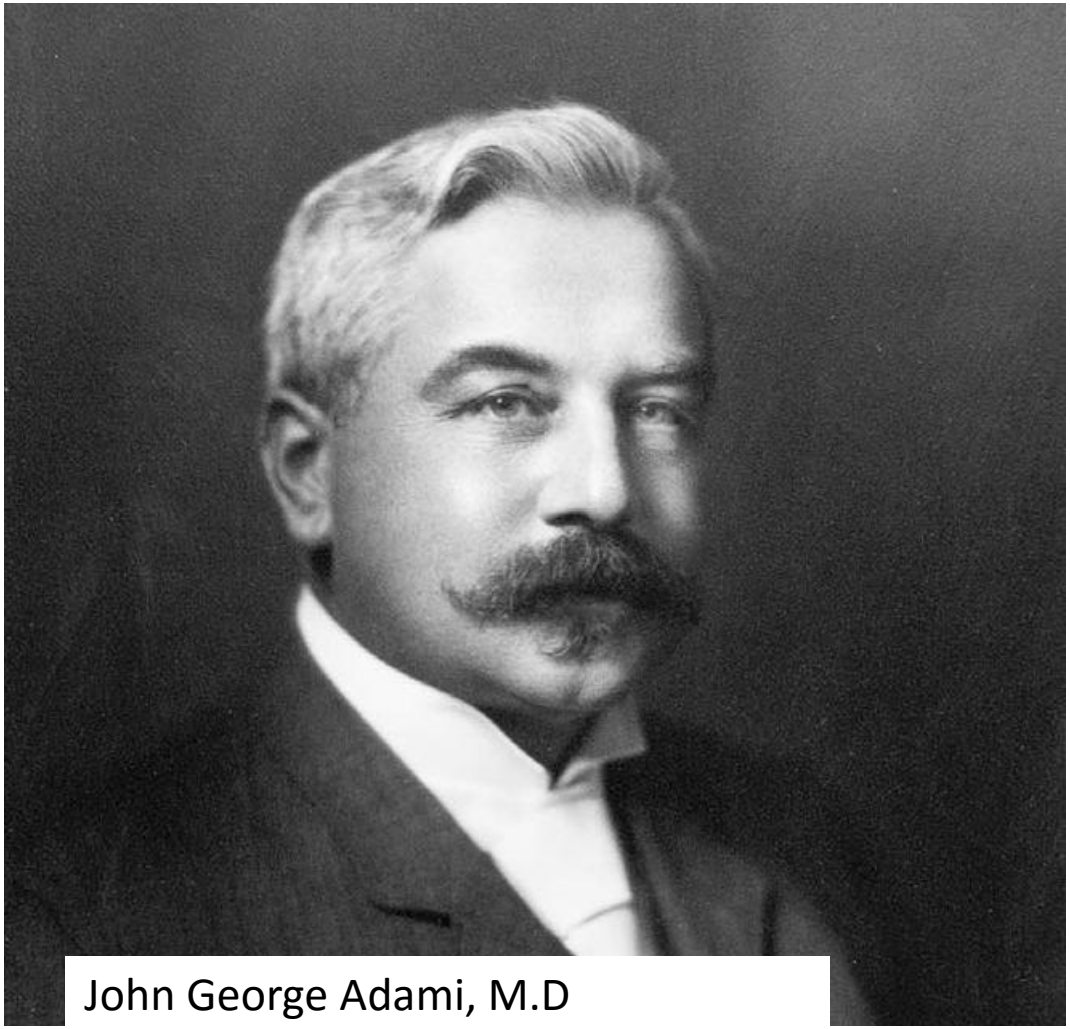

Acinar


Solid


Papillary


Micropapillary

Whence *Lepidic* ?



John George Adami, M.D

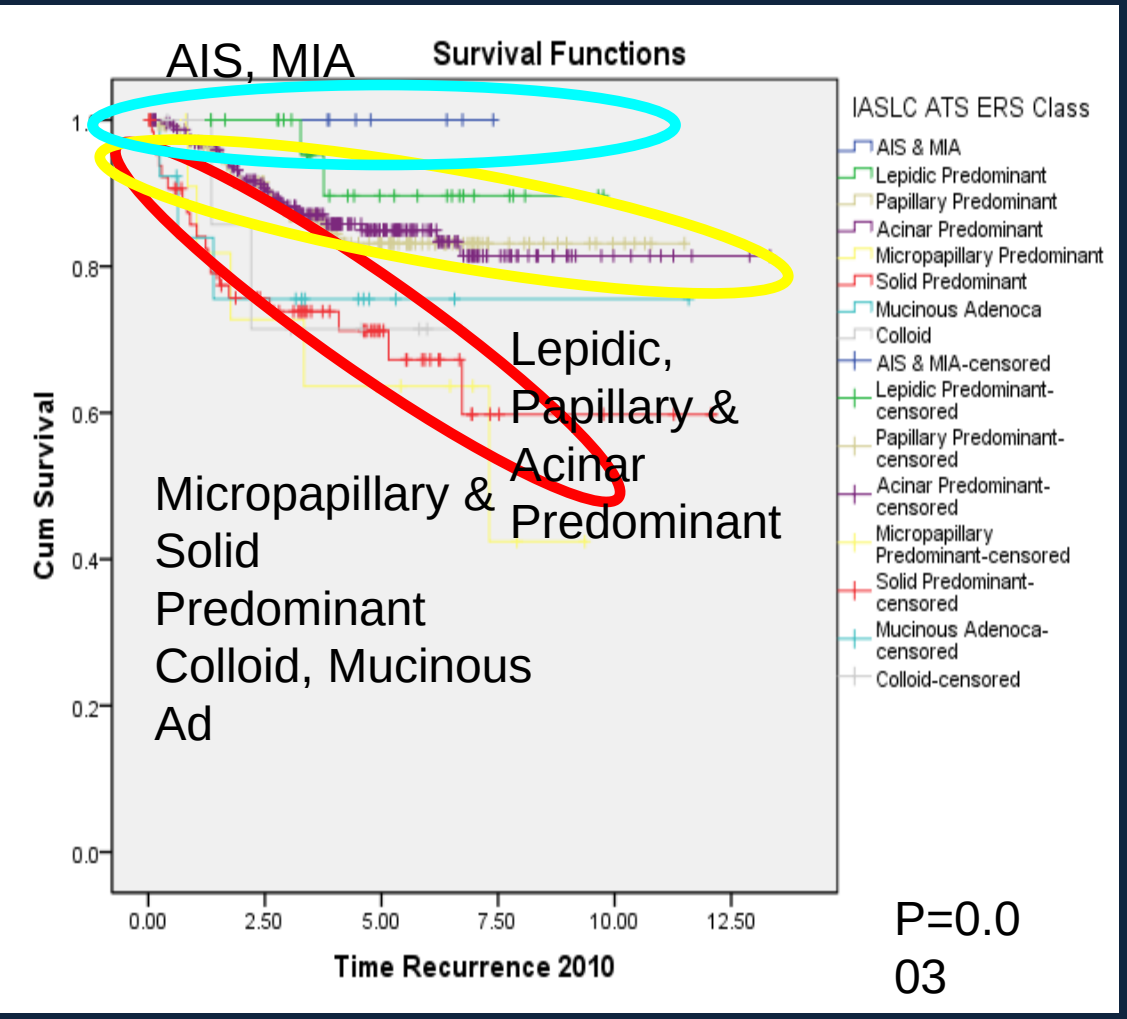
Tumor classification
(Toronto Pathology Society, 1902)
Lepidic: rind, skin,membrane
Tumours that appeared to be derived from surface-lining cells
Hylic: crude undifferentiated material
Tumours that appeared to be derived from connective tissue

Herbert Spencer's
Pathology of the Lung
(1962)
Tumours may grow into the surrounding alveoli "either filling them with a solid mass of malignant cells (hylic) or lining their walls (lepidic)".

Jones KD. Arch Pthol Lab Med, 2013

STAGE I ADENOCARCINOMA (N=514)

RECURRENCE-FREE SURVIVAL (RFS) BY IASLC HISTOLOGIC TYPE



Histologic Type (N)	5 Year RFS %
AIS (1)	100
MIA (8)	100
Lepidic NM (29)	90
Papillary (143)	83
Acinar (232)	85
Inv Mucinous Ad (13)	76
Solid (67)	71
Micropapillary (12)	64
Colloid (9)	71

Yoshizawa, A et al; Modern Pathology 24: 653-664, 2011

2015 WHO ADENOCARCINOMA CLASSIFICATION

- PREINVASIVE LESIONS
  - ATYPICAL ADENOMATOUS HYPERPLASIA
  - ADENOCARCINOMA IN SITU (≤3 cm, formerly BAC pattern) †
    - non-mucinous
    - mucinous
- MINIMALLY INVASIVE ADENOCARCINOMA (≤3 cm, a lepidic predominant tumor with ≤5mm invasion)
    - non-mucinous
    - mucinous

† Size should be specified. AIS and MIA should be completely sampled histologically

IASLC/ATS/ERS Classification of Lung Adenocarcinoma in Resection Specimens (1)

Preinvasinve lesions：前浸潤性病変

- Atypical adenomatous hyperplasia：異型腺腫様過形成
- Adenocarcinoma in situ (<3 cm formerly BAC)：上皮内腺癌
  - Nonmucinous：非粘液産生性
  - Mucinous：粘液産生性
  - Mixed mucinous/nonmucinous：混合型

Minimally invasive adenocarcinoma (<3 cm lepidic predominant tumor with <5 mm invasion)：微少浸潤性腺癌

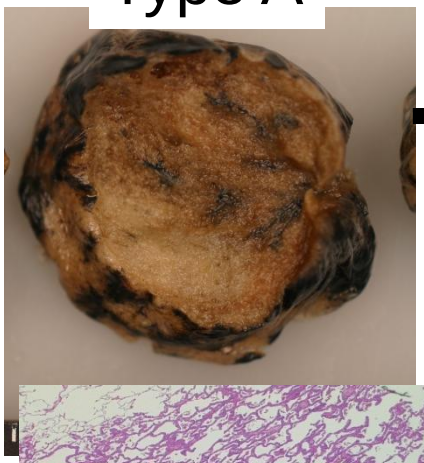
- Nonmucinous：非粘液産生性
- Mucinous：粘液産生性
- Mixed mucinous/nonmucinous：混合型

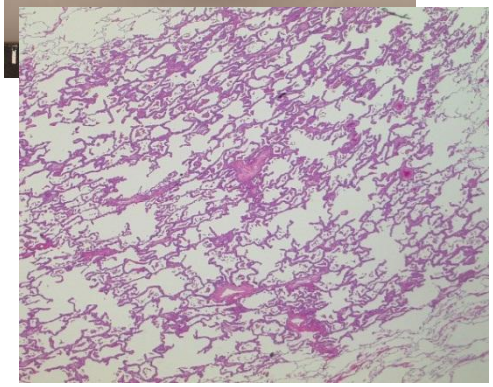
Travis WD, Elisabeth B, Noguchi M, et al. *JTO*, 6;244-285, 2011

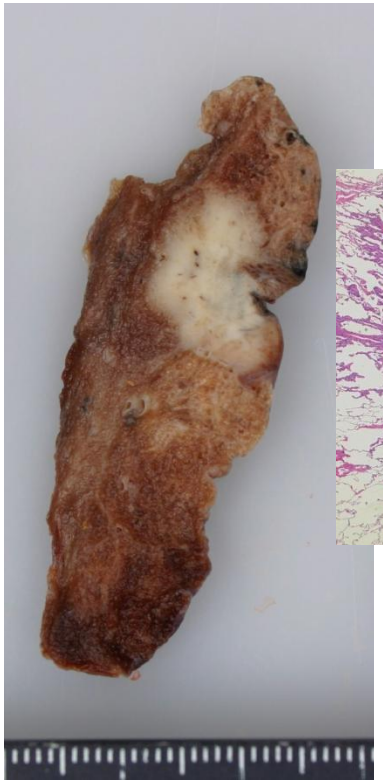
Stepwise progresssion of Lung Adenocarcinoma

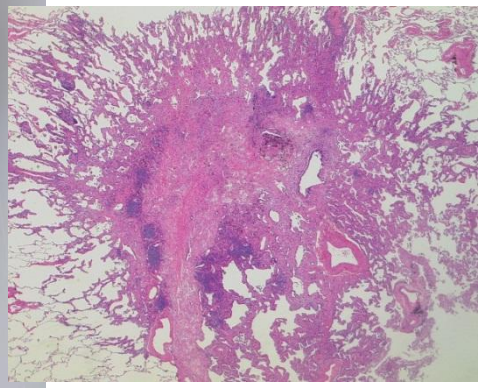
AAH/ Preinvasive lesion → Localized bronchiolo-Aaveolar carcinoma Type A → LBAC with fibroblastic foci Type C → Overt invasive Cancer

Type A









Type C

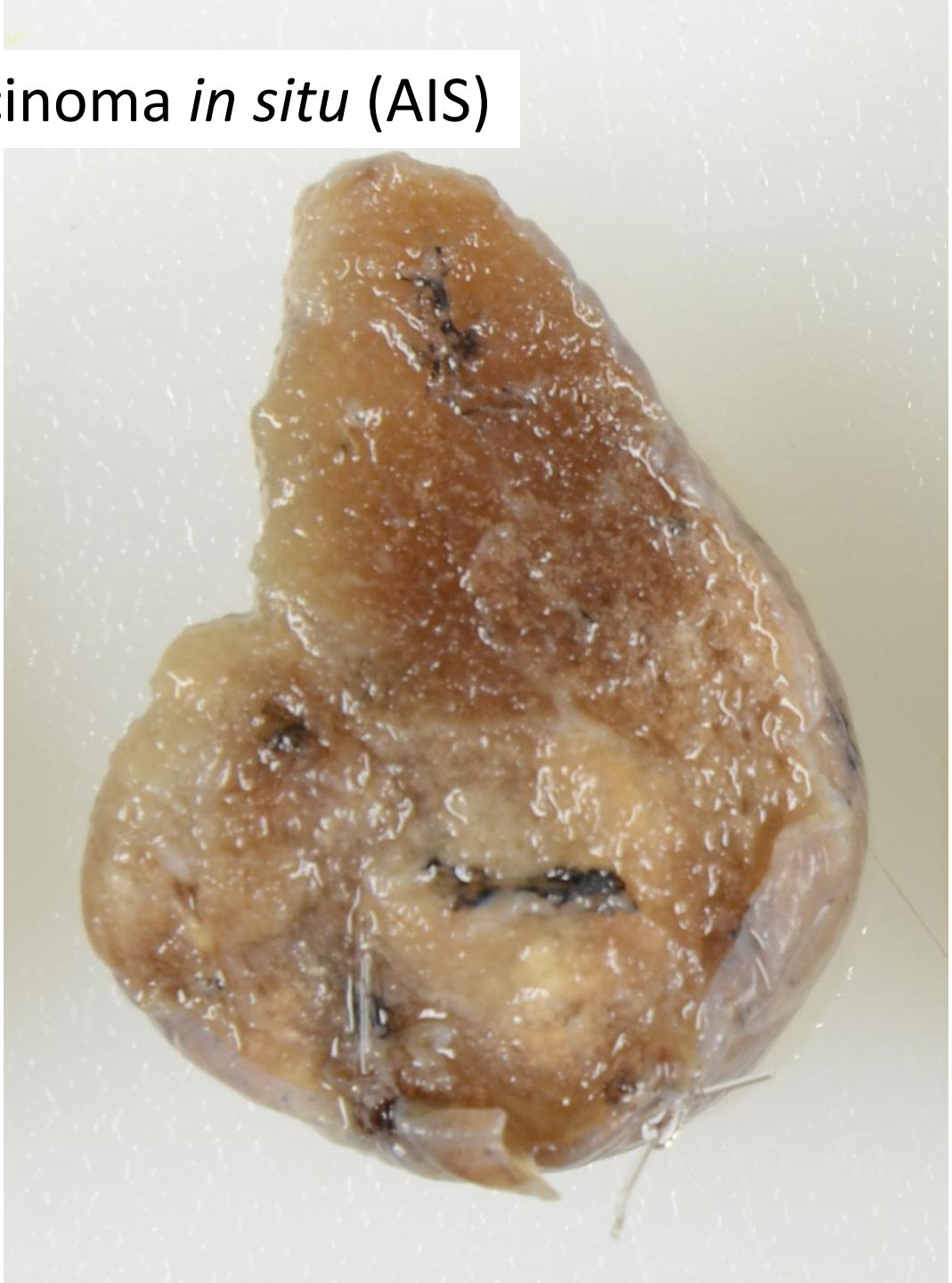
Heterogeneous histological characteristics

Acinar
Papillary
Bronchioloalveolar
Solid

PROPOSAL FOR 8TH EDITION TNM

- *In situ* carcinoma
  - Tis (AIS)
  - Tis (SCIS)
- Minimally invasive adenocarcinoma
  - T1a(mi)
  - If multiple – OK to use T1a(mi)(m)
- Use invasive size for size T-descriptor in subsolid lung adenoca by CT or nonmucinous adenoca with a lepidic component by pathology

Adenocarcinoma *in situ* (AIS)



Adenocarcinoma *in situ* (AIS)

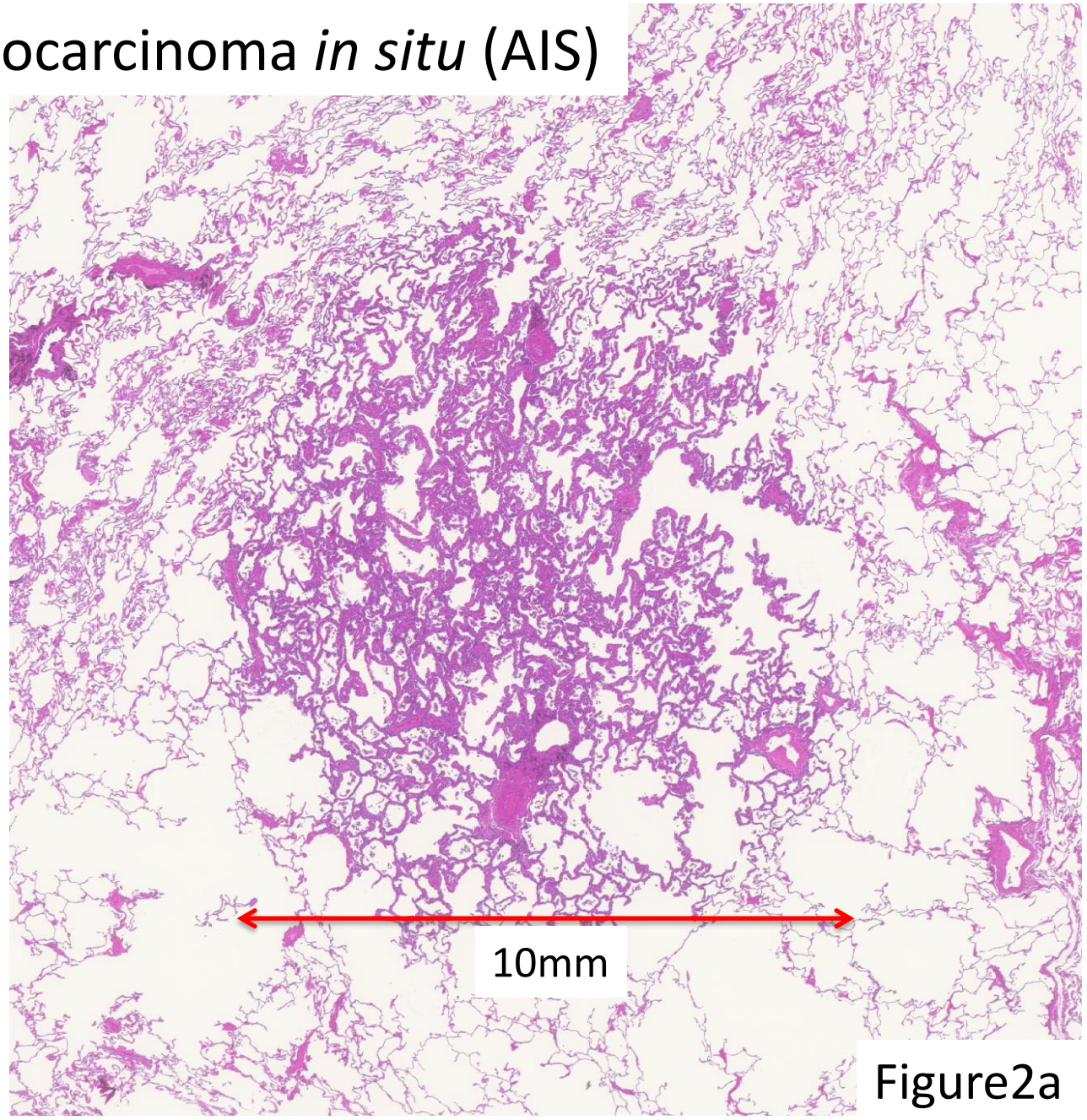
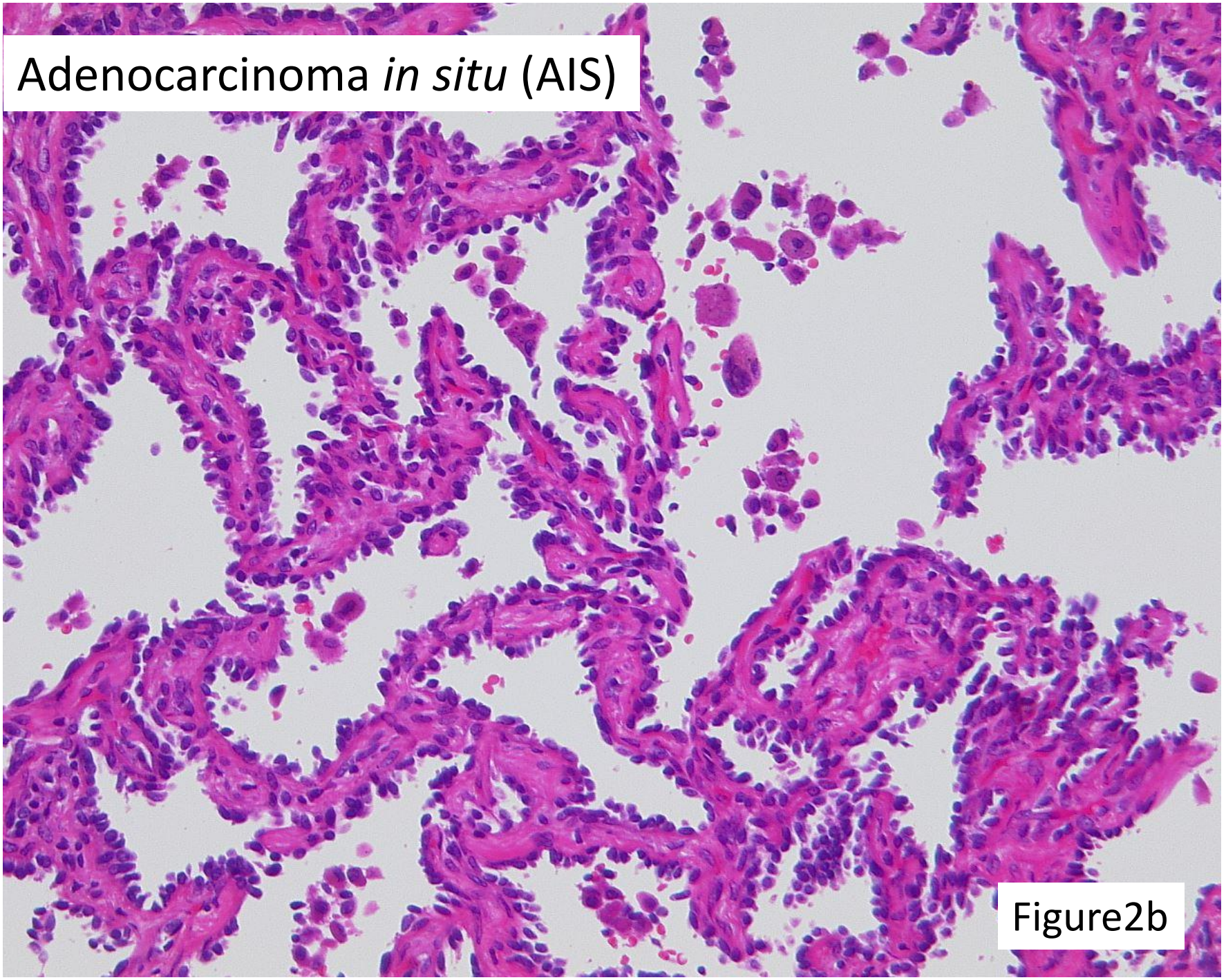


Figure2a


Adenocarcinoma *in situ* (AIS)
Figure2b

Minimally invasive adenocarinoma (MIA)

Minimally invasive adenocarinoma (MIA)

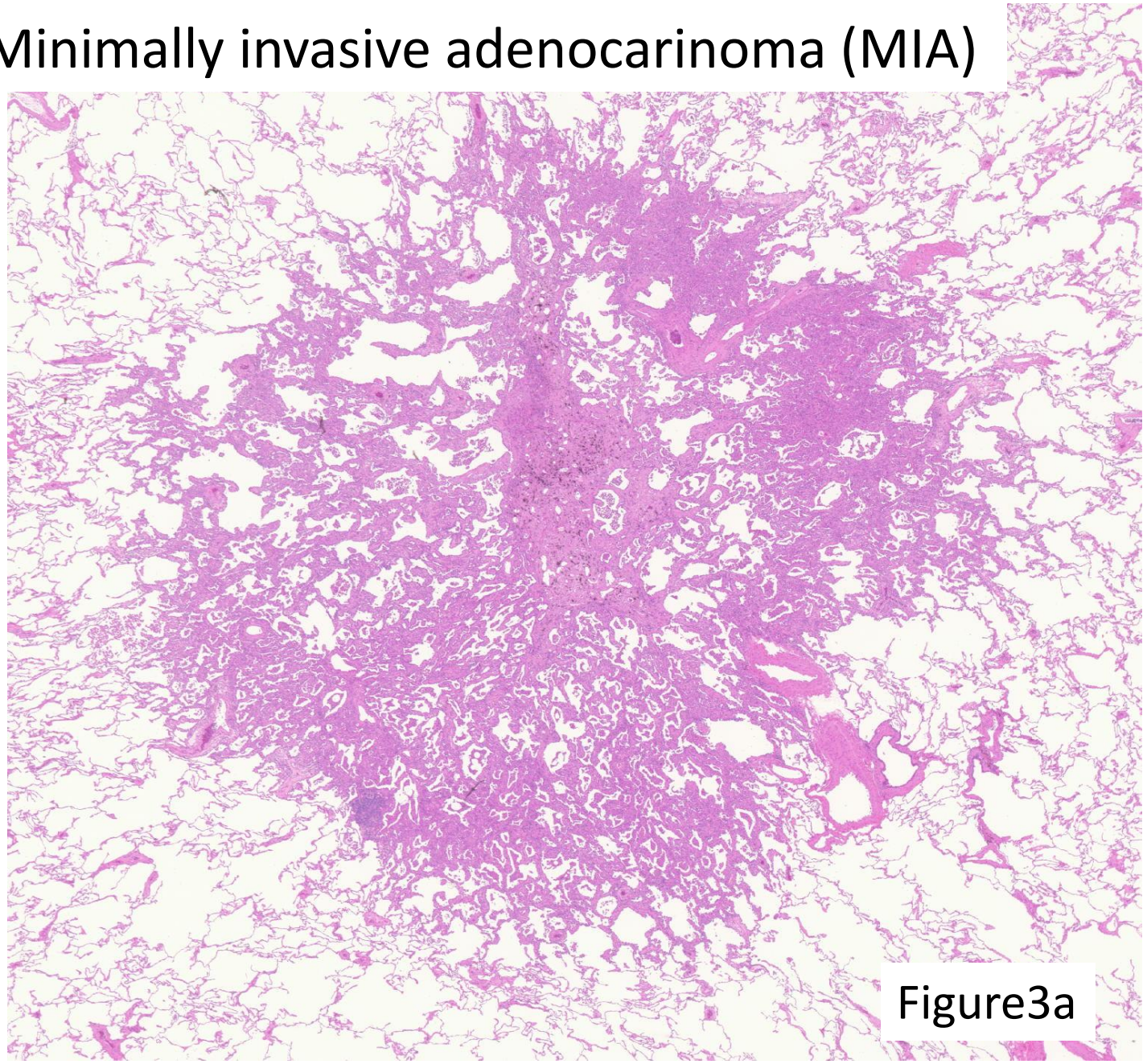
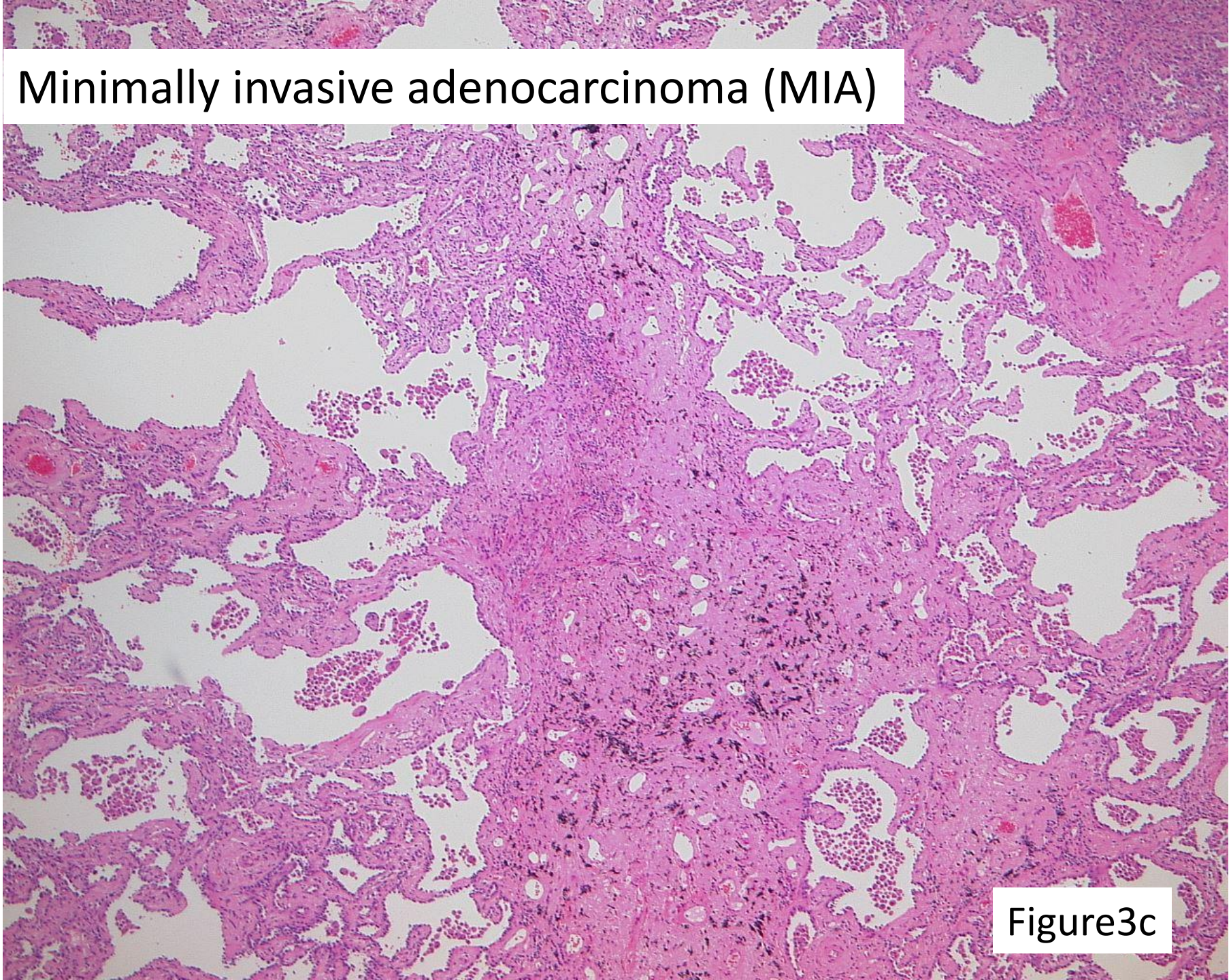


Figure3a

Diagnostic Criteria of Minimally Invasive Adenocarcinoma

1) A small tumor measuring ≤3 cm
2) A solitary adenocarcinoma
3) Predominantly lepidic growth
4) An invasive component ≤0.5 cm in greatest dimension at any one focus
5) Invasive component to be measured includes
    i) any histologic subtype other than a lepidic pattern (such as acinar, papillary, micropapillary, solid, colloidal, fetal or invasive mucinous adenocarcinoma),
    ii) tumor cells infiltrating a myofibroblastic stroma
6) A diagnosis of minimally invasive adenocarcinoma can be excluded if the tumor
    i) invades lymphatics, blood vessels, air spaces or pleura,
    ii) contains tumor necrosis,
    iii) spreads through the alveolar spaces
7) Cell type mostly non-mucinous (type 2 pneumocytes or Clara cells), but in rare cases may be mucinous (tall columnar cells with basal nuclei and abundant cytoplasmic mucin, sometimes resembling goblet cells).

Table 3


Minimally invasive adenocarcinoma (MIA)
Figure3c

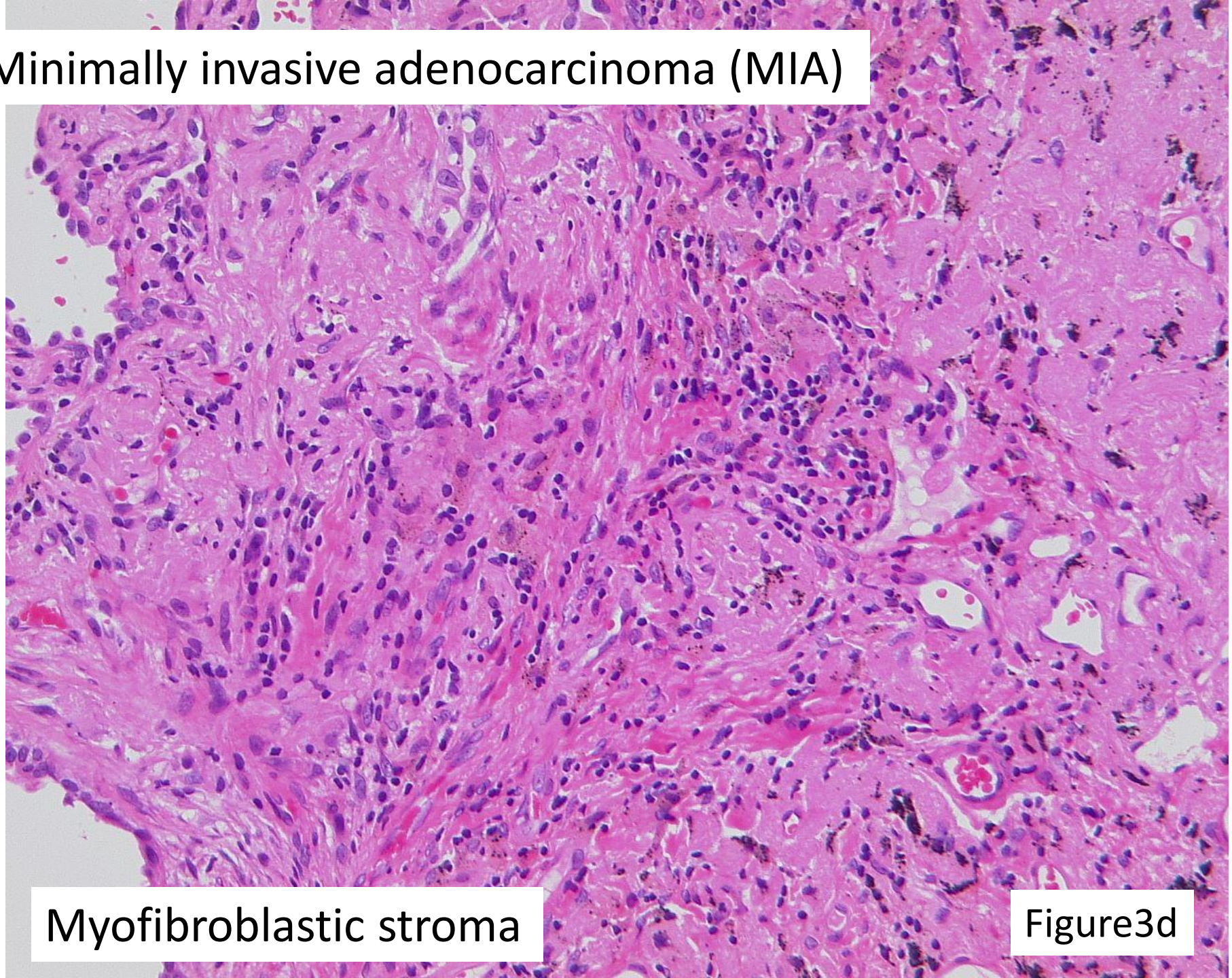
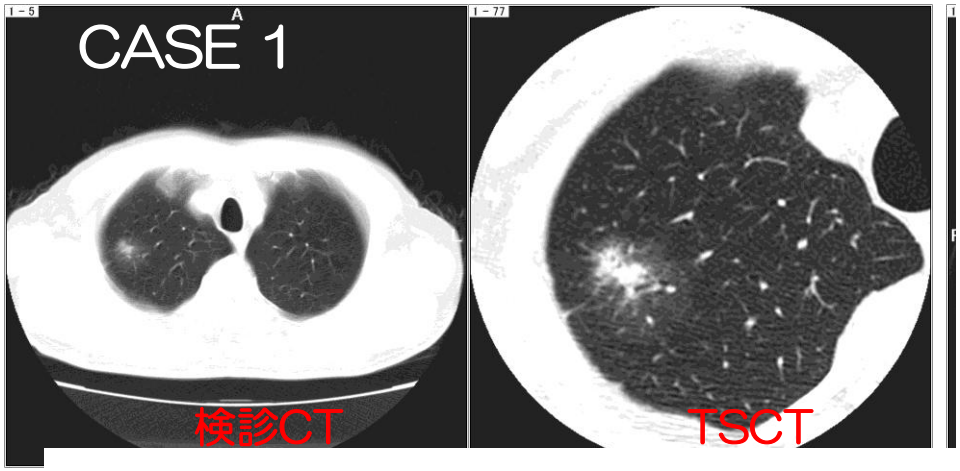

Minimally invasive adenocarcinoma (MIA)
Myofibroblastic stroma

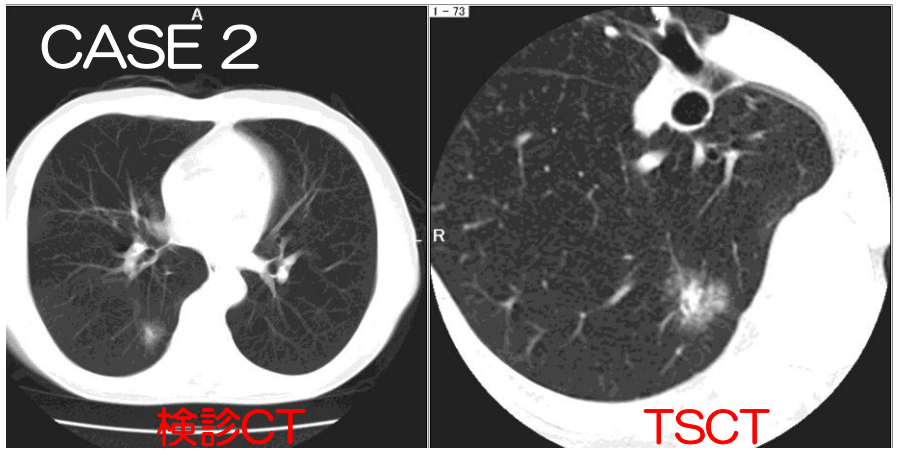
Figure3d

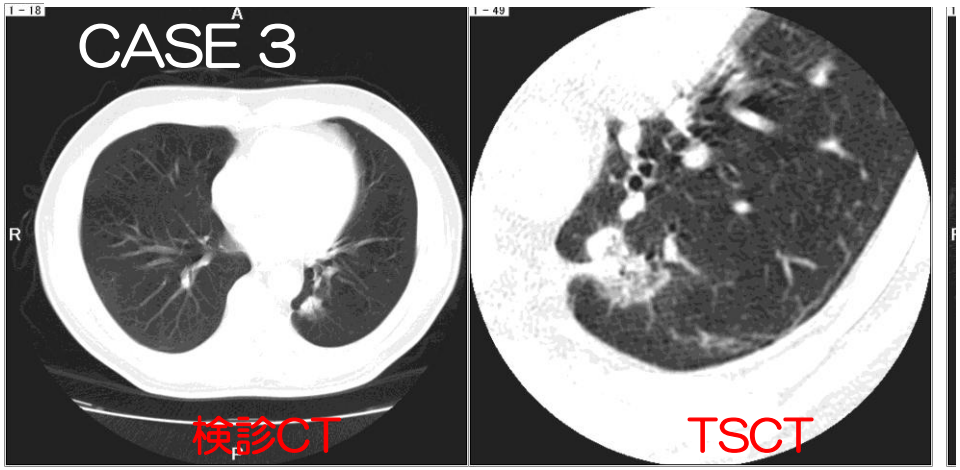
日立健康管理センタ
初回CT検診発見肺がん

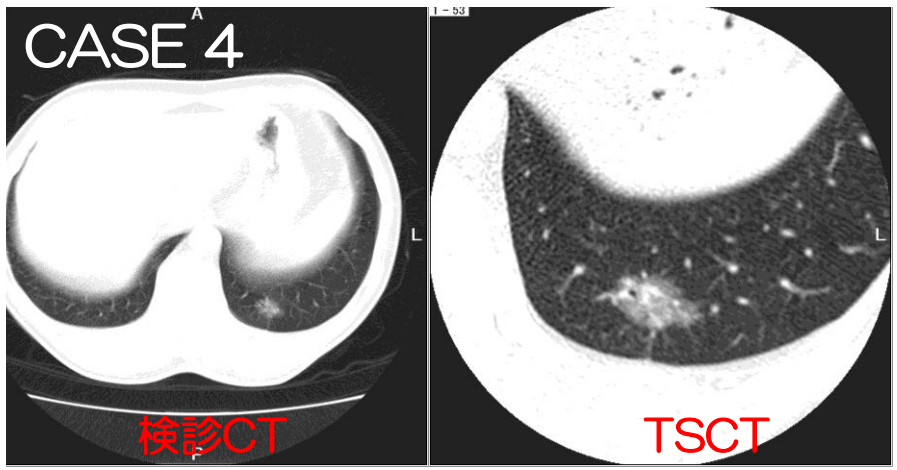
高分化腺がん（全例）pT1NOMO, Stage ⅠA

58歳 男性	59歳 女性
62歳 男性	51歳 男性





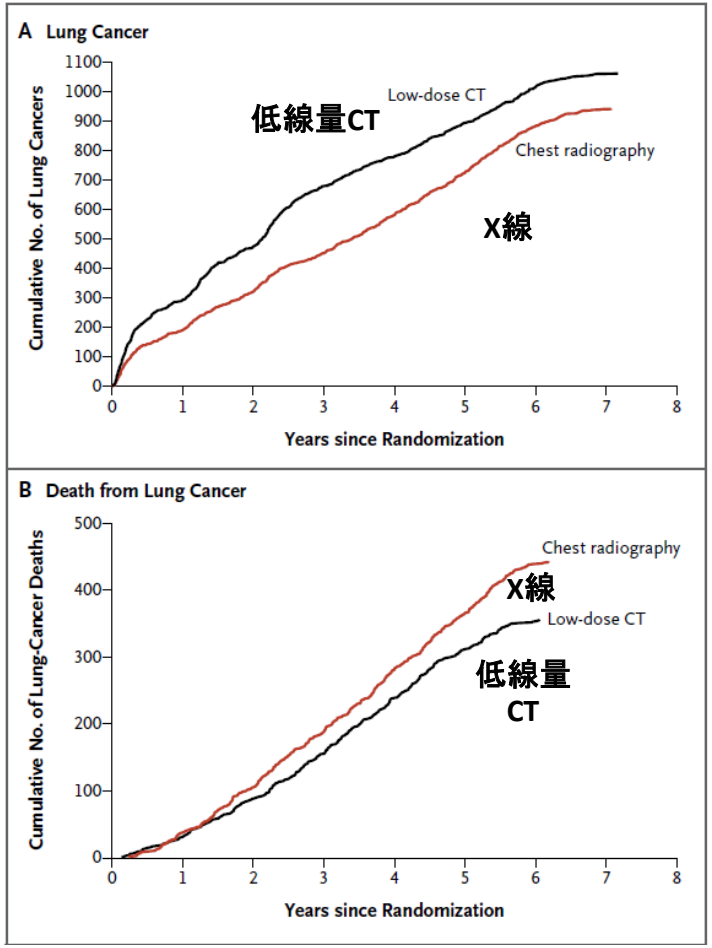




TSCT : Thin Section CT

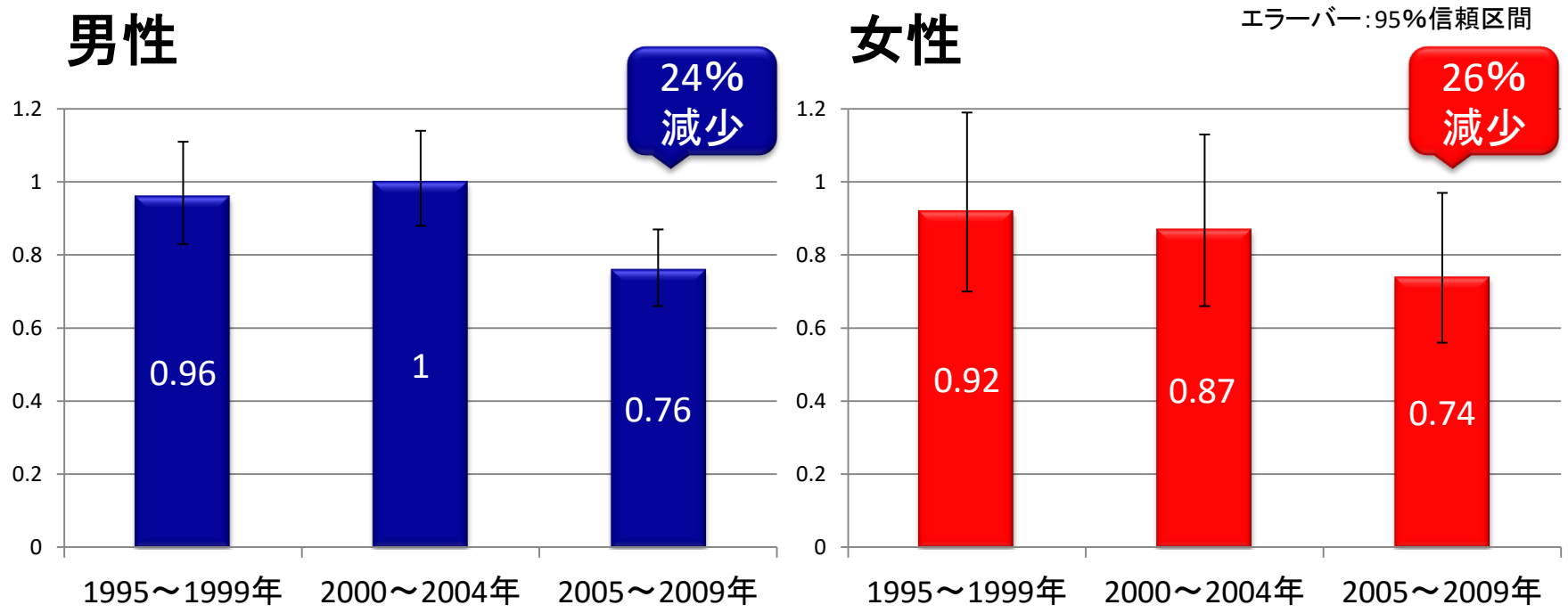
アメリカで示された低線量CT検診の有効性

- NLST（National Lung Screening Trial）
  - 胸部X線と低線量CT検診の無作為化比較試験。
  - 53,454名の参加、3年間連続の検診、6.5年経過観察
  - 低線量CT検診により20%の（1000人当たり3人）肺がん死亡減少。



National Lung Screening Trial Research Team, Reduced lung-cancer mortality with low-dose Computed tomographic screening. *N Engl J Med*. 365:395-409, 2011

標準化死亡比
（実際の死亡数÷期待死亡数）推移



	1995～1999年	2000～2004年	2005～2009年
期待死亡数	201.4	227.6	267.1
実死亡数	194	228	203

63人
減少

	1995～1999年	2000～2004年	2005～2009年
期待死亡数	61.8	65.2	71.3
実死亡数	57	57	53

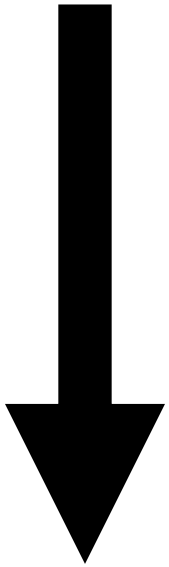
18人
減少

Nawa T et al, Lung Cancer, 78:225-228, 2012

がん研究開発費
「肺野限局性すりガラス様陰影の
自然史解明のための前向き研究」
（柿沼小班）

主任研究者：柿沼 龍太郎
分担研究者：柿沼 龍太郎、大松 広伸、岡見 次郎、古泉 直也、児玉 憲
近藤 哲郎、末久 弘、新田 哲久、松隈 治久,村田 喜代史、森 清志
画像中央診断委員会：芦澤 和人、栗山 啓子、村山 貞之
病理中央診断委員会：野口 雅之、前島 亜希子、松野 吉宏
統計解析：山地 大樹

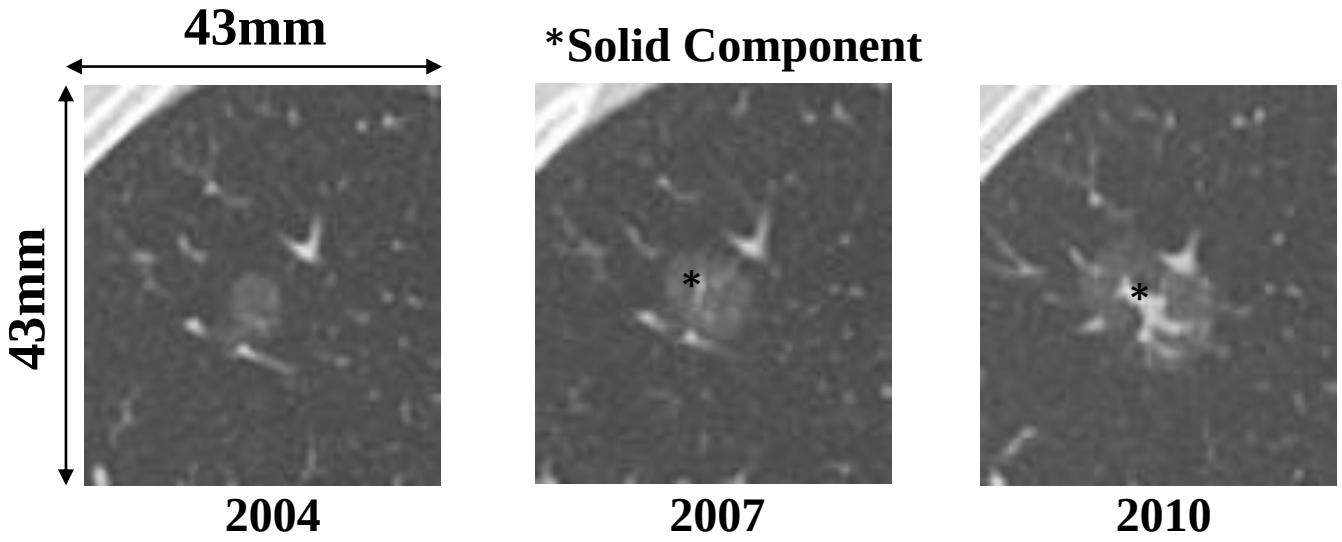
同意取得
846人1,325結節



- 炎症　3人11結節
- 即手術　5人5結節
- 最初からsolid　1人1結節
- 3cmより大　2人2結節
- 縦隔solid 9mm　1人1結節
- 経過観察不十分　1人3結節
- 転院　1人1結節
- 同意撤回　1人1結節
- 器質化　1人1結節

評価対象
833人1,299結節

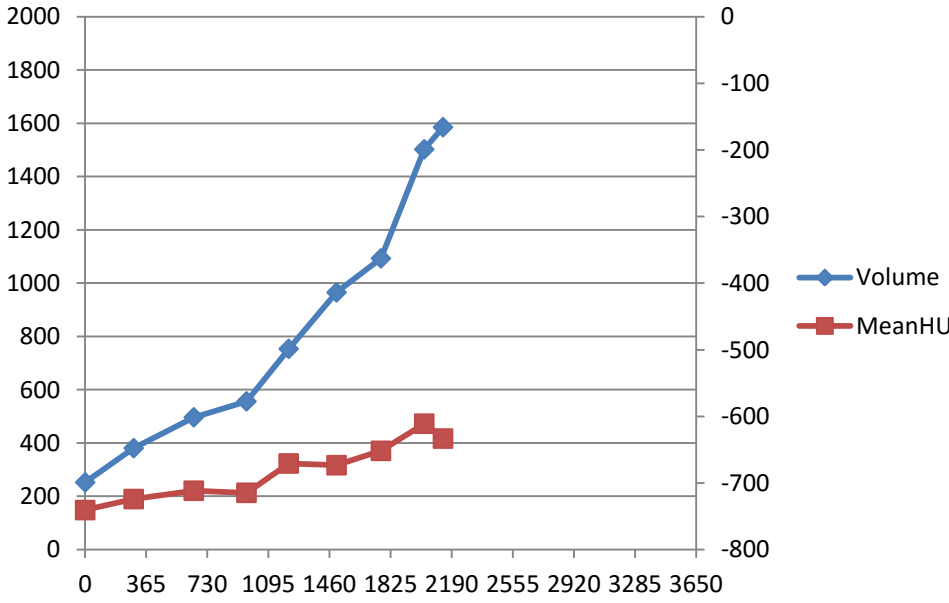
Case #1: 62-Year-Old Female, Never Smoker
Thin-Section CT



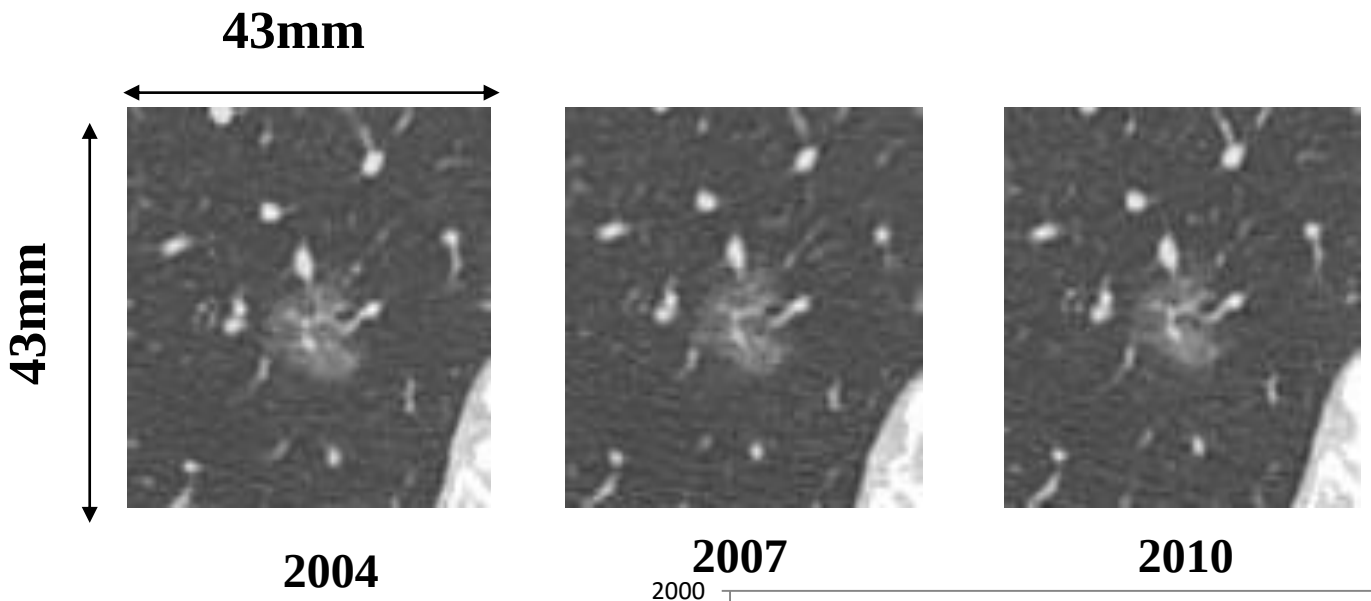
Growing Type

Adenocarcinoma, Minimally Invasive

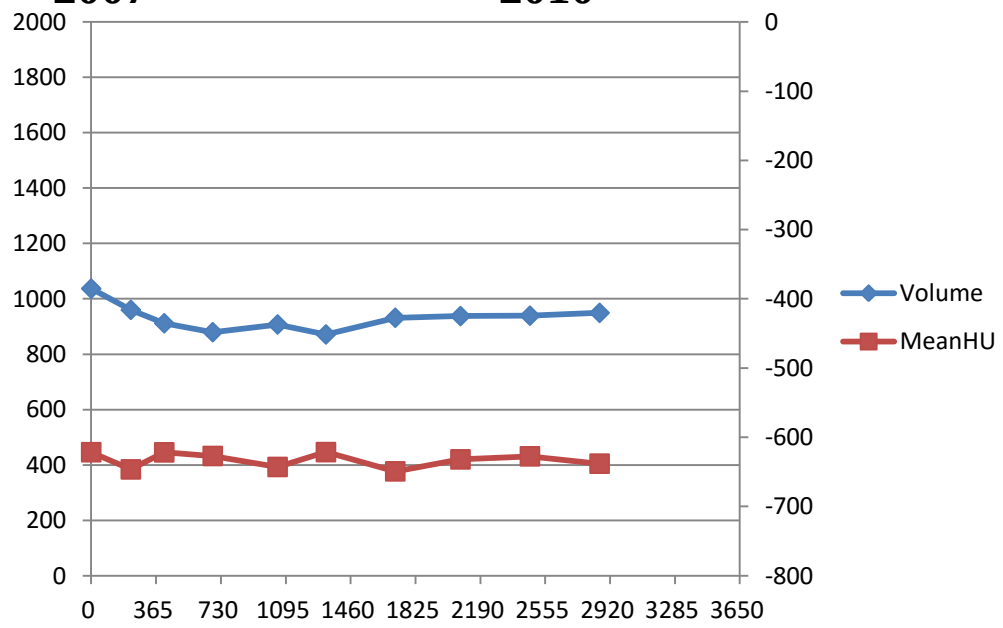
Resected after Six Years Follow-Up



Case #2: 68-Year-Old Female, Never Smoker
Thin-Section CT



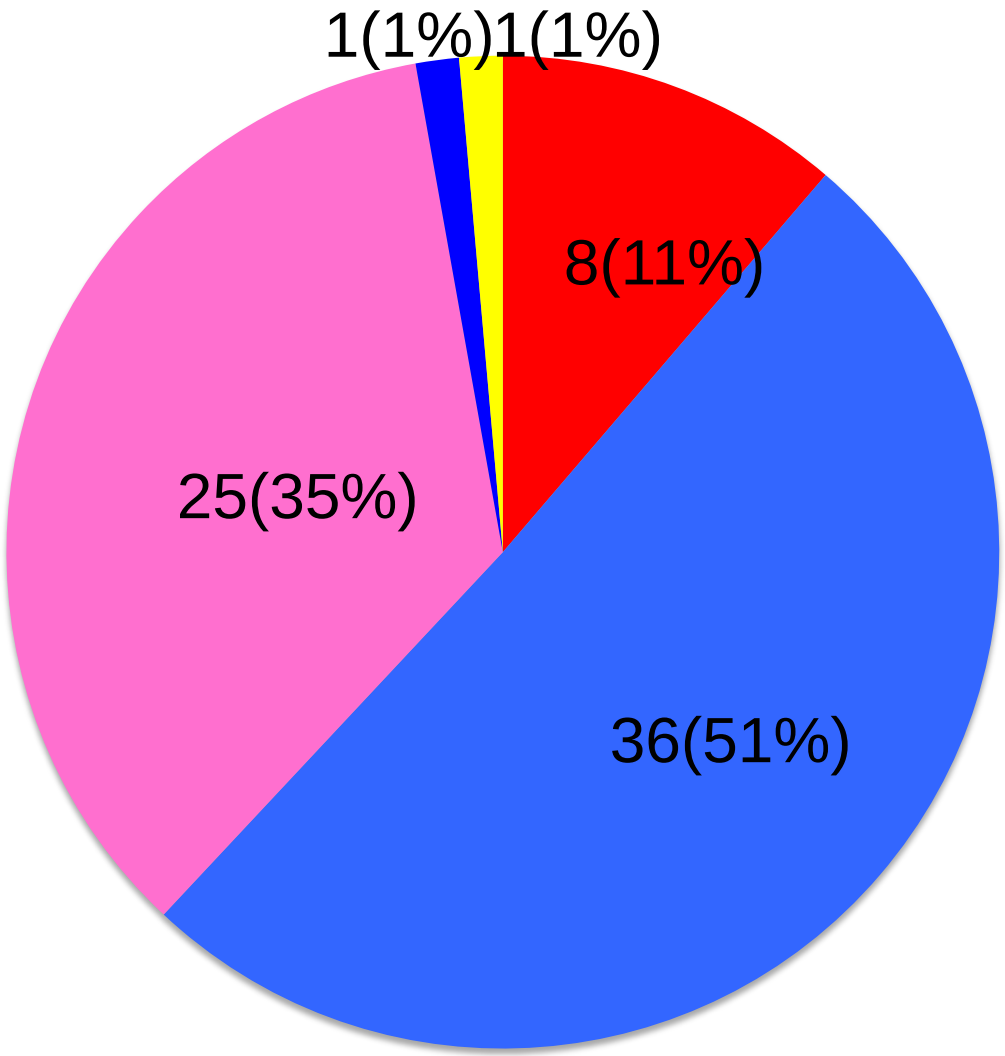
Stable Type



平均3.5年の経過観察の結果GGNはどのように変化したか？

	Pure GGN	Hetero GGN	Part Solid GGN	Total
number	1047	81	101	1229
Pure GGN	978 (93.4%)			978
Hetero GGN	13 (1.3%)	65 (80.2%)		78
Part Solid GGN	56 (5.3%)	16 (19.8%)	101 (100%)	173

病理中央診断の結果



N=71

- LPA
- MIA
- AIS
- AAH
- Indefinite neoplasia

Noguchi M, Matsuno Y, and Maeshima K

LPA: lepidic predominant invasive adenocarcinoma
MIA: minimally invasive adenocarcinoma
AIS: adenocarcinoma in situ
AAH: atypical adenomatous hyperplasia

	Path Diag.	Pure GGN	Hetero GGN	Part Solid GGN	
Operation		57 /1047(5.4%)	11/81 (13.6%)	24/101 (23.6%)	92
Pure GGN		**36 (63.2%)**			**36**
	AAH	5			5
	AIS	22			22
	MIA	9			9
	Invasive	0			0
Hetero GGN		**1 (1.8%)**	**6 (54.5%)**		**7**
	AAH	0	0		0
	AIS	0	2		2
	MIA	1	4		5
	Invasive	0	0		0
Part Solid GGN		**20 (35.1%)**	**5 (45.5%)**	**24 (100%)**	**49**
	AAH	1	0	1	2
	AIS	7	0	1	8
	MIA	9	2	15	26
	Invasive	3 (15%)	3 (60%)	6 (25%)	12

Multistep carcinogenesis of lung adenocarcinoma

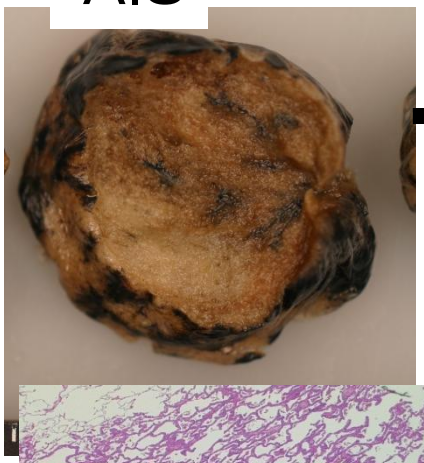
AAH/ Preinvasive lesion

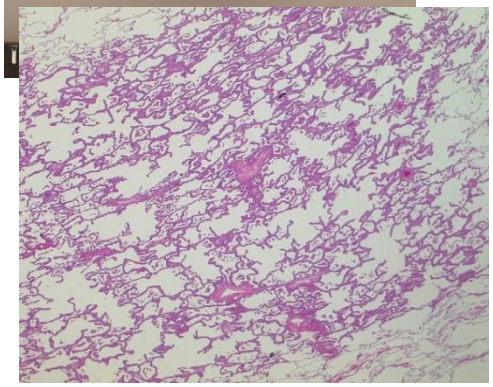
AIS/ Adenocarcinoma *In situ*

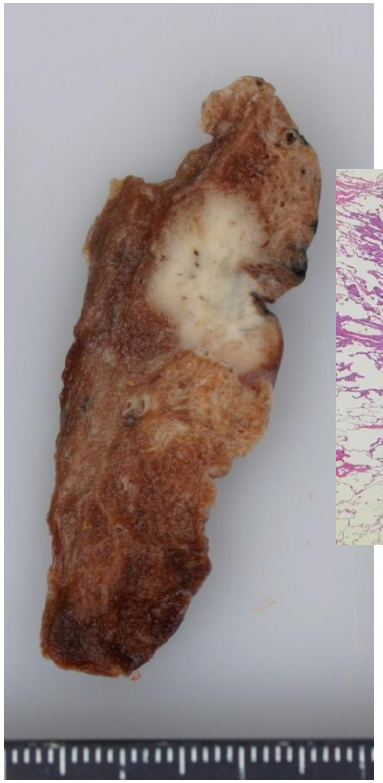
Minimally Invasive Cancer

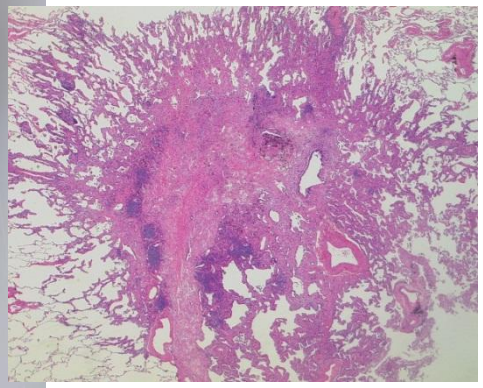
Overt invasive Cancer

AIS







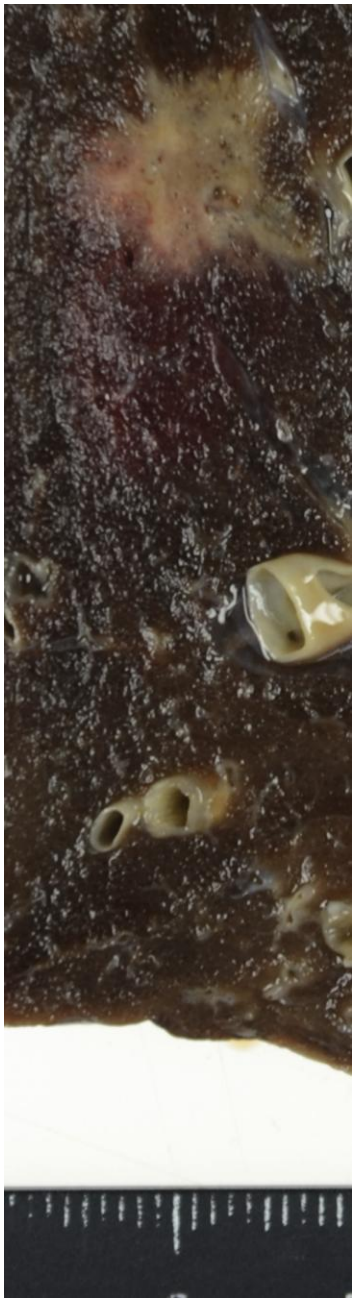


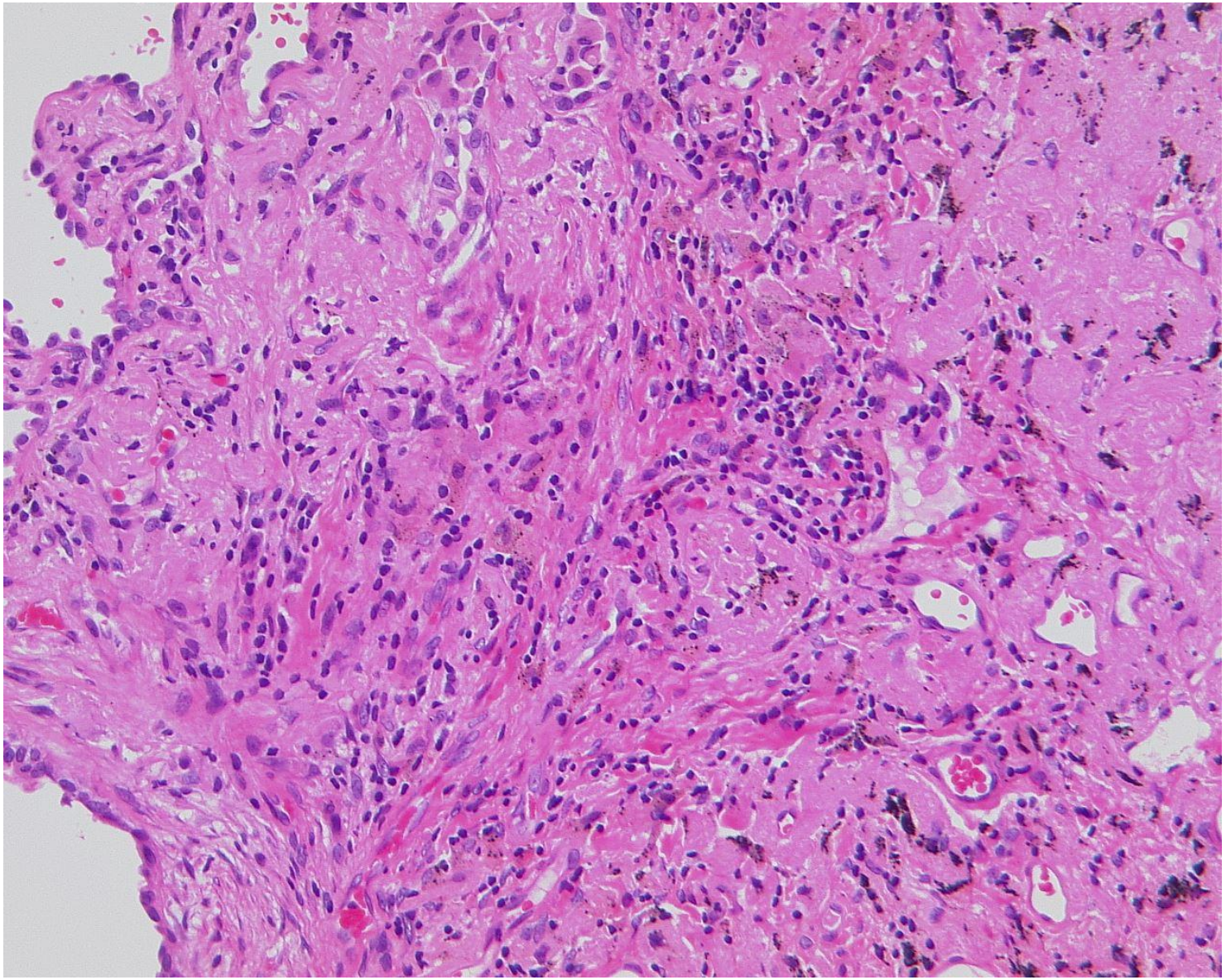
MIA

Heterogeneous histological characteristics

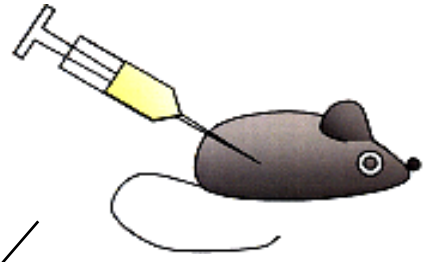
Lepidic
Acinar
Papillary
Solid
Micropapillary

Biological significance of fibroblastic proliferation?

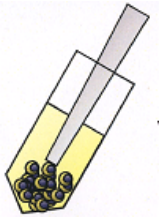




Immunizing swine fetal lung to get fetal antigen which can be used for biomarker of lung adenocarcinoma

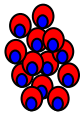


マウス腹腔に4回免疫

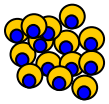


ホモジナイズ
ソニケーション

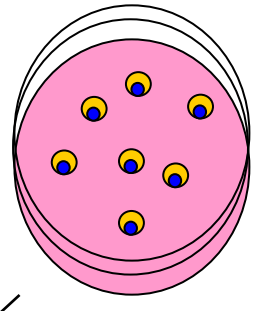
マウス血清を用いた
抗体価測定
ブタ胎児肺組織染色像



リンパ球

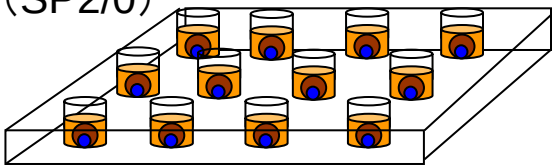


ハイブリドーマ

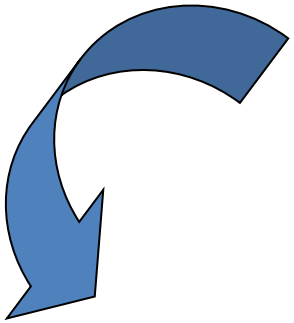


マウスミエローマ
（SP2/0）

HAT培地選択



1個のコロニーを1 wellずつ
増殖培地で培養



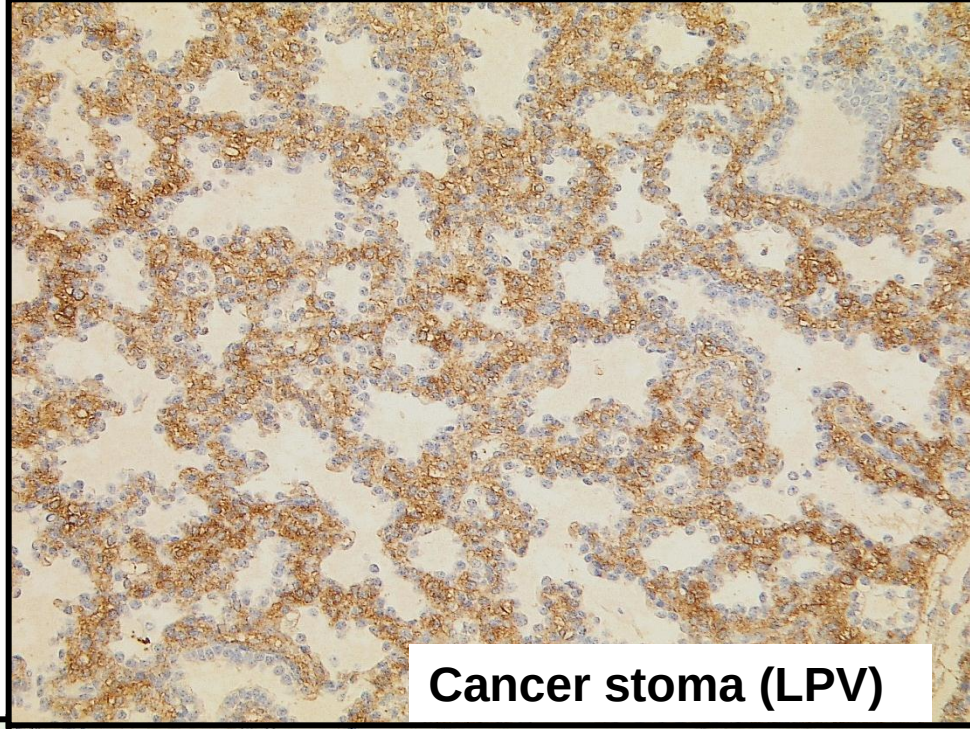
上清回収

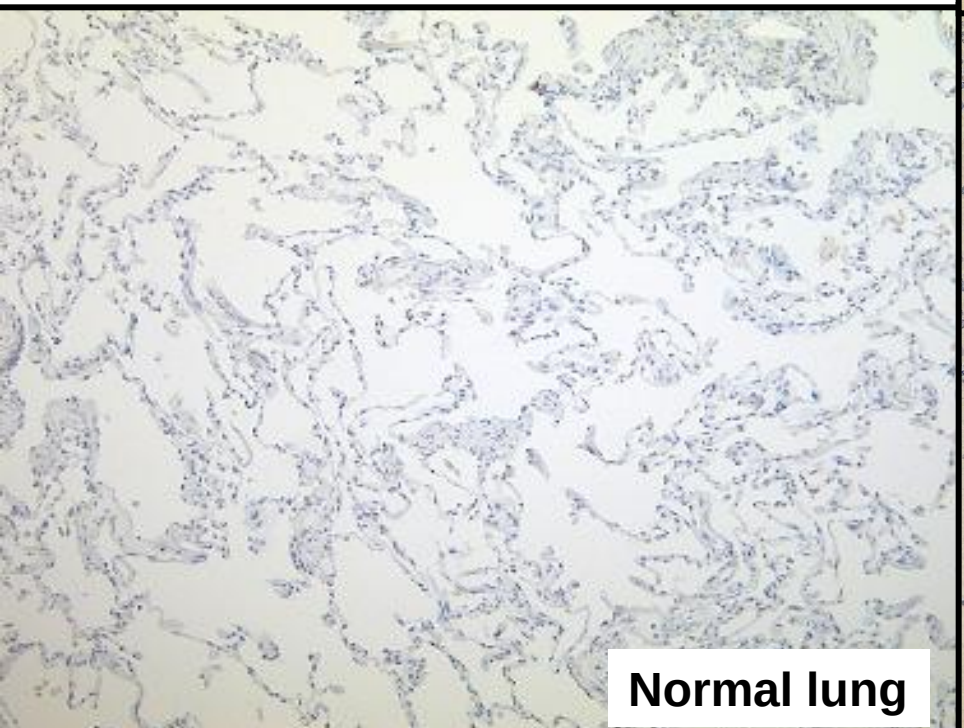
Swine fetal lung: positive
Human adenocarcinoma: positive

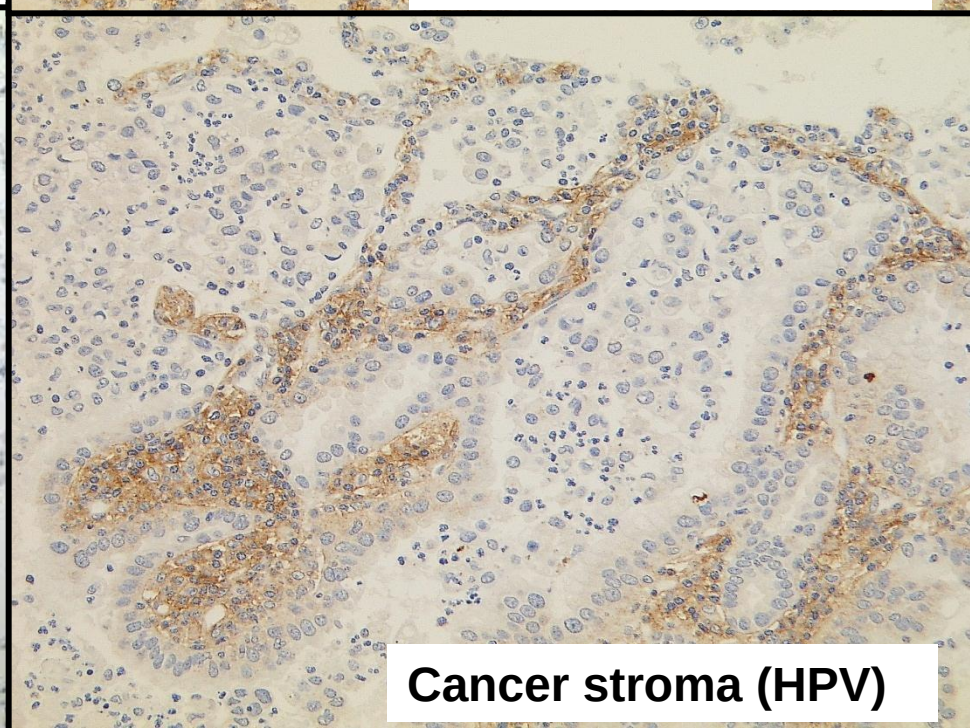
⇒ **candidates of new biomarker**

I found a clone that reacts against adenocarcinoma stoma specifically from 196 clones

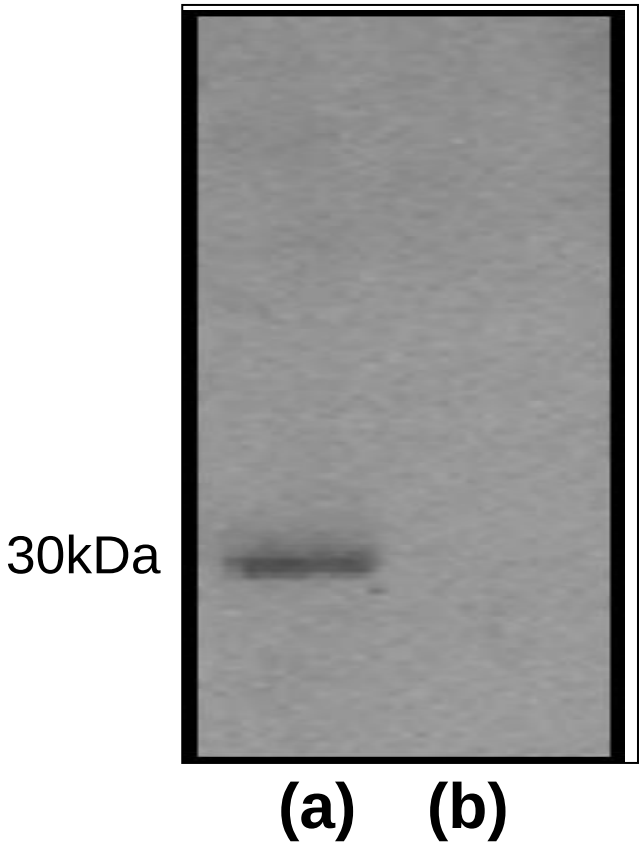
LS MS/MS analysis revealed this hybroidoma recognizes **DDAH2: Dimethylarginine dimethylaminohydrolase 2**



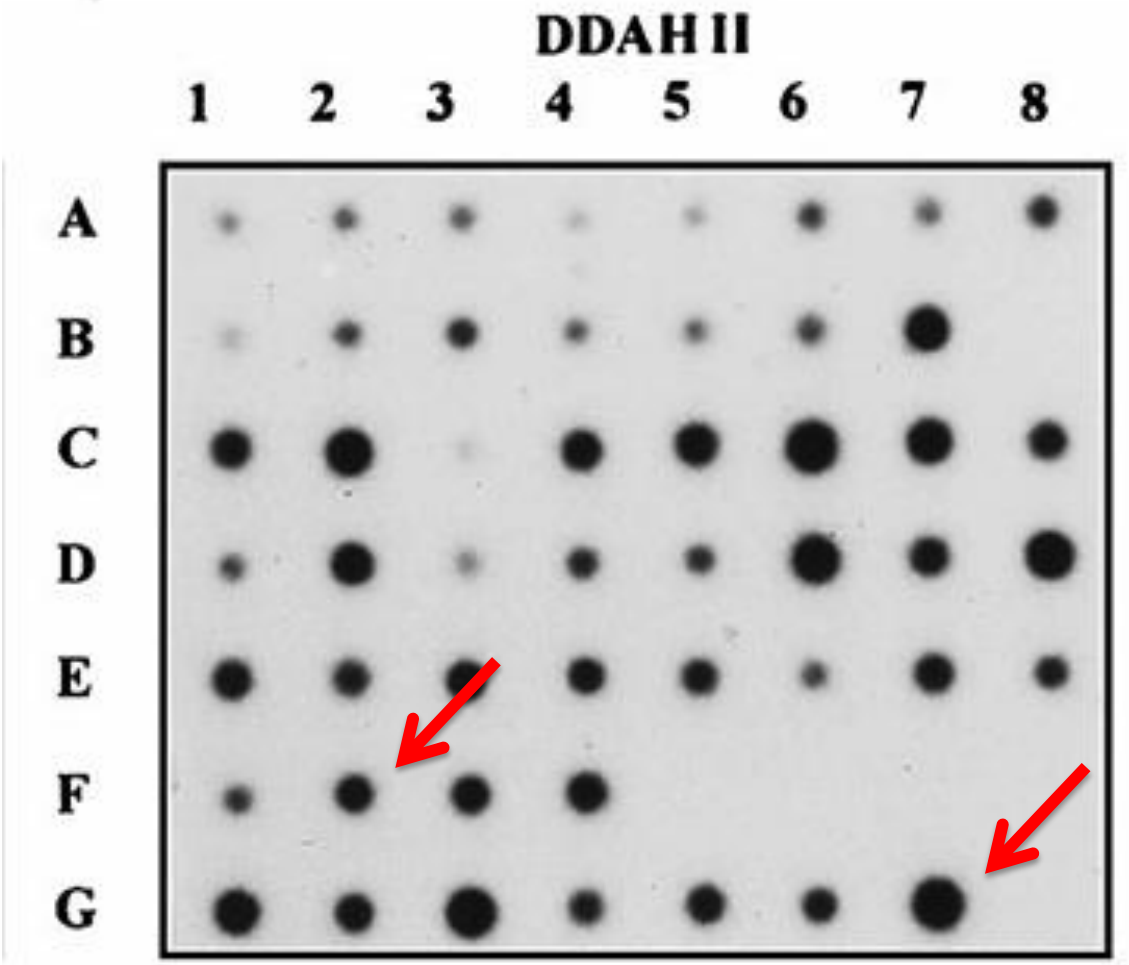




LC-MS/MS analysis revealed that the antibody recognosed DDAH2 **(Dimethylarginine dimethylaminohydrolase 2)**.



(a): lung adenocarcinoma (b):Control



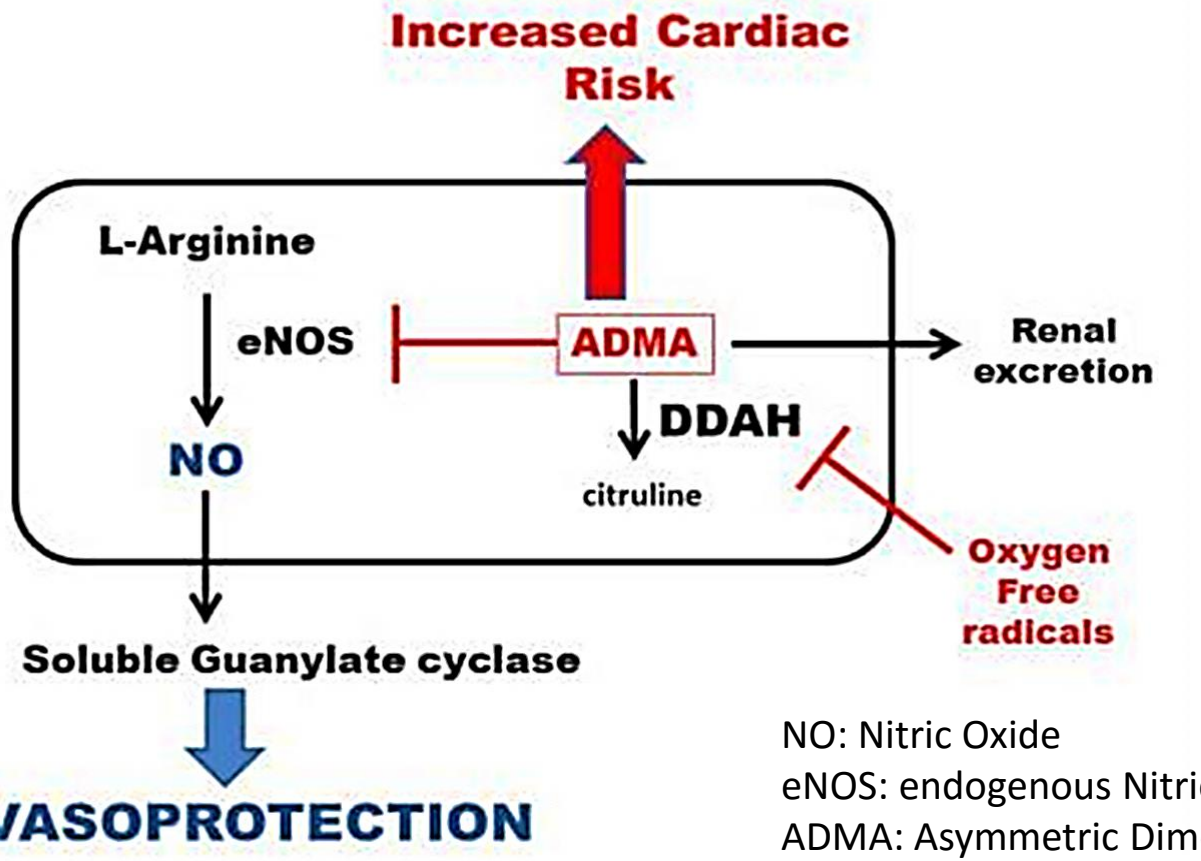
F-2 : Lung (Adult) **G-7: Fetal Lung**

Tran CT *et al*. Genomics(2000); 68(1): 101–105

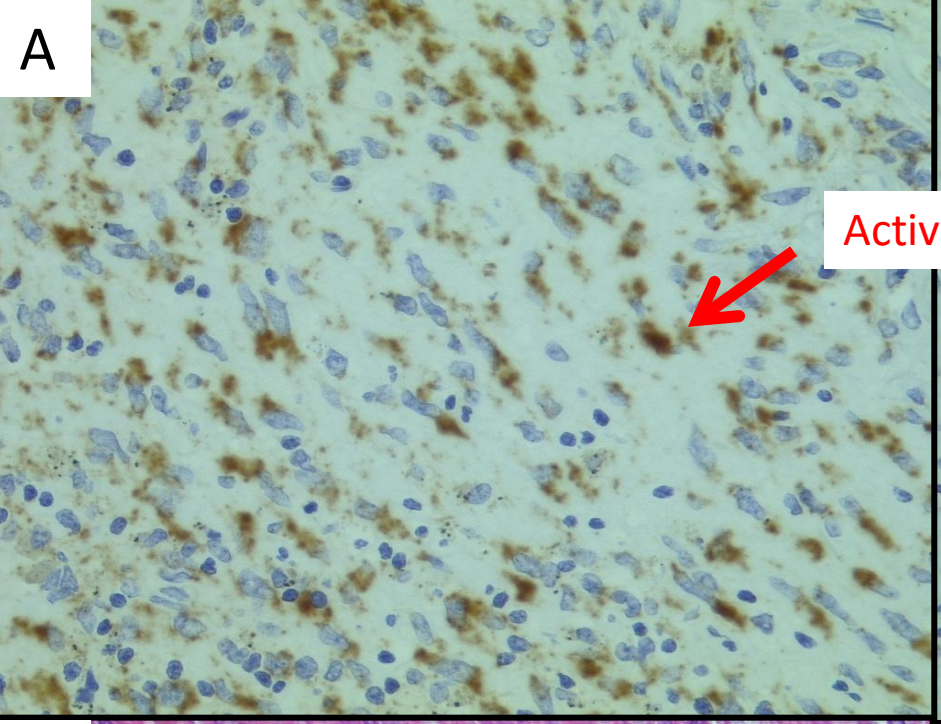
DDAH2

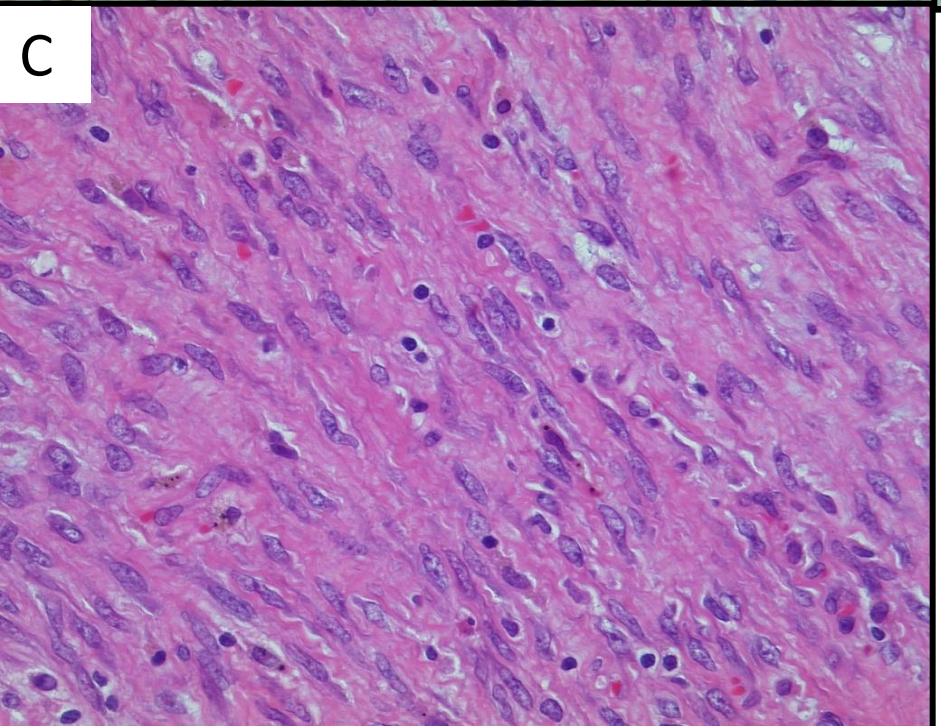
DDAH: Dimethylarginine dimethylaminohydrolase

- DDAH has two isoform, DDAH1 and DDAH2.
- DDAH2 stimulates NO production and is associated with angiogenesis.



NO: Nitric Oxide
eNOS: endogenous Nitric Oxide Synthase
ADMA: Asymmetric Dimethylarginine





In situ hybridization

Activated fibroblast revealed positive reaction against DDAH2 mRNA probe.

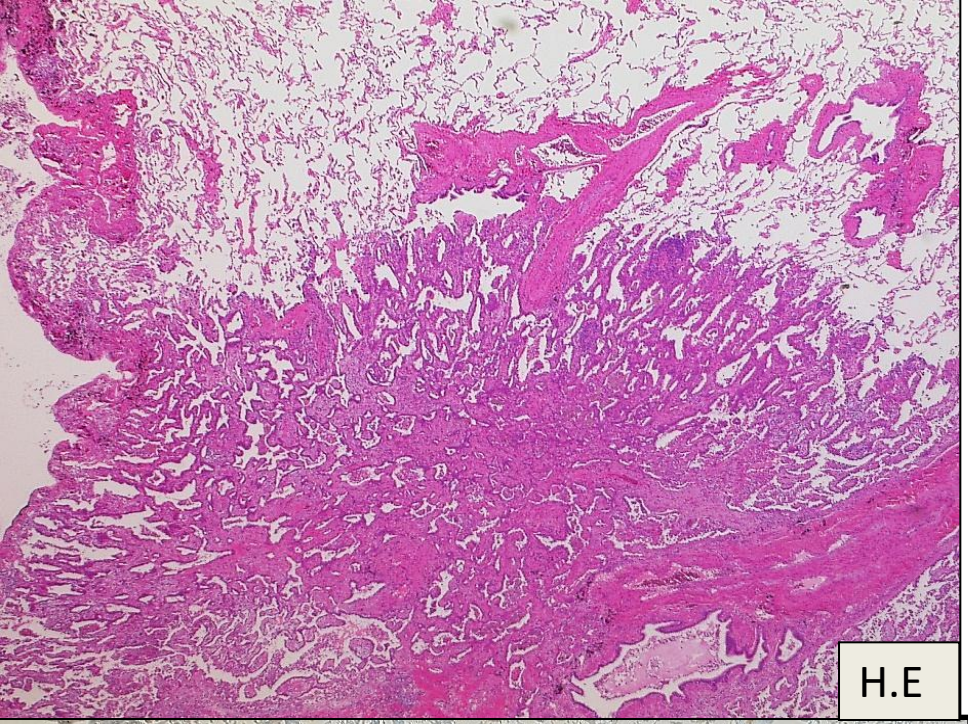
(A) DDAH2 mRNA probeを用いたin situ hybridization(anti-sense)

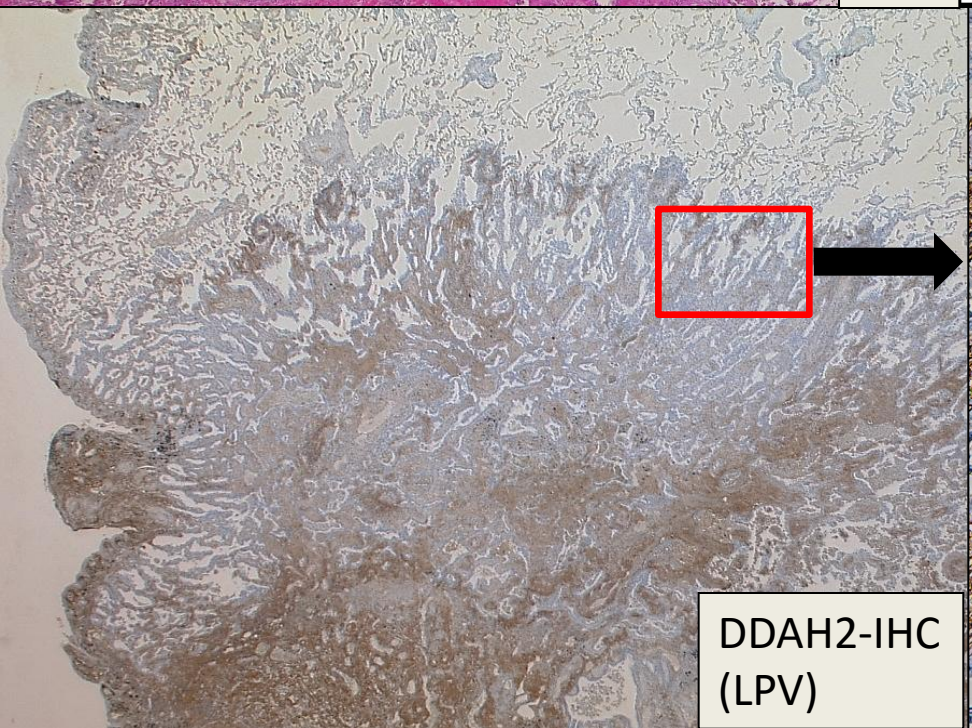
(B) ISH negative control(sense)

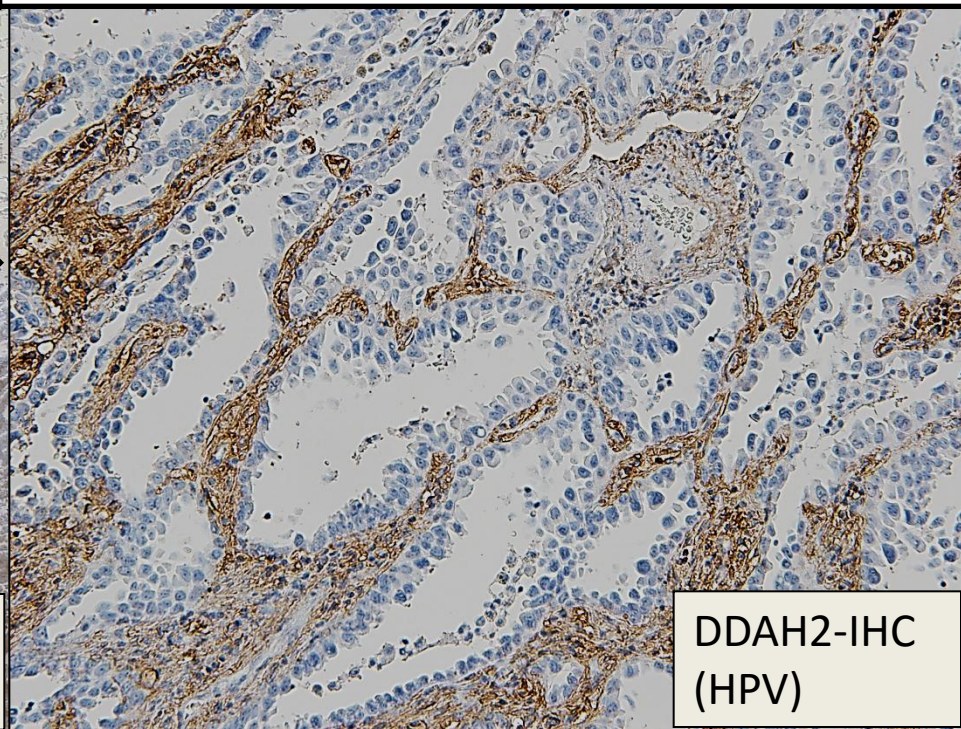
(C) HE

Immunohistochemistry

- Tumor stroma is positive for DDAH2 but tumor cells are negative



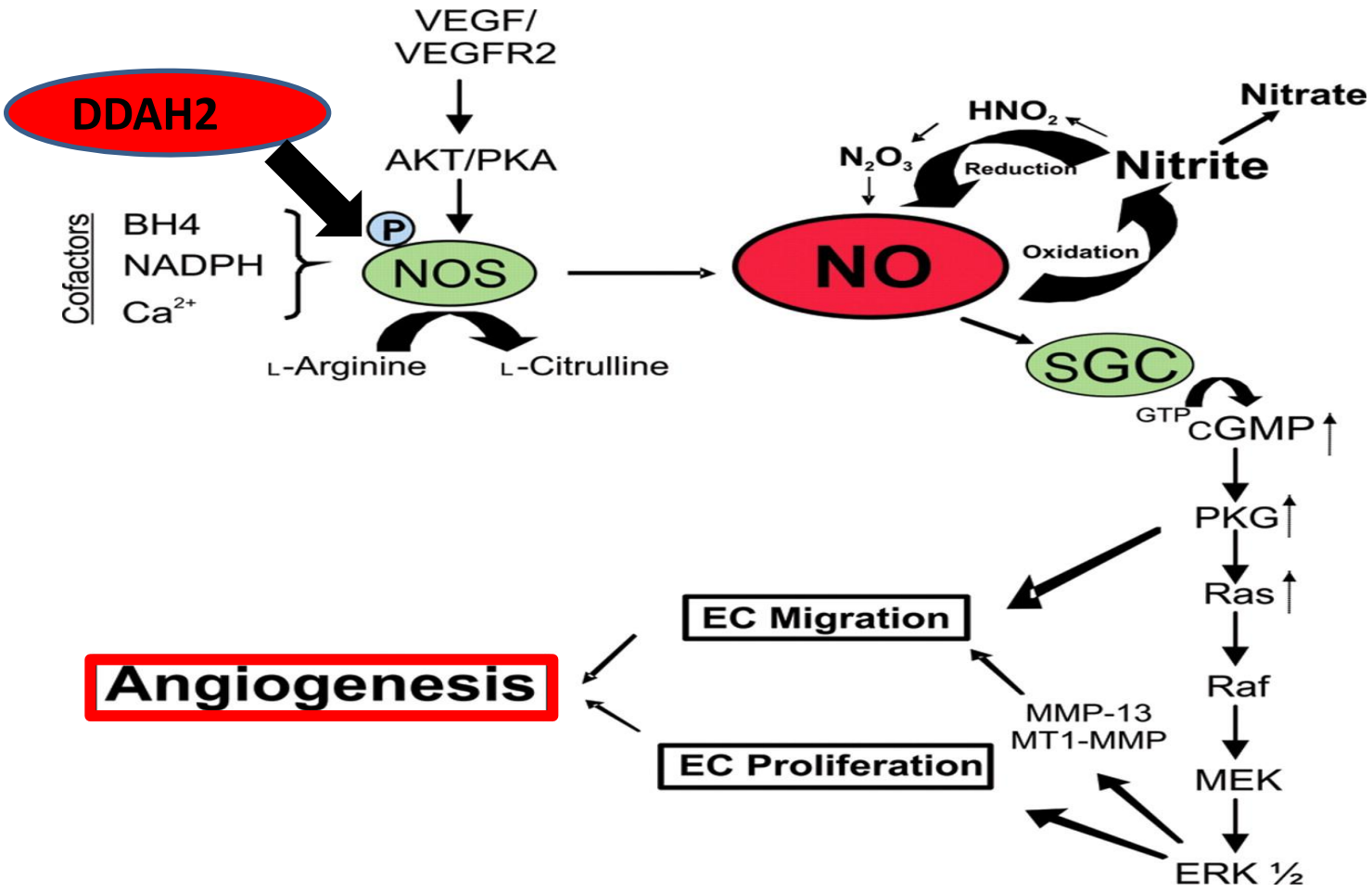




Immunohistochemistry of DDAH2

Histological subtype (New WHO)	Positive rate (%)
Preinvasive lesion	21/47(46%)
Atypical adenomatous hyperplasia (AAH)	2/14
Adenocarcinoma in situ (AIS)	19/33
Minimally invasive adenocarcinoma (MIA)	**11/11(100%)**
Invasive adenocarcinoma	**74/75 (99%)**
Lepidic predominant	40/41
Acinar predominant	7/7
Papillary predominant	8/8
Micropapillary predominant	1/1
Solid predominant	18/18

Angitogenesis pathway



Bir S C *et al*. Cardiovasc Res 2012;95:7-18

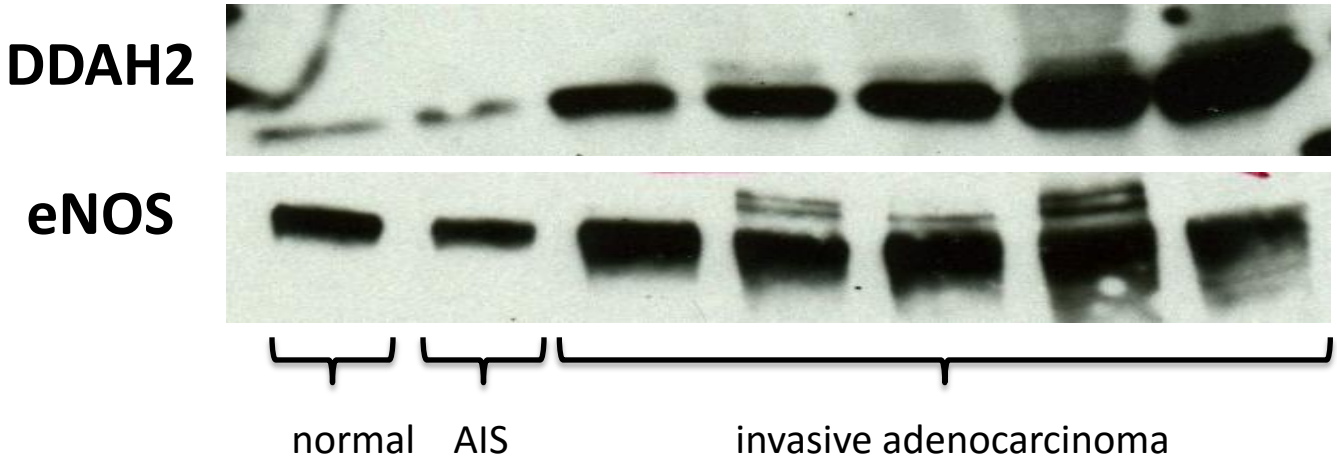
eNOSによって**NO**が産生されると、血管内皮細胞の遊走や増殖、管腔形成などが生じ、血管新生が促進される。

DDAN2 and eNOS expression in adenocarcinoma of the lung

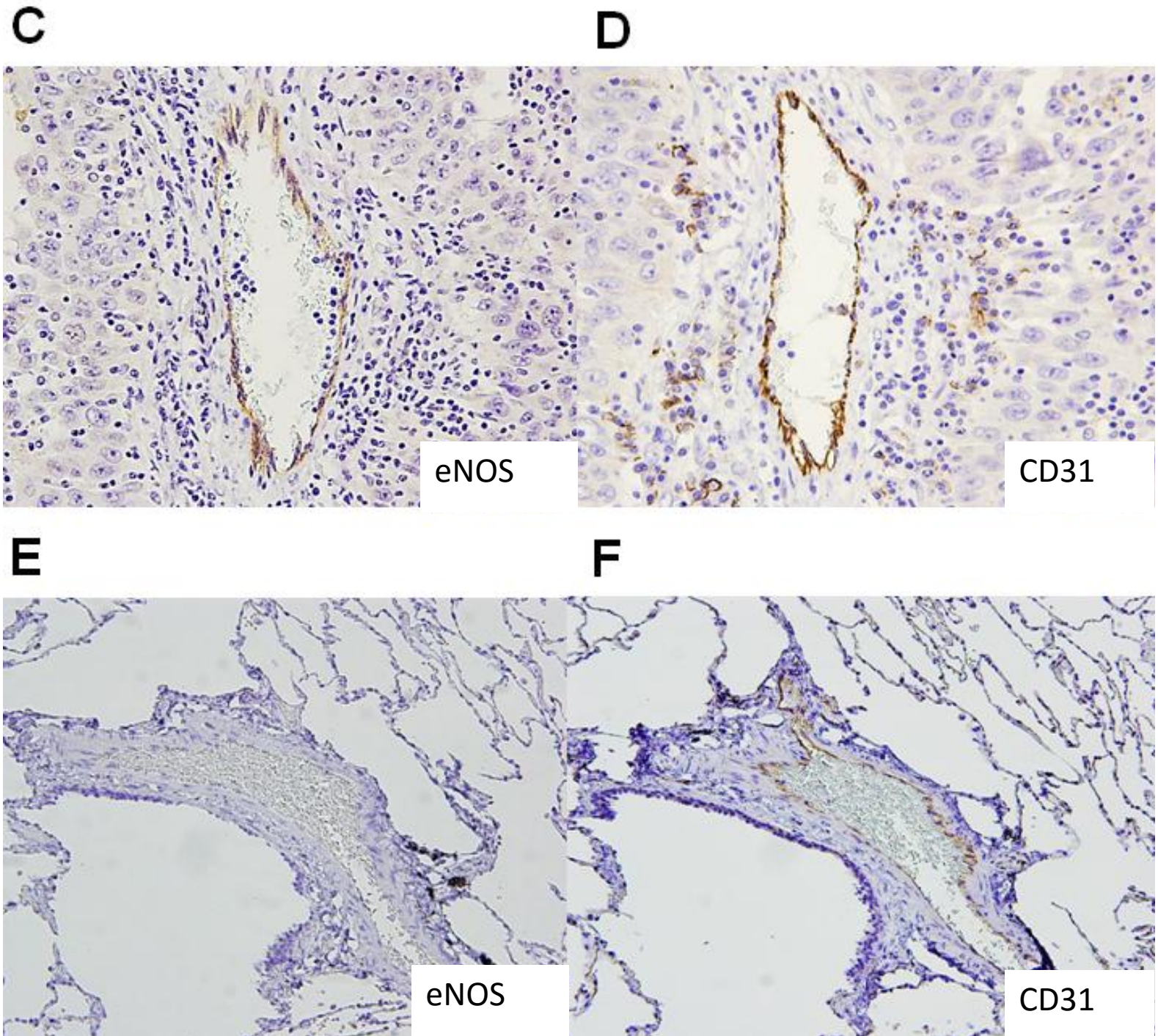
（1）Materials

- ①normal(n=1)、②AIS(n=1)、③Invasive adenocarcinoma(n=5)

_（2）Western blotting

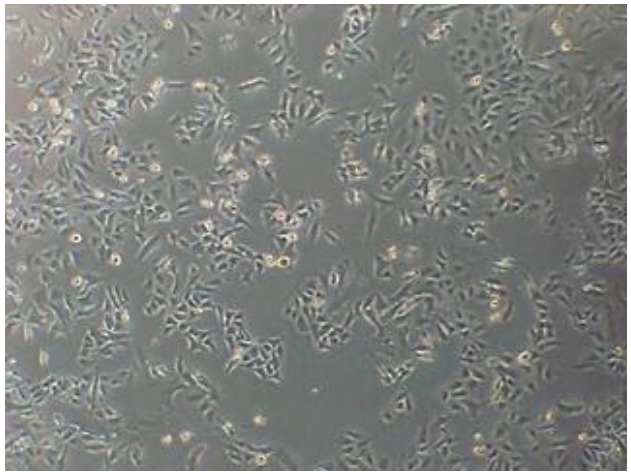


正常肺や上皮内癌と比べてDDAH2が高発現している浸潤性腺癌では、eNOSの発現も有意に亢進していた。

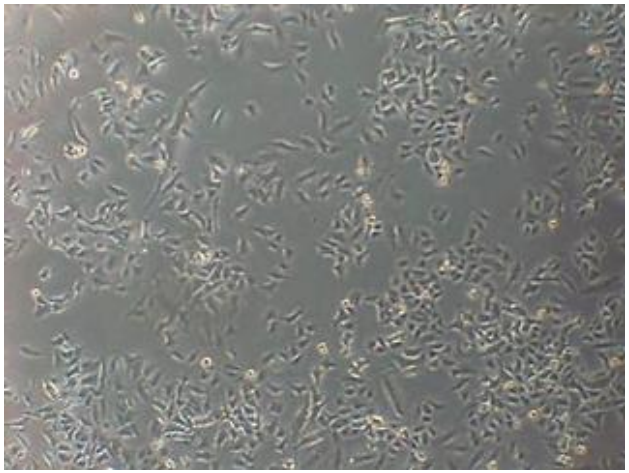

C
D
eNOS
CD31
E
F
eNOS
CD31

HUVEC Proliferation assay

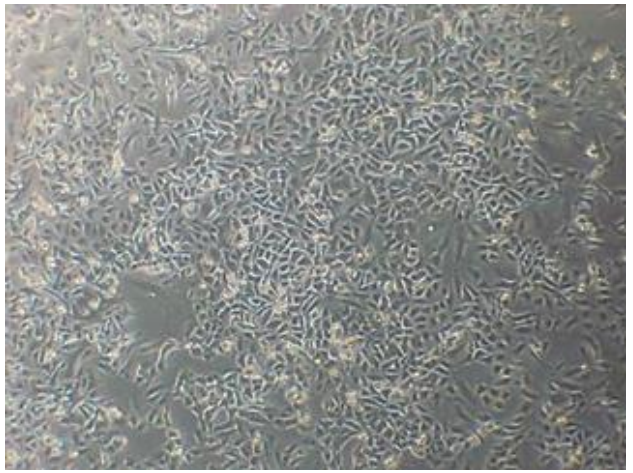
Control (0μg/ml)

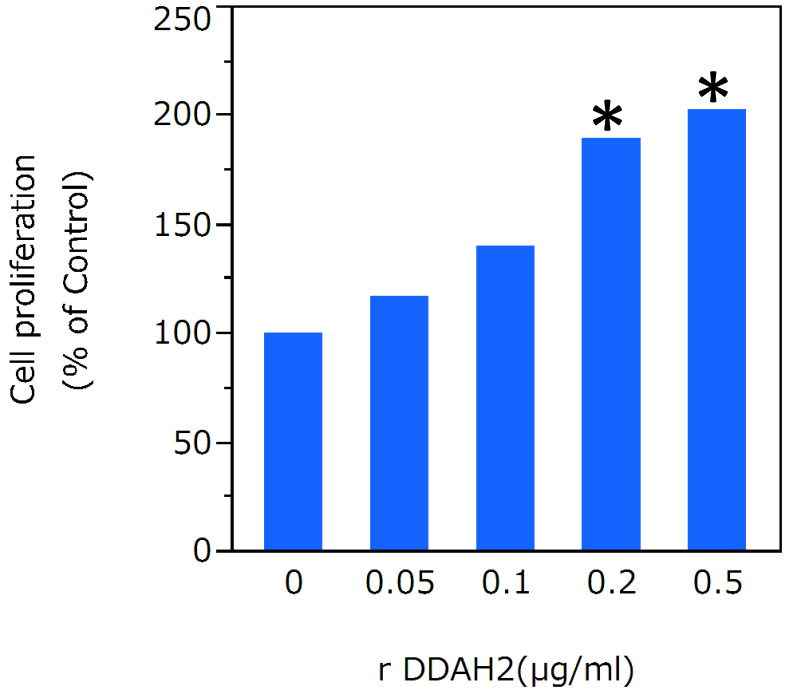


rDDAH2 0.10μg/ml



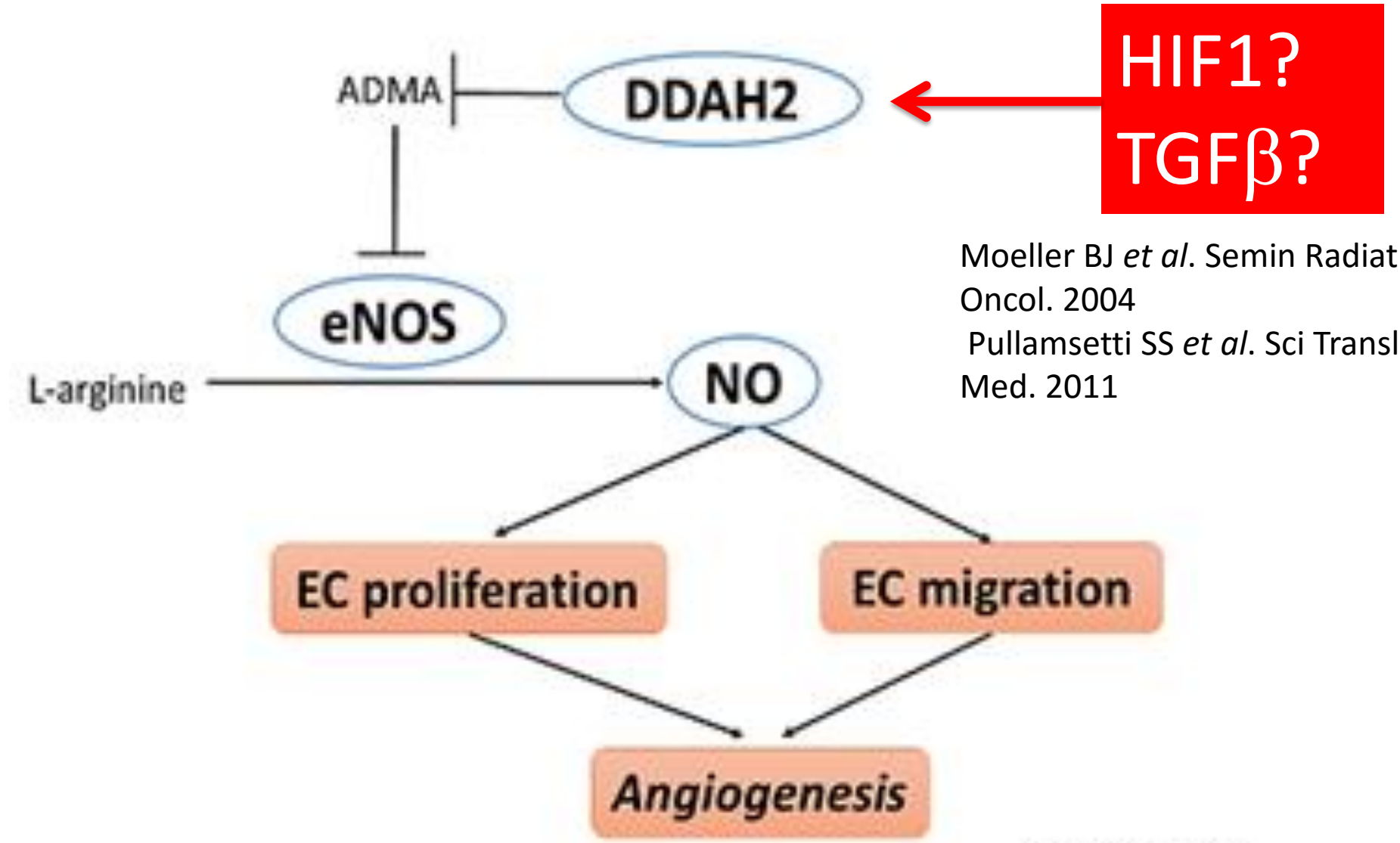
rDDAH2 0.50μg/ml





*p＜0.05 (Controlと比較)

血管内皮細胞株(HUVEC)の増殖はDDAH2の添加によって濃度依存的に促進した。



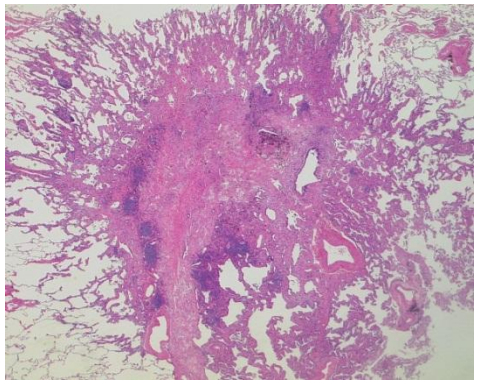
Moeller BJ *et al*. Semin Radiat Oncol. 2004

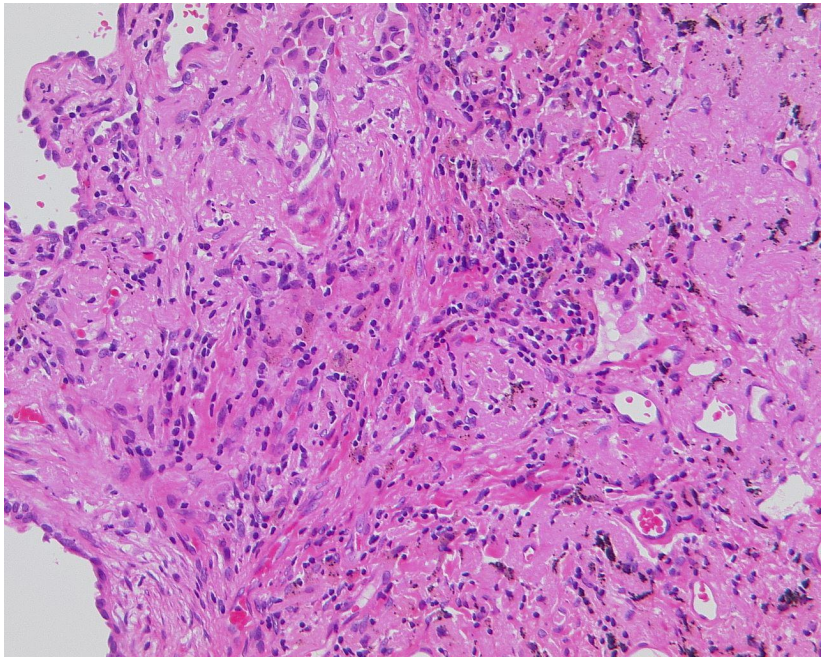
Pullamsetti SS *et al*. Sci Transl Med. 2011

EC: Endothelial Cell

Multistep carcinogenesis

AAH/ Preinvasive lesion → AIS/ Adenocarcinoma *In situ* → Minimally Invasive Cancer → Overt invasive Cancer





DDAH2

Heterogeneous histological characteristics

- Lepidic
- Acinar
- Papillary
- Solid
- Micropapillary