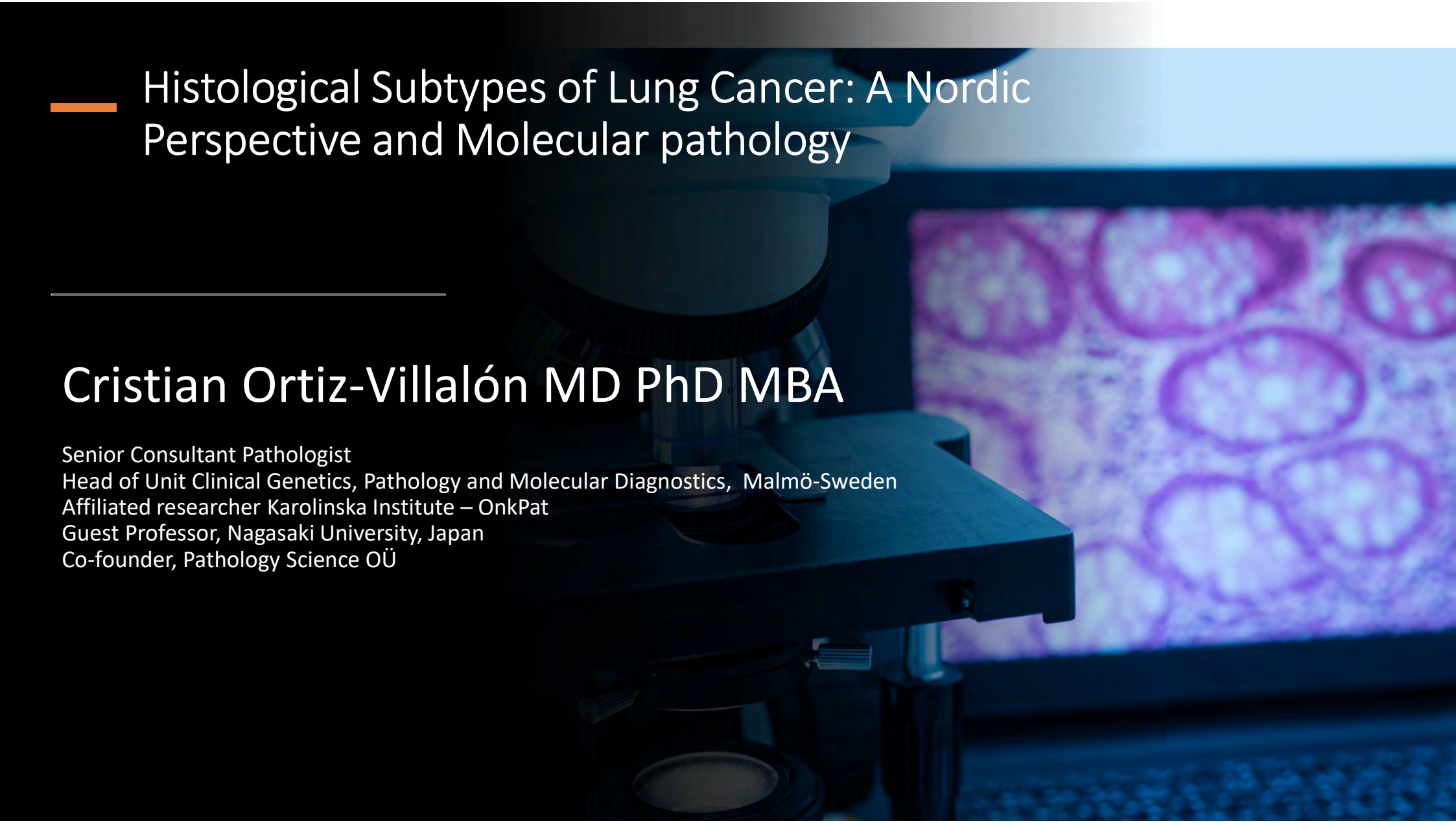
Histological Subtypes of Lung Cancer: A Nordic Perspective and Molecular pathology



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Disclosures

- Has worked as a Senior Director for AstraZeneca, holding shares
- Speaker for Boehringer Ingelheim, Smart in Media, Deciphex, Roche, Bayer
- Scientific advisor for Deciphex, Smart in Media, Lumito.
- Co-founder of Pathology Science OÜ

KVAST
Remissutgåvor
Allmänna dokument
Equalis
Referensbilder
Swedac
KVAST styrgrupp
Bröstpatologi
Digital patologi
Endokrin patologi
Exfoliativ cytologi
Övrigt: utskärningsmallar, SNOMED etc
Okänd primärtumör ("CUP")
Gastrointestinal patologi
Gynekologisk patologi

Thoraxpatologi

Medlemmar i arbetsgruppen

Kvalitetsindikatorer

Nationella vårdprogram

KVAST-dokument

Gamla (ej aktuella) KVAST-dokument

XI. Rekommenderade klassifikationssystem

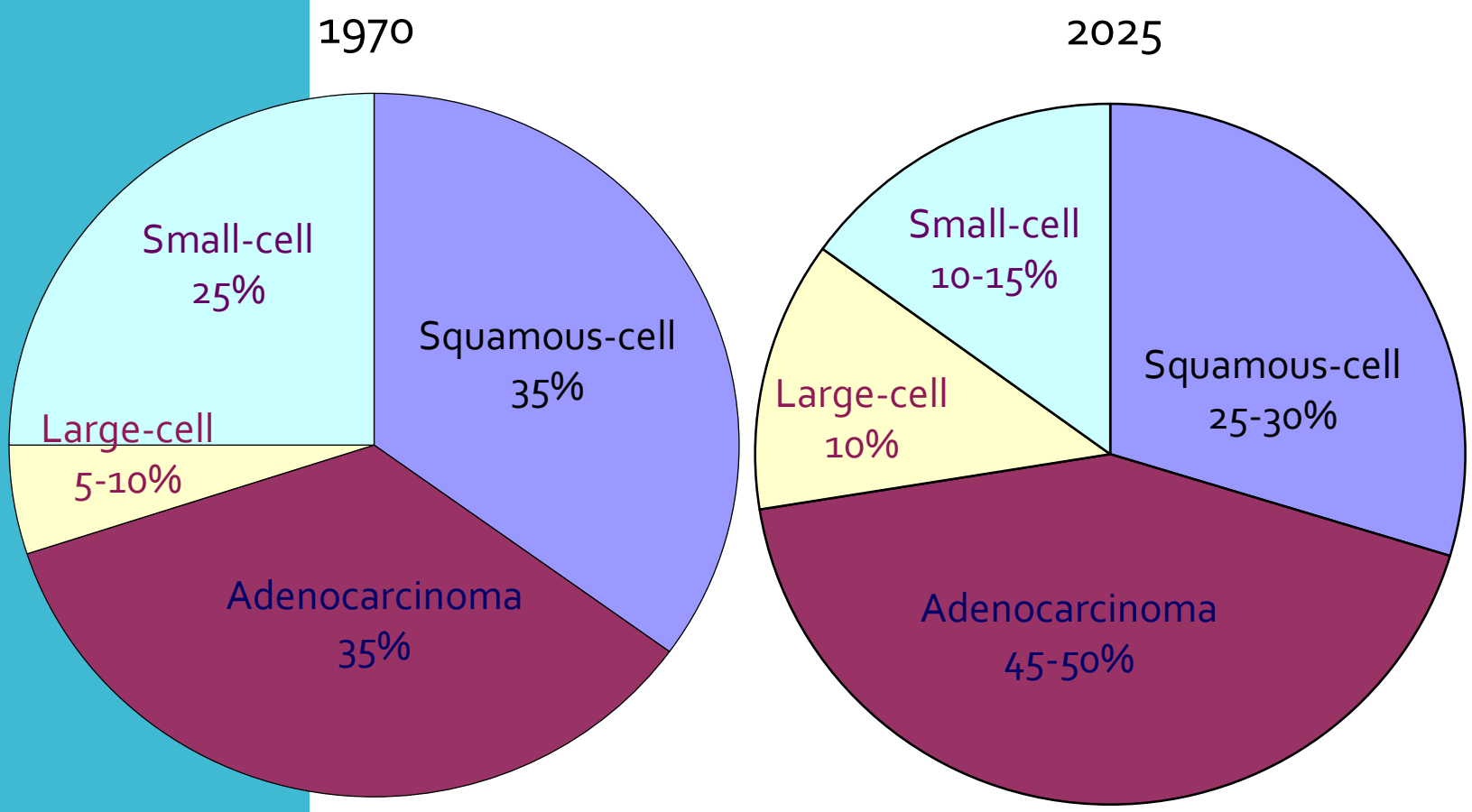
För histologisk indelning av lungtumörer ska senaste WHO-klassifikation användas, idag klassifikationen från 2021. En relevant publikation från IASLC rörande diagnostiska immunfärgningar vid lungcancer publicerades 2019 (Yatabe förstanamn). För stadieindelning ska TNM9 användas. I Appendix 3 och 4 finns sammanfattning av klassifikation/nomenklatur respektive stadieindelning.

Histologic Classification of Malignant Epithelial Lung Tumors

(2021 WHO Classification, Simplified Version)

- Adenocarcinoma
- Squamous cell carcinoma
- Large cell carcinoma
- Neuroendocrine cancer
- Sarcomatoid carcinoma
- Other epithelial tumours

Lung Cancer Histology



Novelties of 2004-2021 WHO “blue book”

The importance of small biopsies has changed over the time……

For CLINICIANS

For PATHOLOGISTS

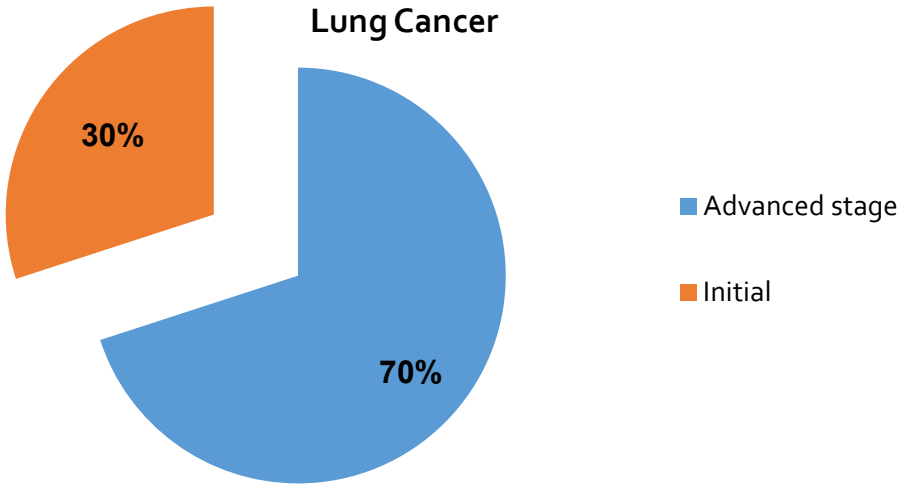
For resections

1967 HE
1981 HE & Mucin
1999 HE, Mucin & IHC
2004 HE, Mucin, IHC & Genetics

- Wide use of IHC through the classification
- A new classification for small biopsy/cytology samples
  As in 2011/2013 IALSCT/ERS classification
- Emphasis on genetics for predictive biomarkers in advanced lung cancer
- A different approach to lung adenocarcinoma (AIS, MIA… PATTERNS)
  As in 2011 IALSC/ATS/ERS classification
- The issue of large cell carcinoma, squamous carcinoma and NET
- Reclassifying other tumors (NUT Hamartoma, myxoid sarcoma…)

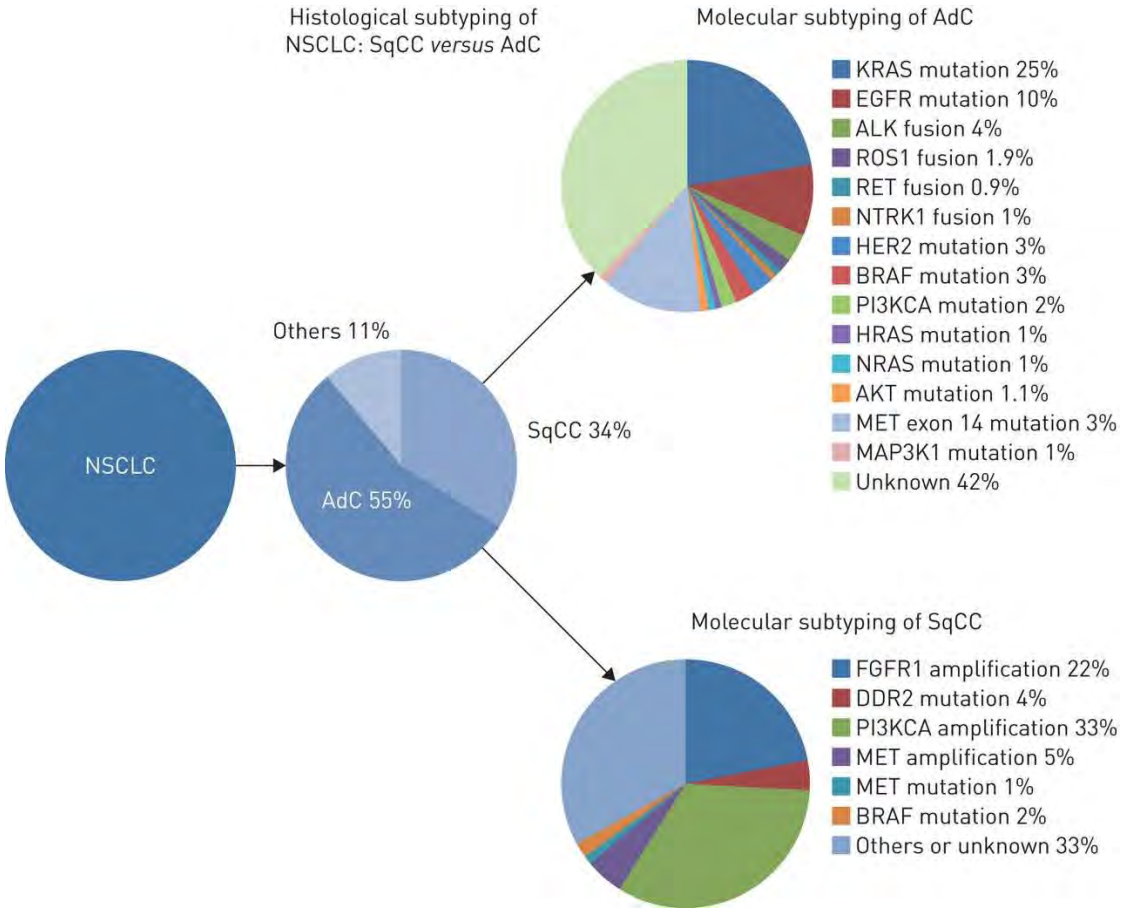
NSCLC: 70% in Advanced Stage

Small material (biopsy/cytolology)



2011 Feb;6(2):244-85. doi: 10.1097/JTO.0b013e318206a221.
Sigel RA Ca Cancer J Clin 2012:62: 10
C.A. Ridge, Epidemiology of lung cancer Semin Interven Radiol, 30 (2013)

Histological and molecular subtypes in nonsmall cell lung carcinoma (NSCLC).



Lukas Bubendorf et al. Eur Respir Rev 2017;26:170007

Type of material

While the number of tests over the past decade has increased, sample size (predominantly small biopsies or cytological specimens) has decreased.

Small Samples: NSCC Subtyping

- Morphological criteria:

Glandular differentiation and/or mucin → **ADC**
Intercellular bridges and/or keratinization → **SCC**

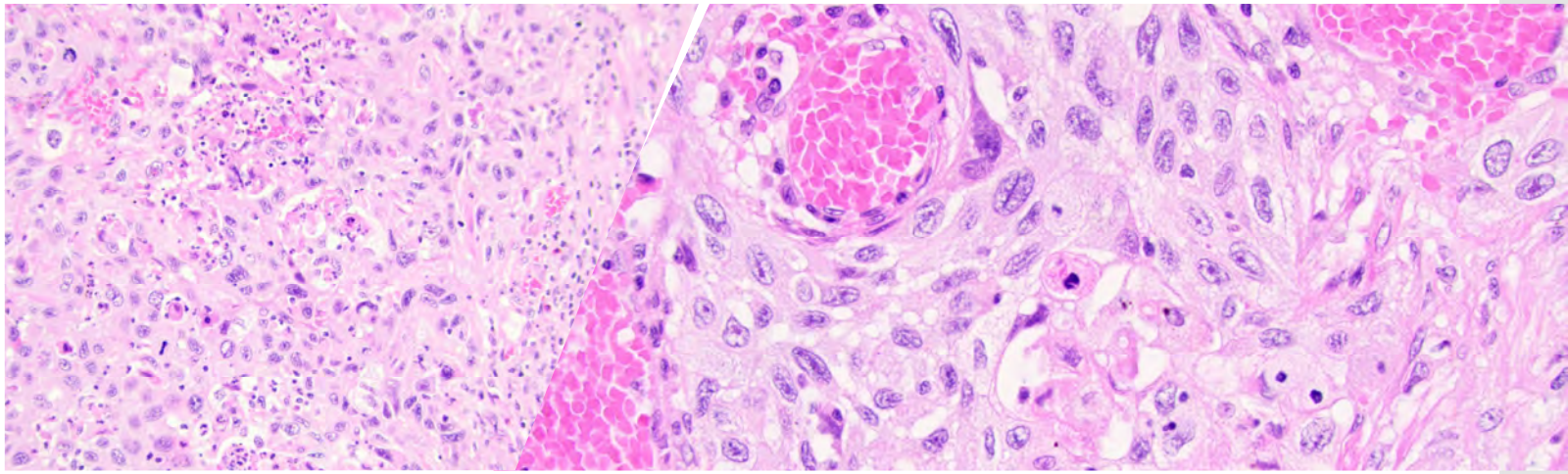
- Established morphological criteria absent – do IHC:

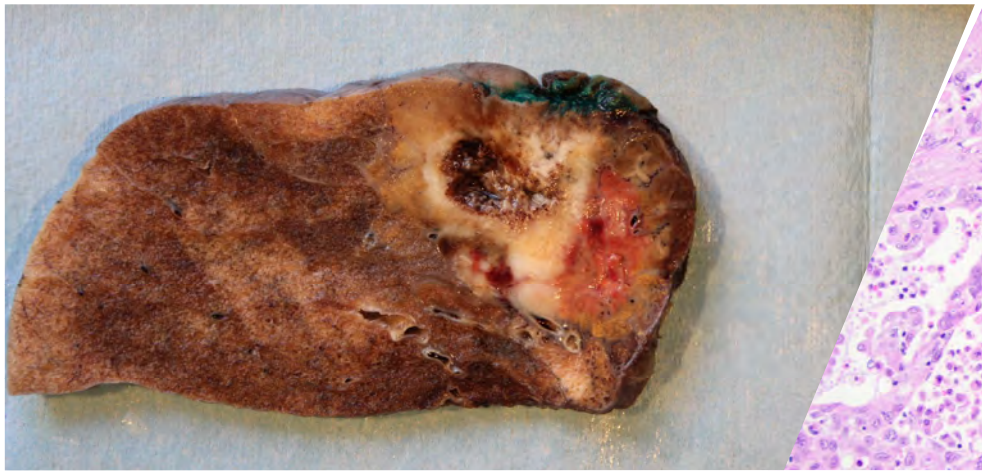
TTF1+ → **NSCC, favor ADC**
p40+ → **NSCC, favor SCC**
Inconclusive → **NSCC, NOS**

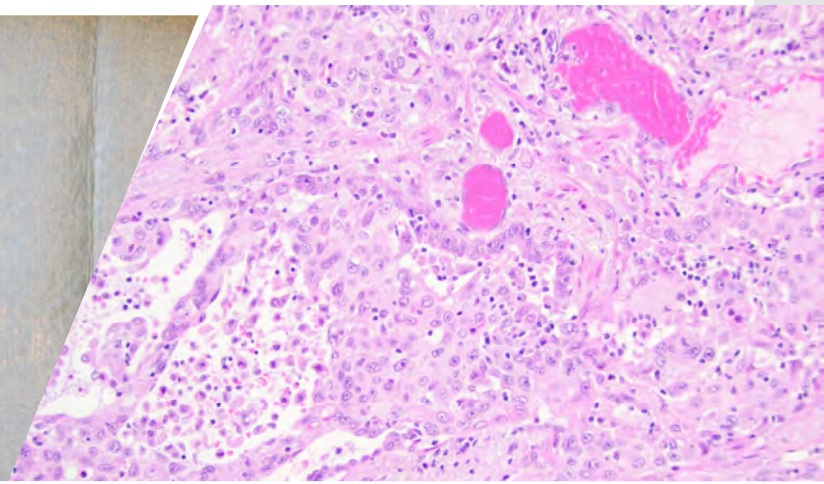
Terminology in small biopsy and cytology versus resection specimens for **squamous cell carcinoma**

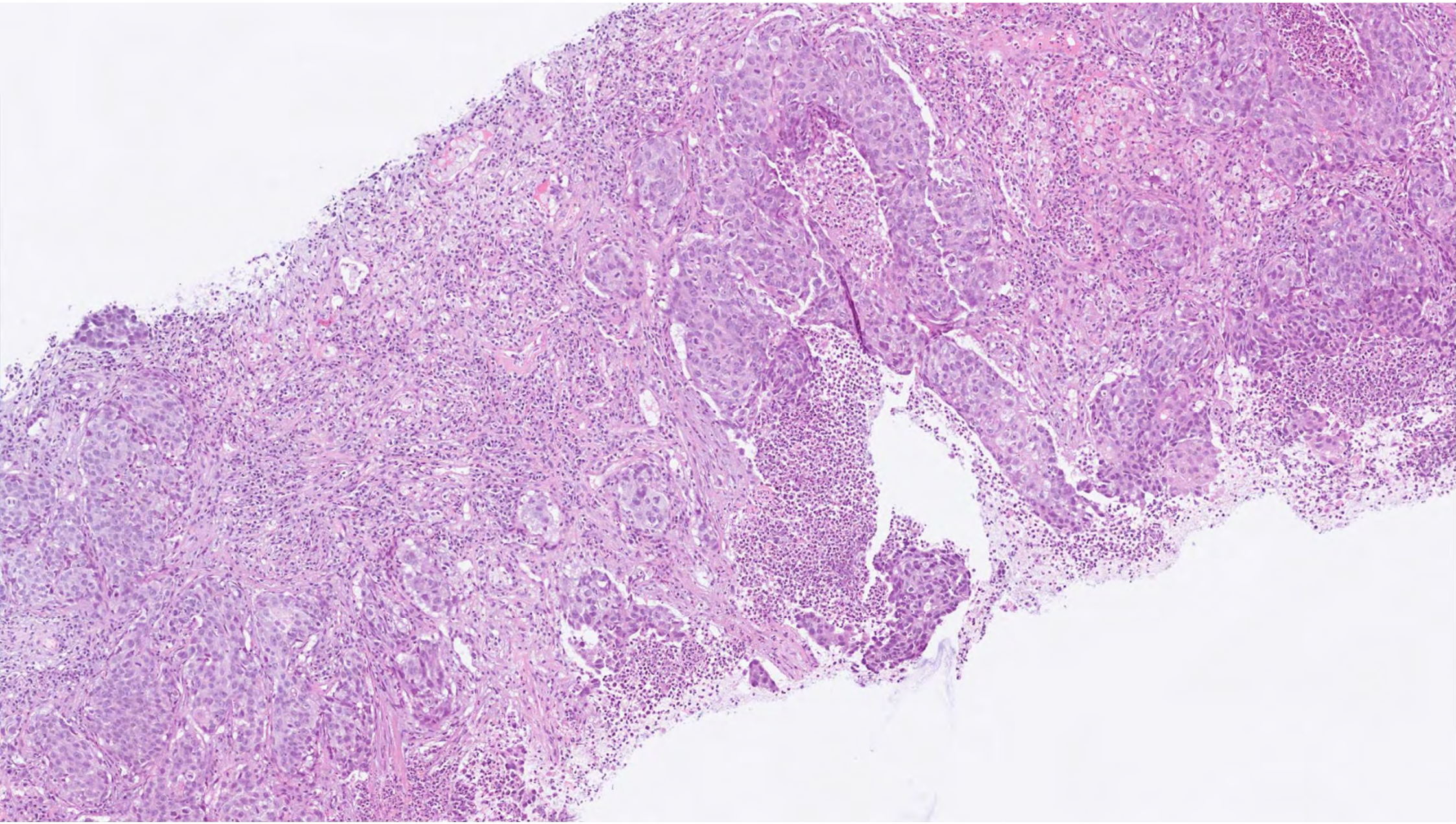
Keratinization
Intercellular bridges
NSCLC with IHC P40 (+)

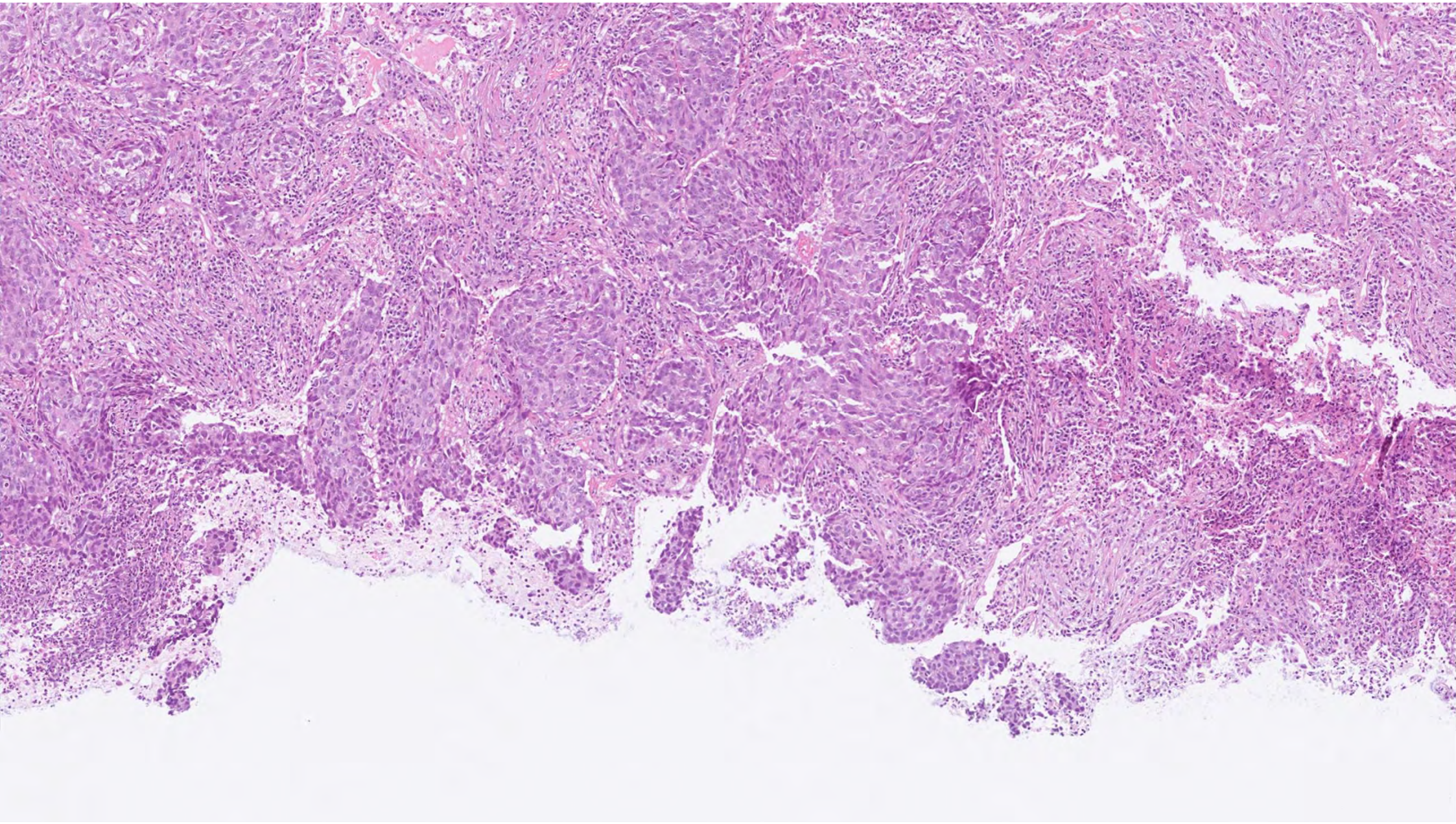
Morphology/stains	Terminology for small biopsies and cytology specimens	Terminology for resection specimens
Morphological squamous cell patterns clearly present	Squamous cell carcinoma	Squamous cell carcinoma

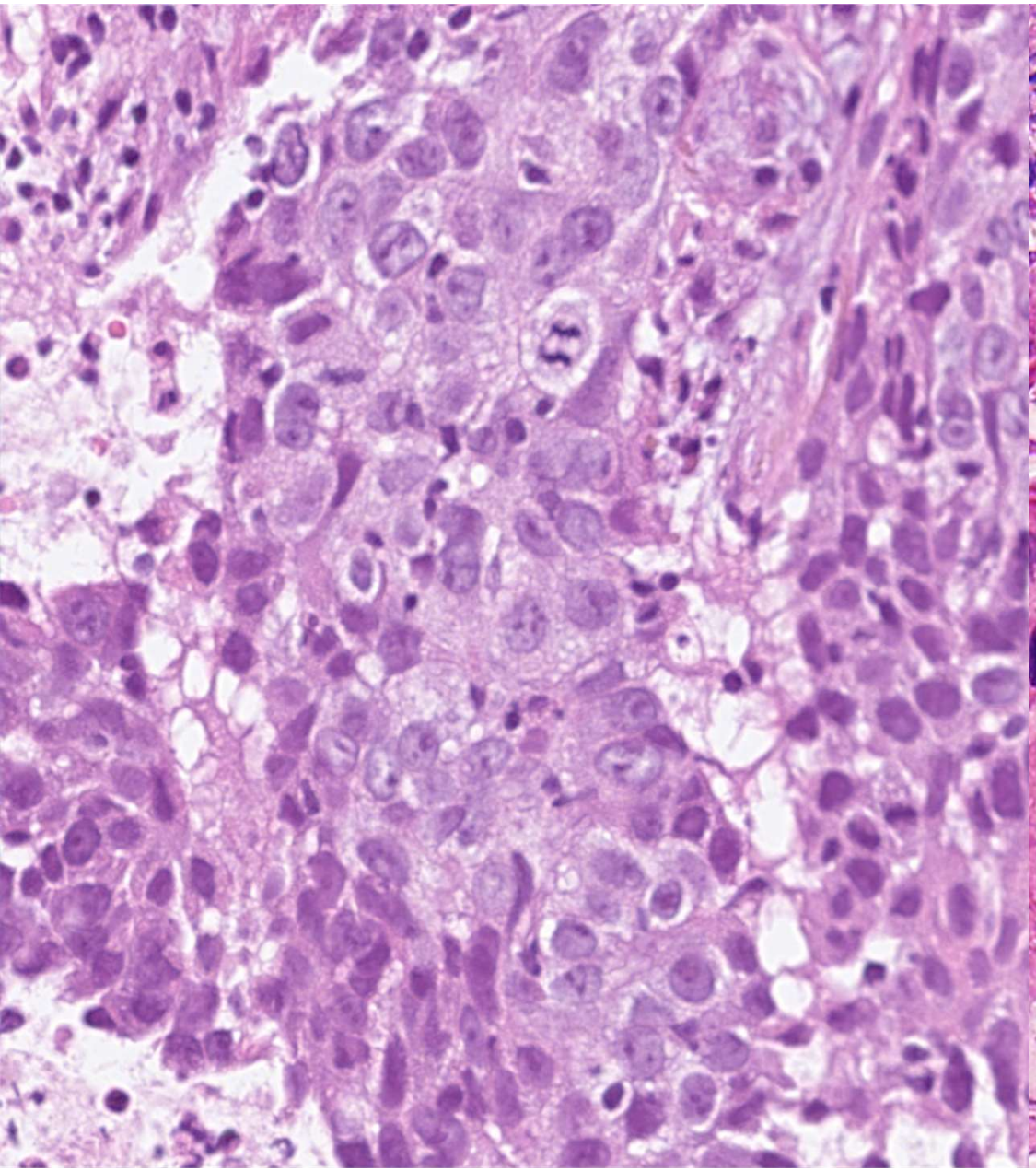
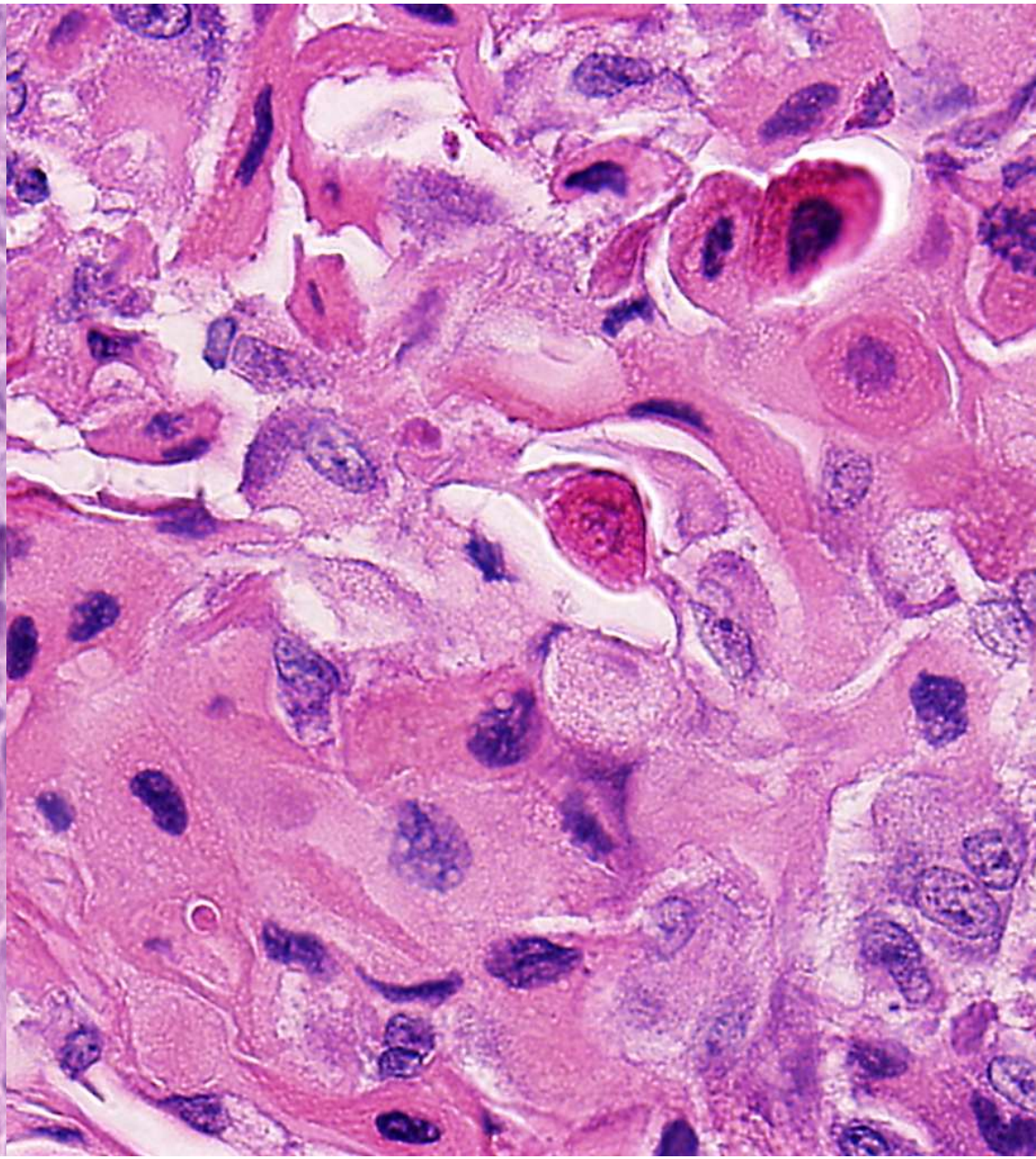






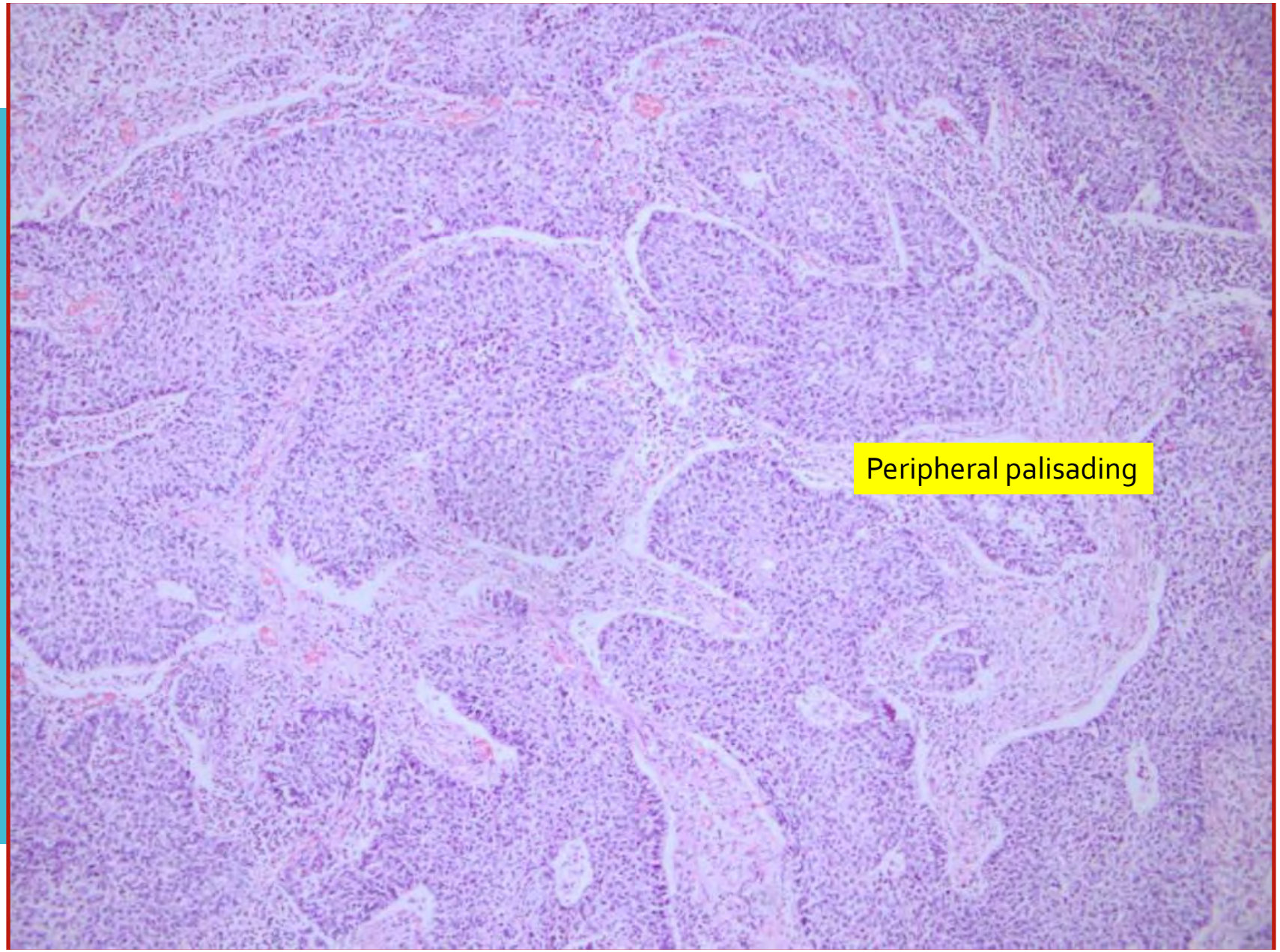


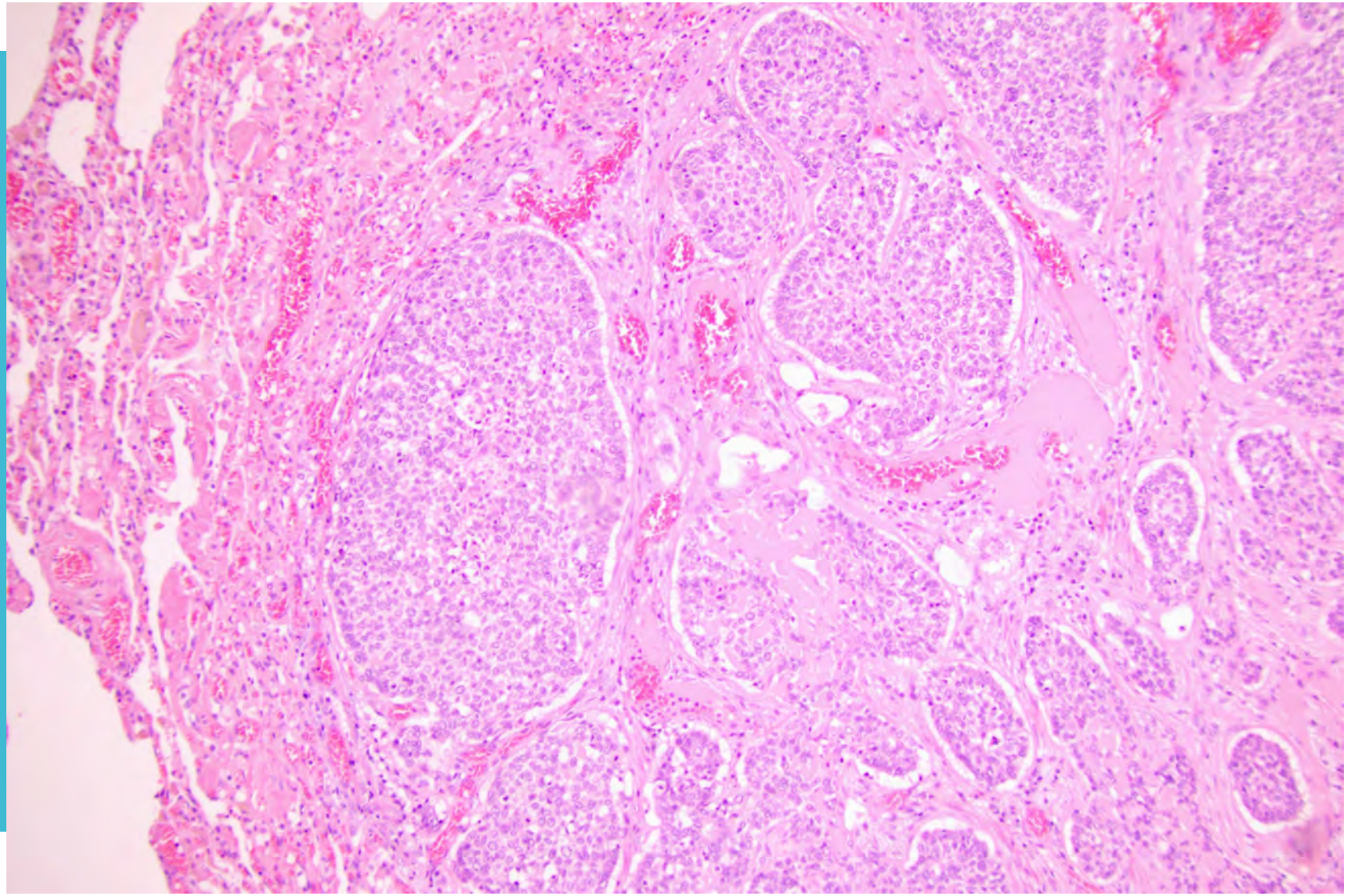


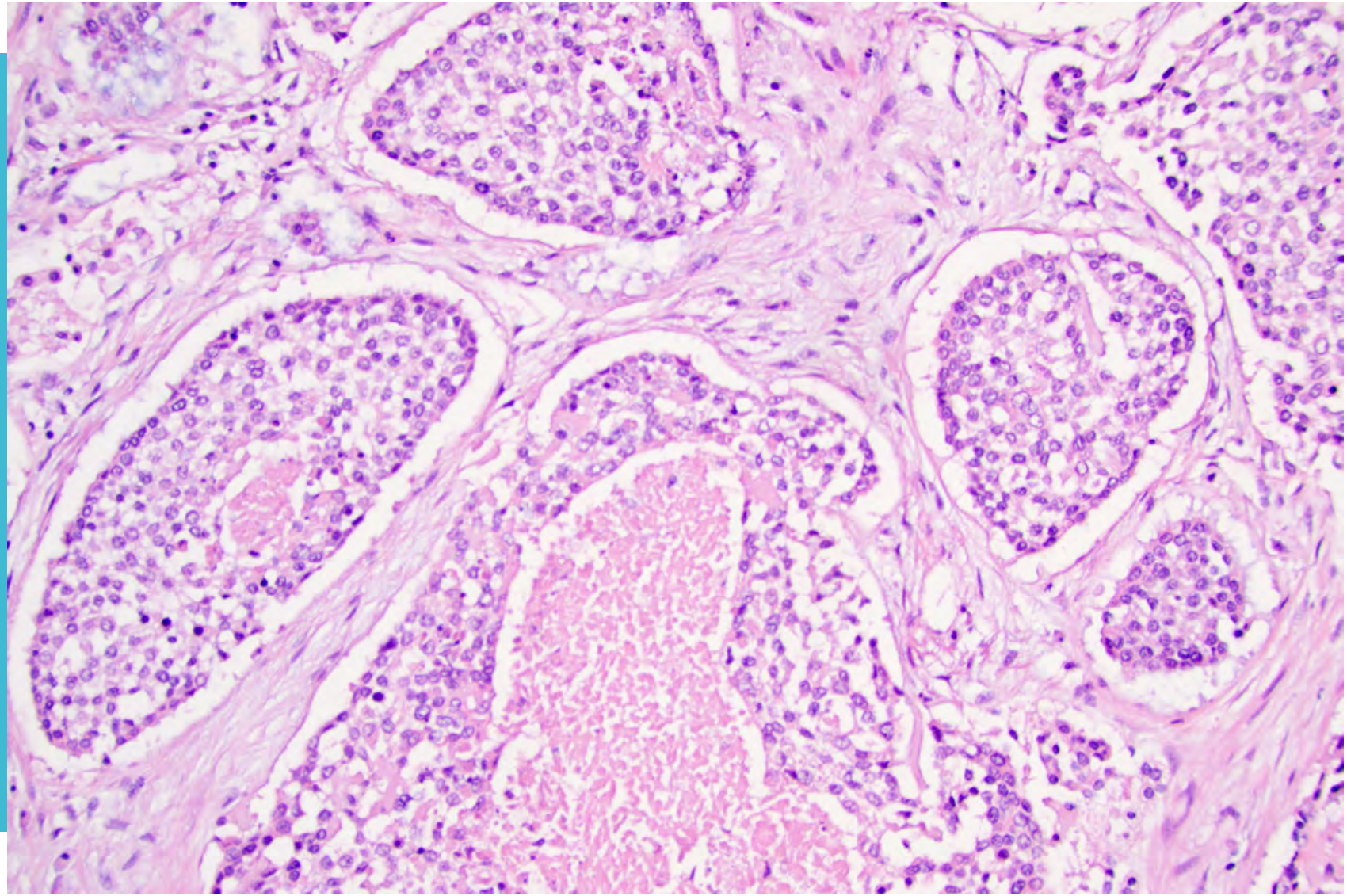



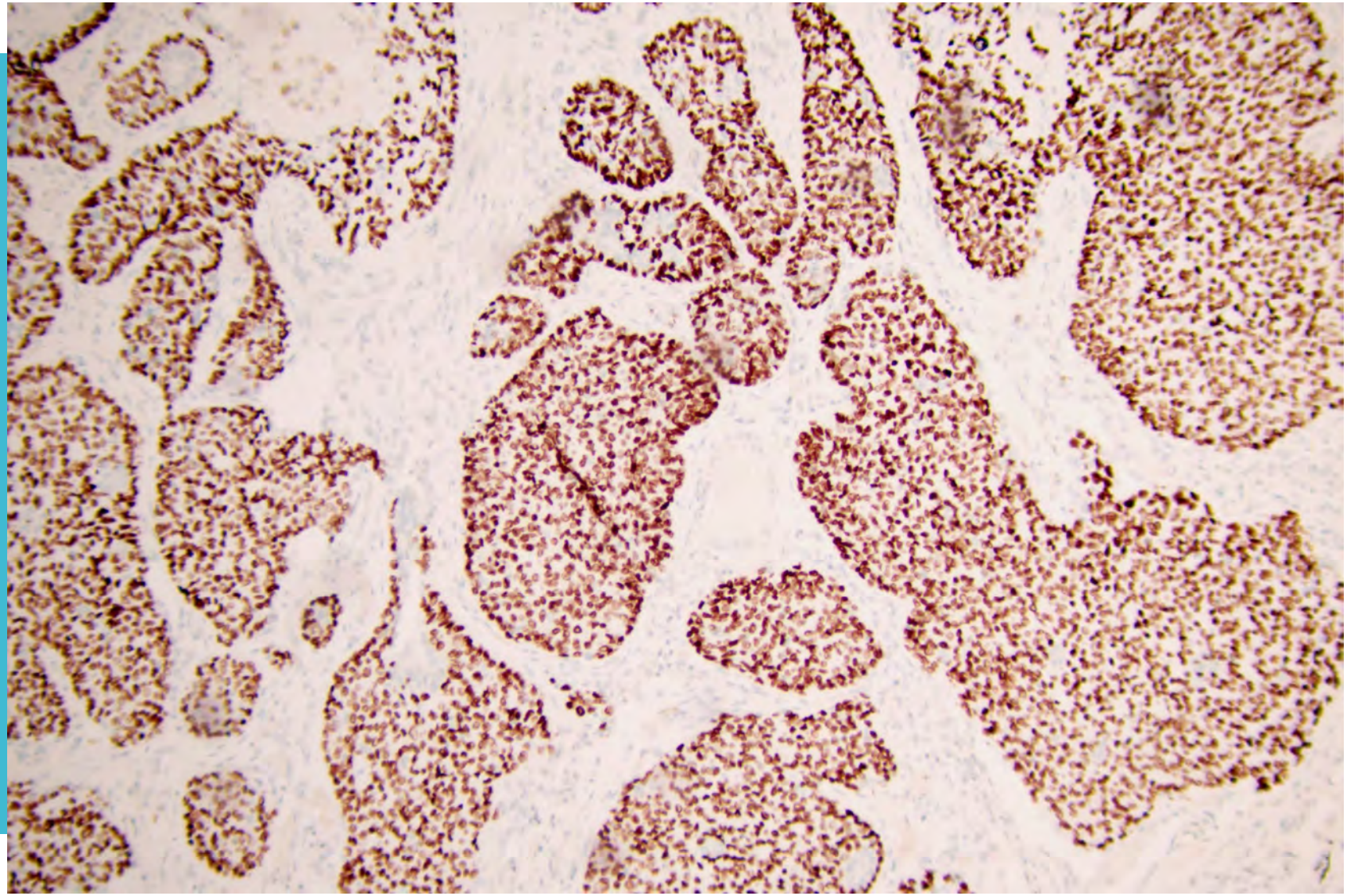
Basaloid squamous cell carcinoma

- Poorly diff cancer with proliferation of small cells with lobular architecture and peripheral palisading.
- The cells lack squamous morphology
- IHC for SCC (+)


Peripheral palisading

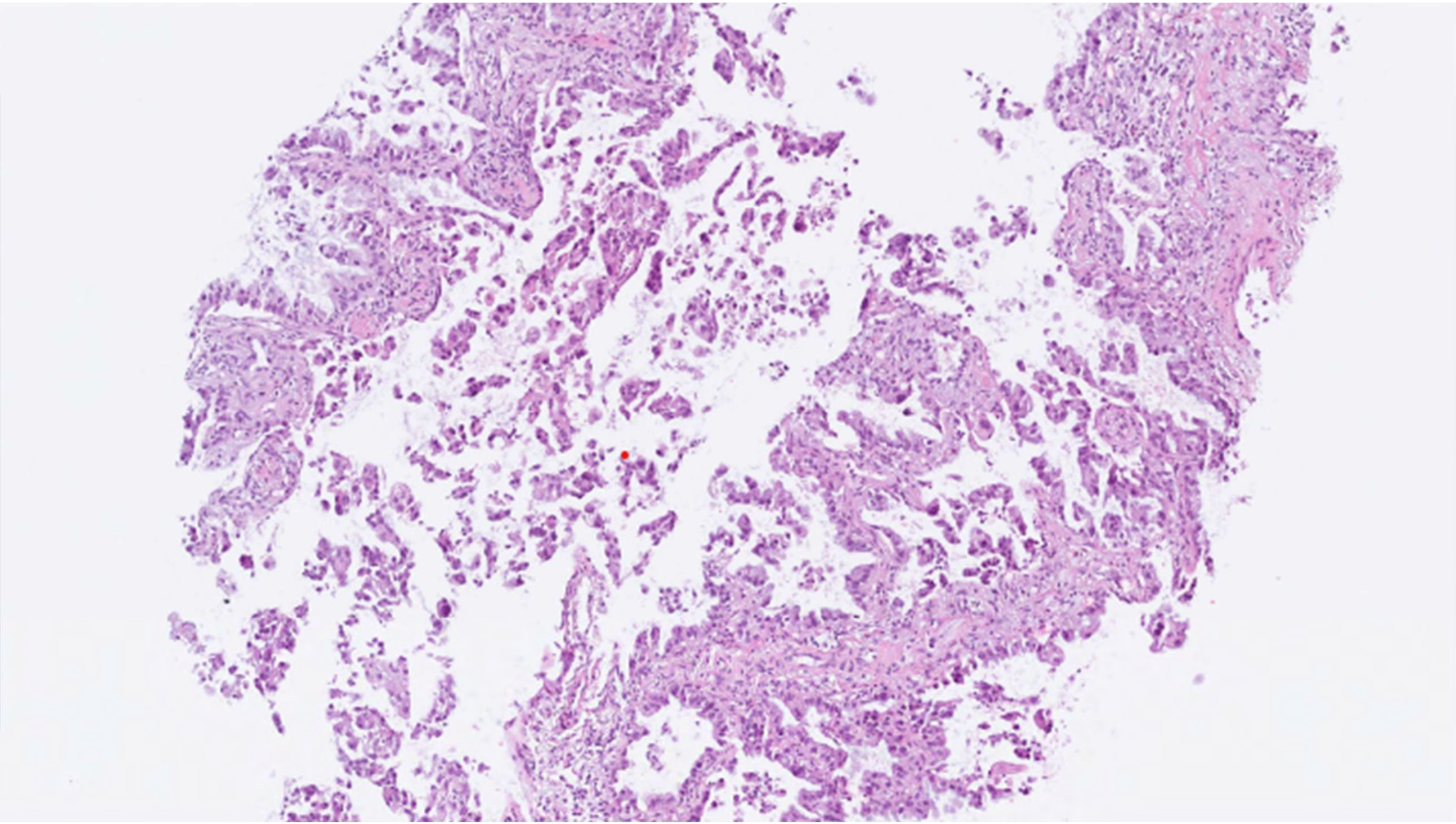


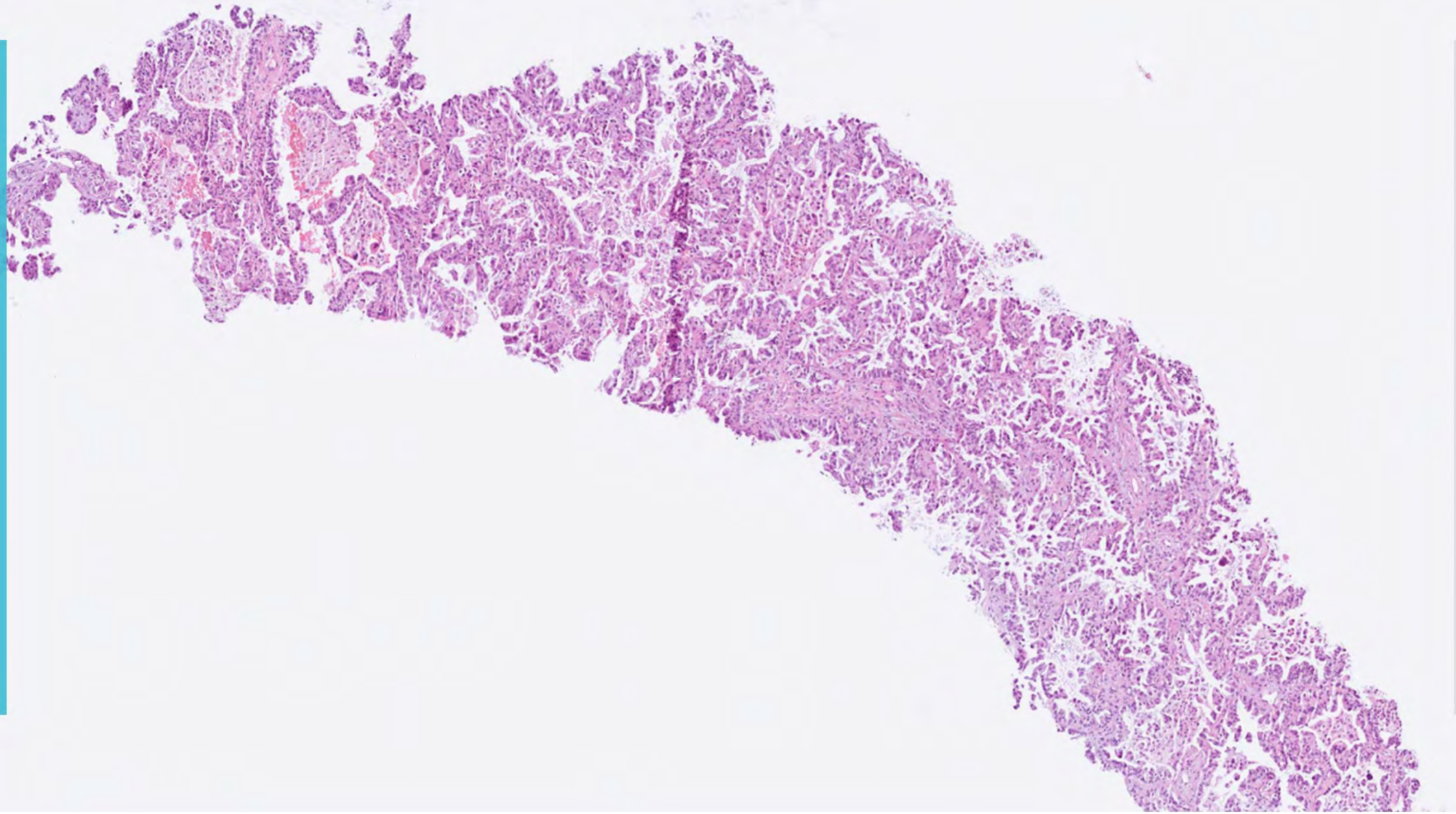


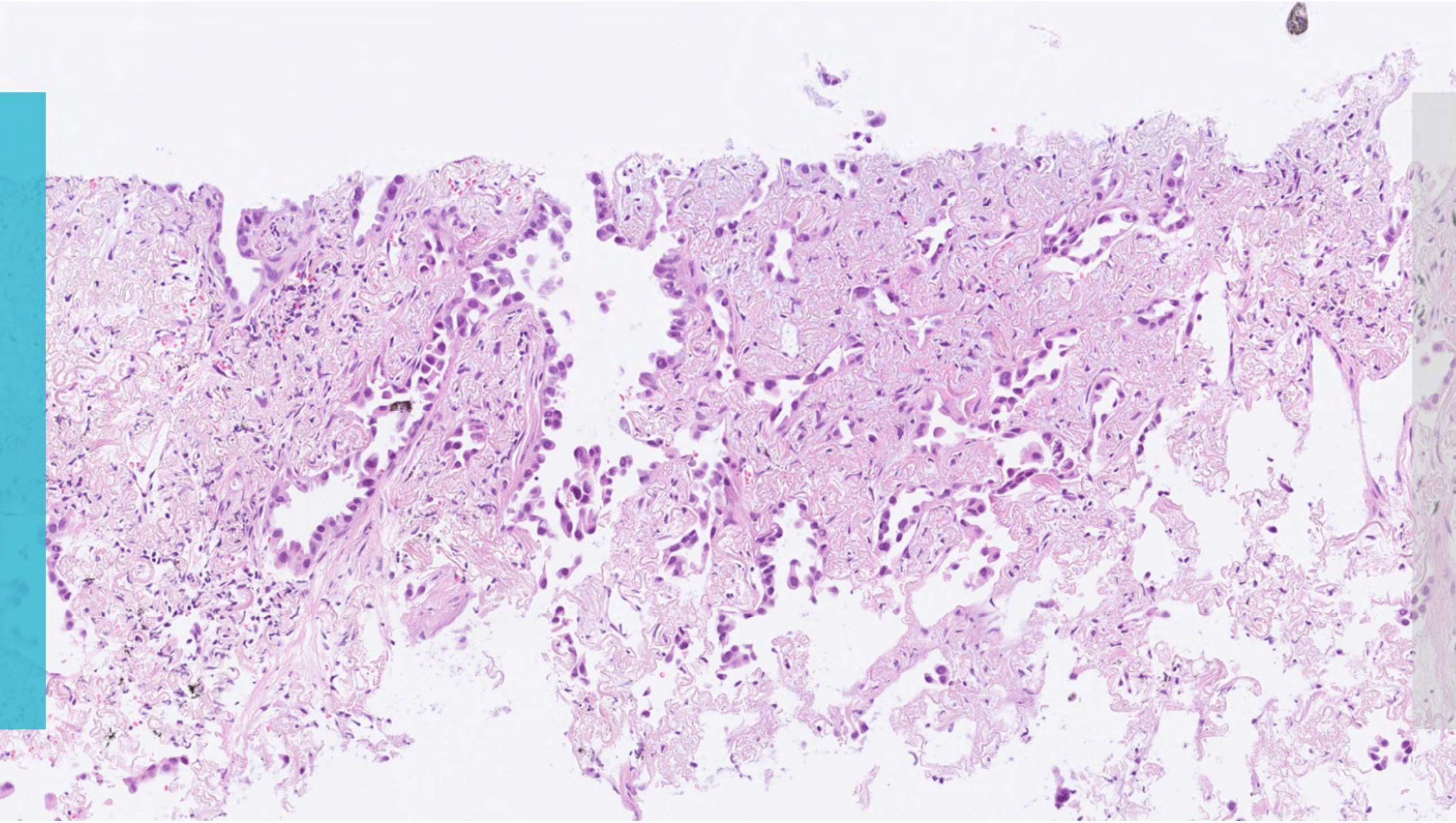


Terminology in **small biopsy** and cytology versus resection specimens for **adenocarcinoma**

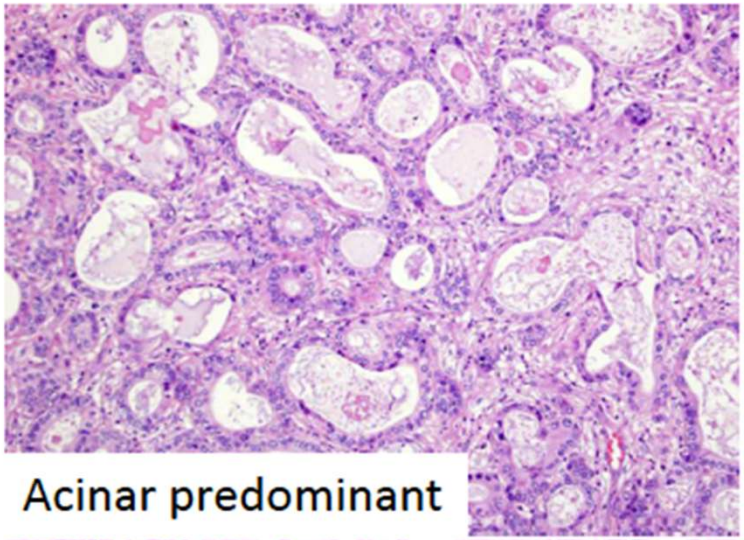
Morphology/stains	Terminology for small biopsies and cytology specimens	Terminology for resection specimens
Morphological adenocarcinoma patterns clearly present	Adenocarcinoma (patterns)	ADC predominant pattern (L,A,P,S,M)
	Adenocarcinoma with lepidic pattern (if pure, list the differential diagnosis on the right and add a comment that an invasive component cannot be excluded)	Minimally invasive adenocarcinoma, adenocarcinoma in situ, or an invasive adenocarcinoma with a lepidic component
	Invasive mucinous adenocarcinoma (list the patterns; use the term "mucinous adenocarcinoma with lepidic pattern" if pure lepidic pattern and mention the differential diagnosis listed on the right)	Invasive mucinous adenocarcinoma Minimally invasive adenocarcinoma or adenocarcinoma in situ, mucinous type
	Adenocarcinoma with colloid features	Colloid adenocarcinoma
	Adenocarcinoma with fetal features	Fetal adenocarcinoma
	Adenocarcinoma with enteric features	Enteric adenocarcinoma



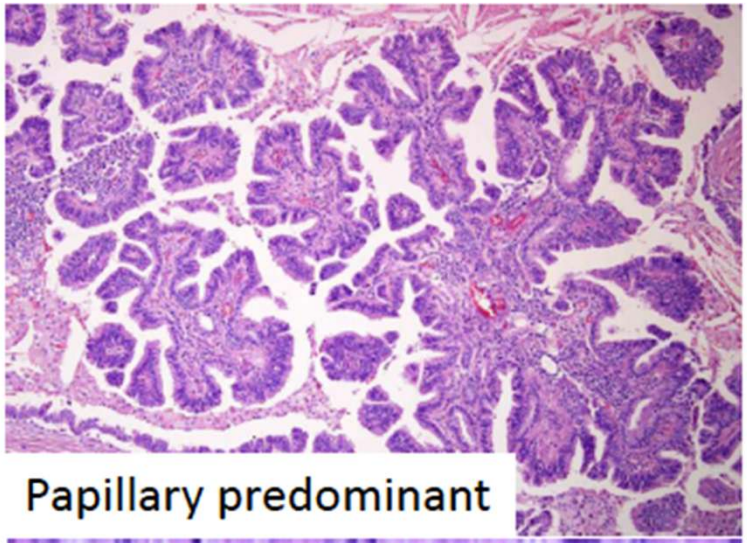




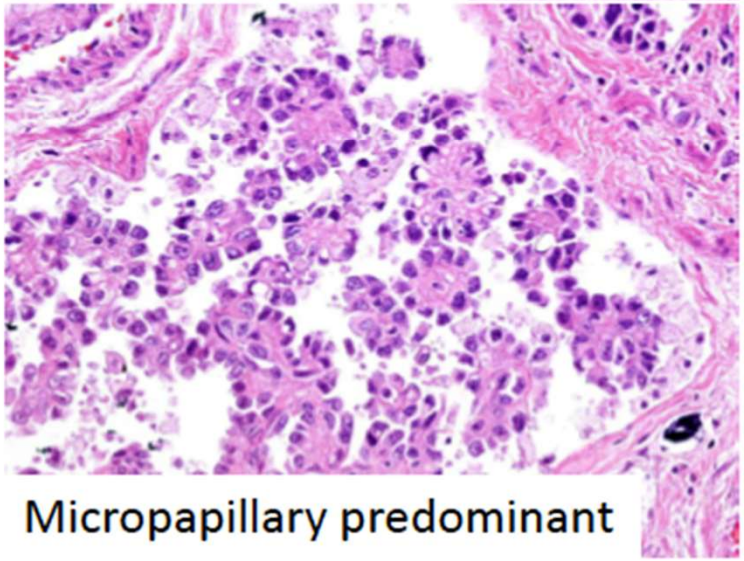
Invasive Adenocarcinoma



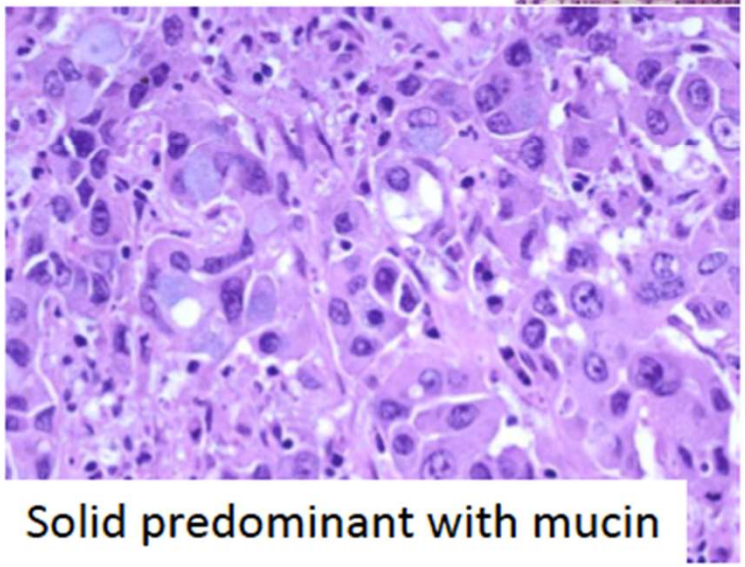
Acinar predominant



Papillary predominant

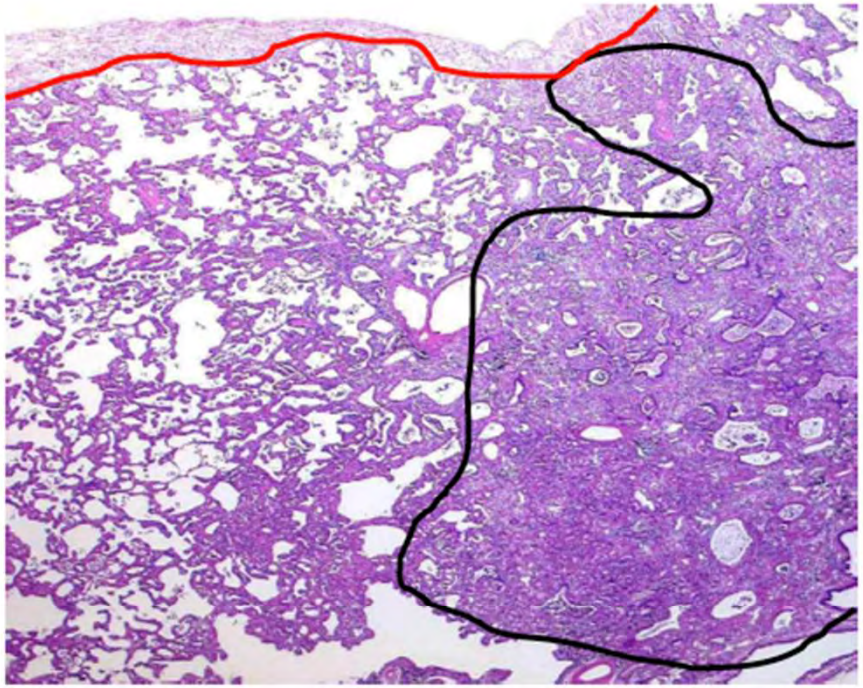


Micropapillary predominant

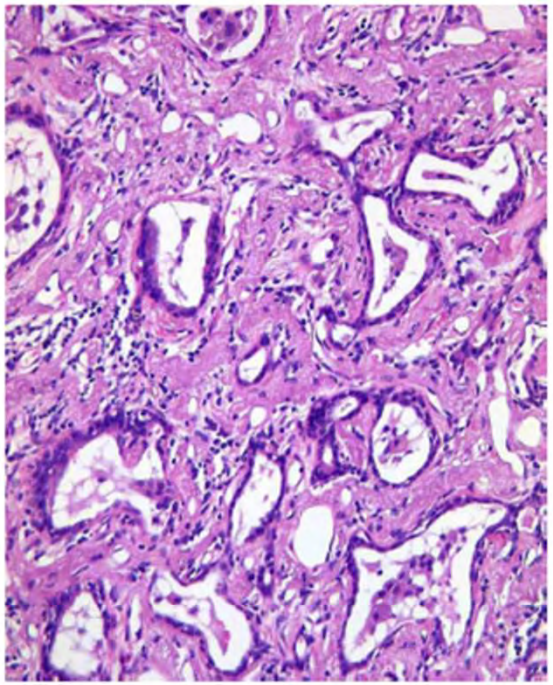


Solid predominant with mucin

Minimally invasive Adenocarcinoma

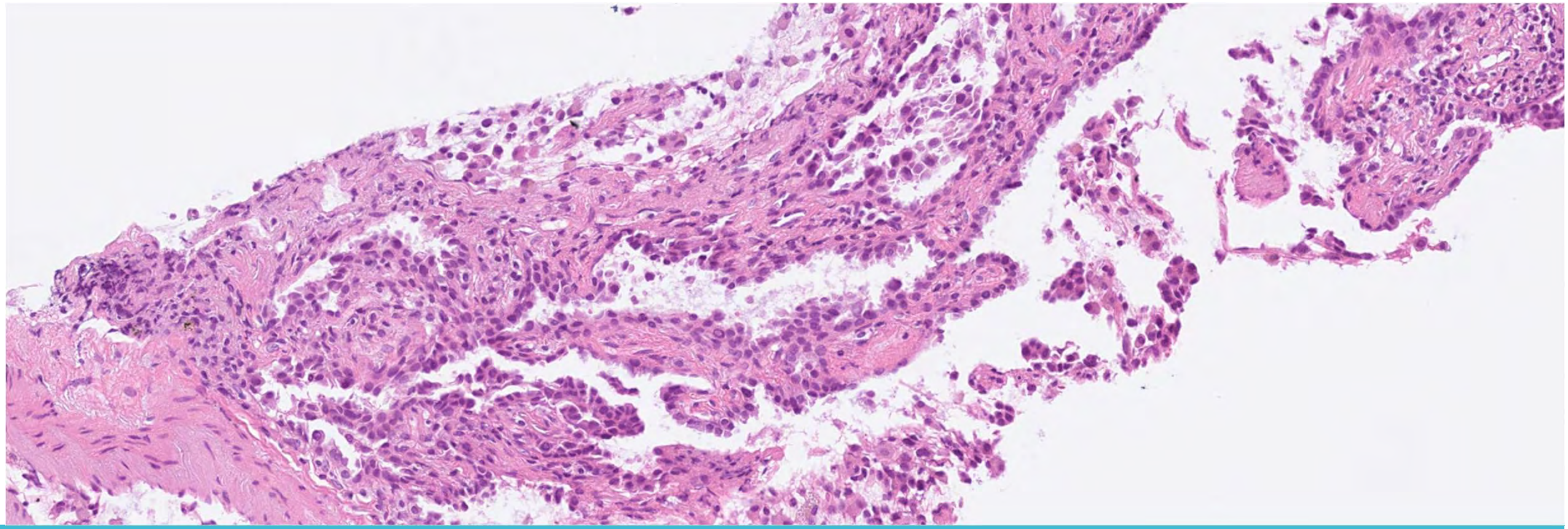


≤ 3cm
lepidic predominant

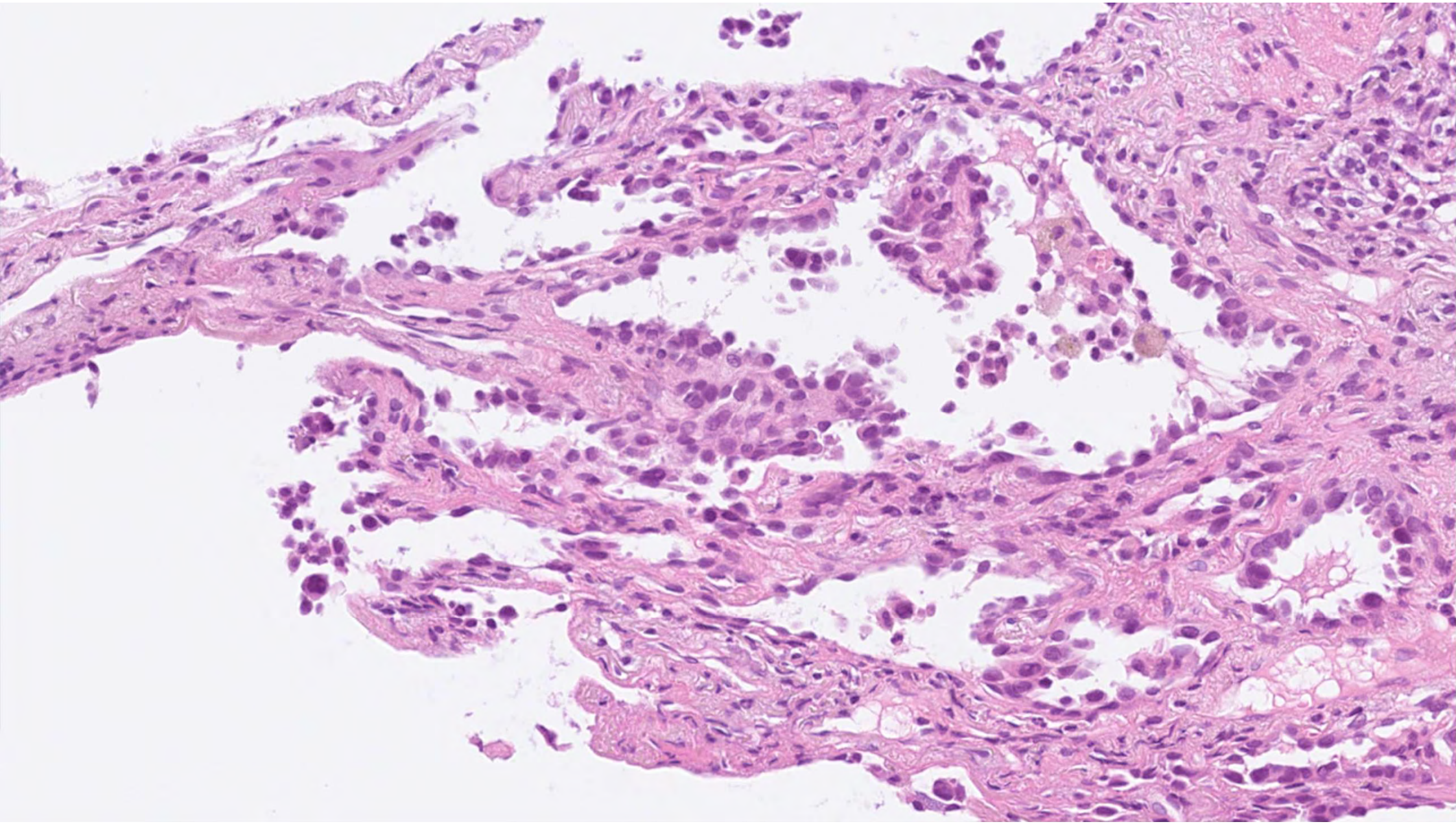


≤ 0.5cm invasion:
– invasive subtypes
– tumor cells infiltr. stroma

no invasion of lymphatics, blood vessels or pleura, no tumor necrosis



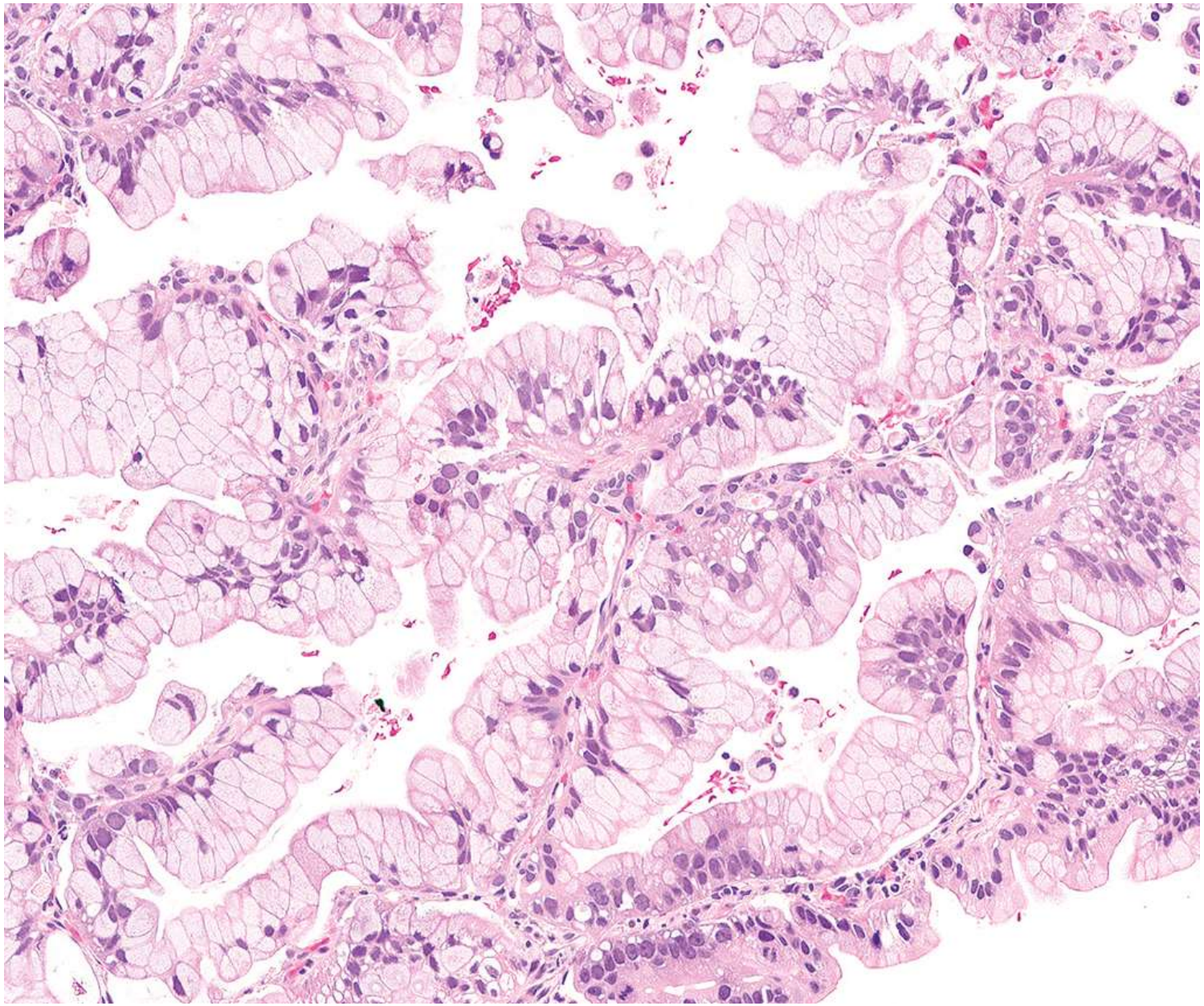
Adenocarcinoma with lepidic pattern (if pure, list the differential diagnosis on the right and add a comment that an invasive component cannot be excluded)



Resection specimens

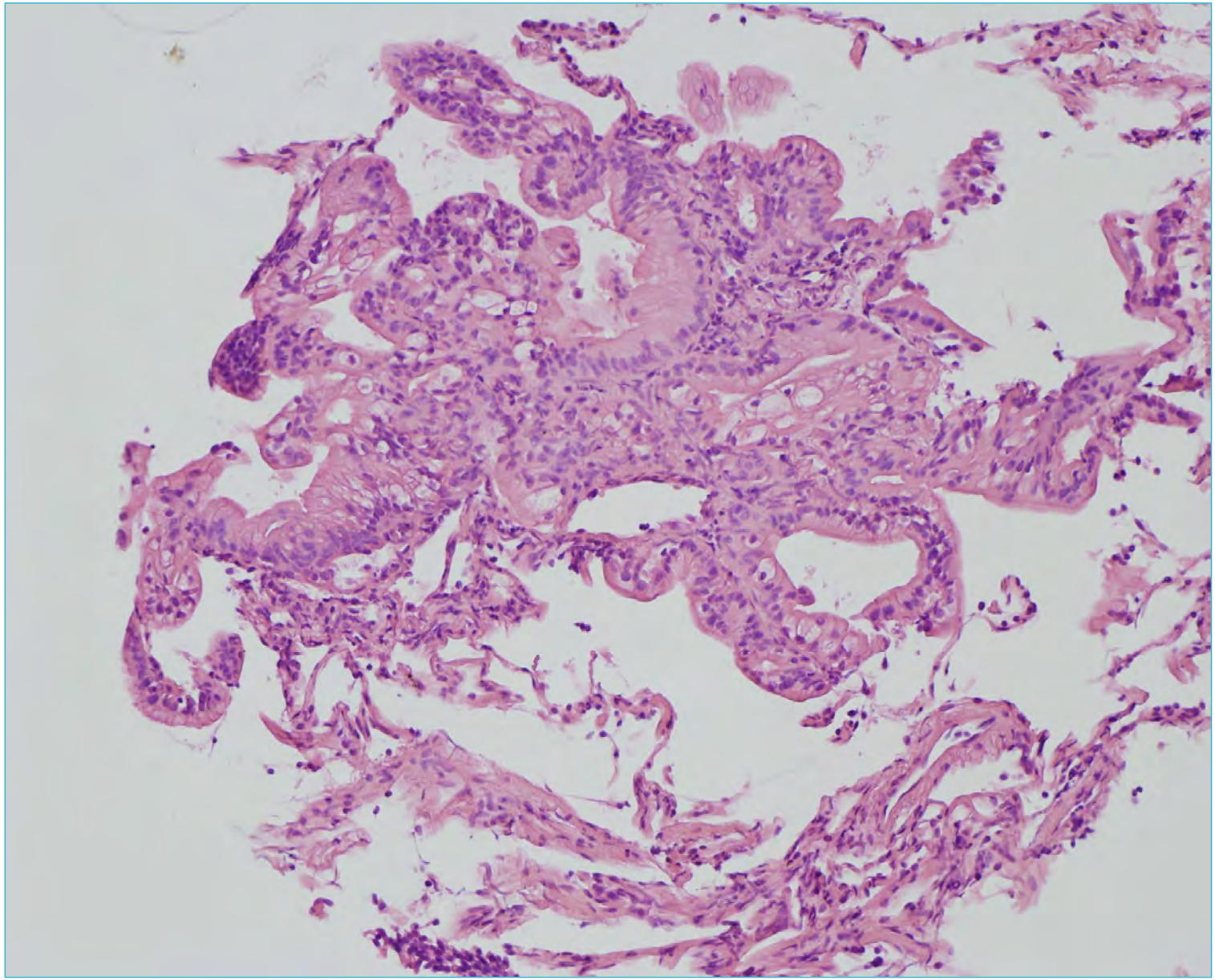
Invasive mucinous adenocarcinoma

Minimally invasive adenocarcinoma or adenocarcinoma in situ, mucinous type



Small biopsies

Invasive mucinous adenocarcinoma (list the patterns; use the term "mucinous adenocarcinoma with lepidic pattern" if pure lepidic pattern and mention the differential diagnosis)

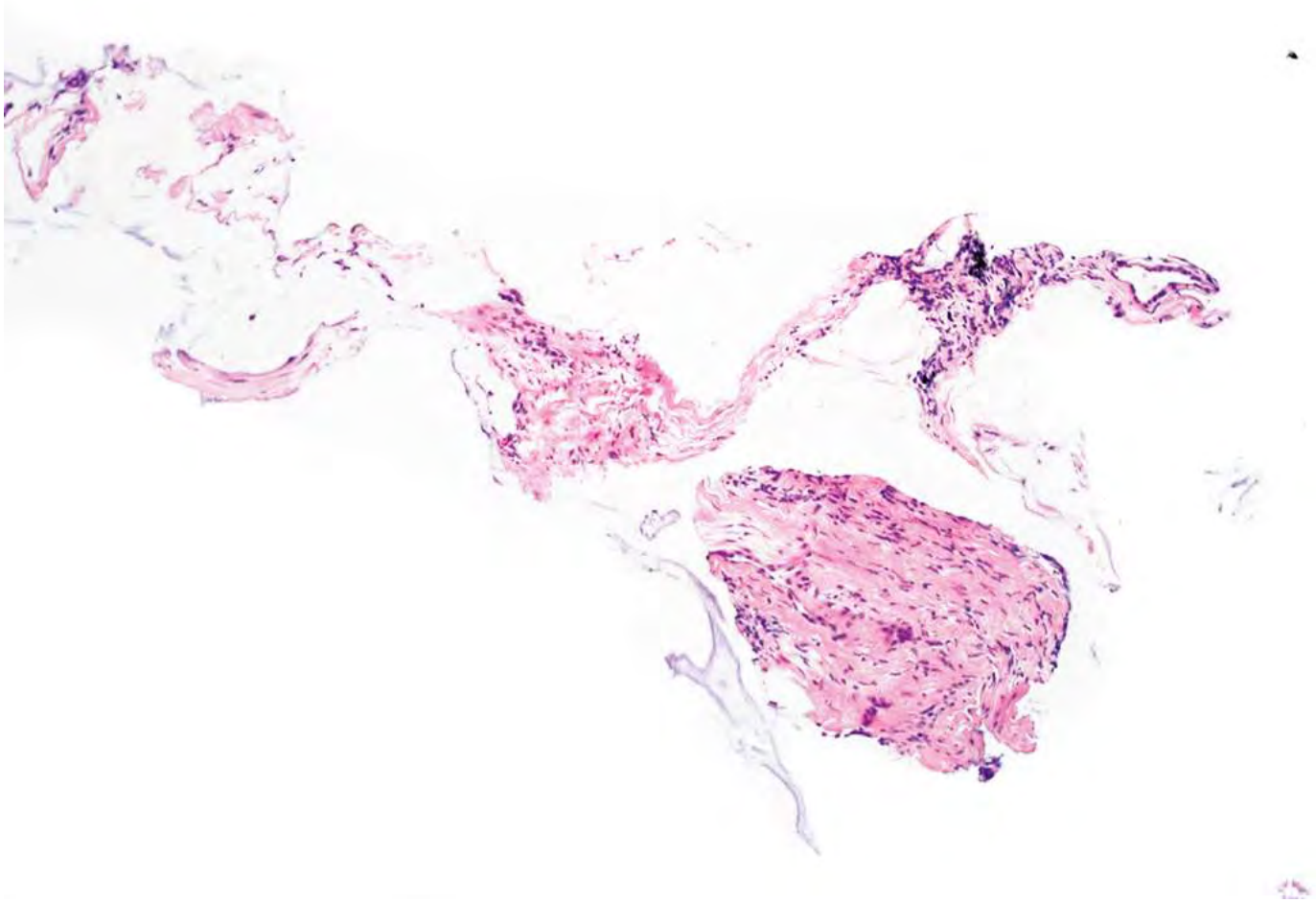


Small biopsies

Adenocarcinoma with colloid features

Specimens

Colloid adenocarcinoma

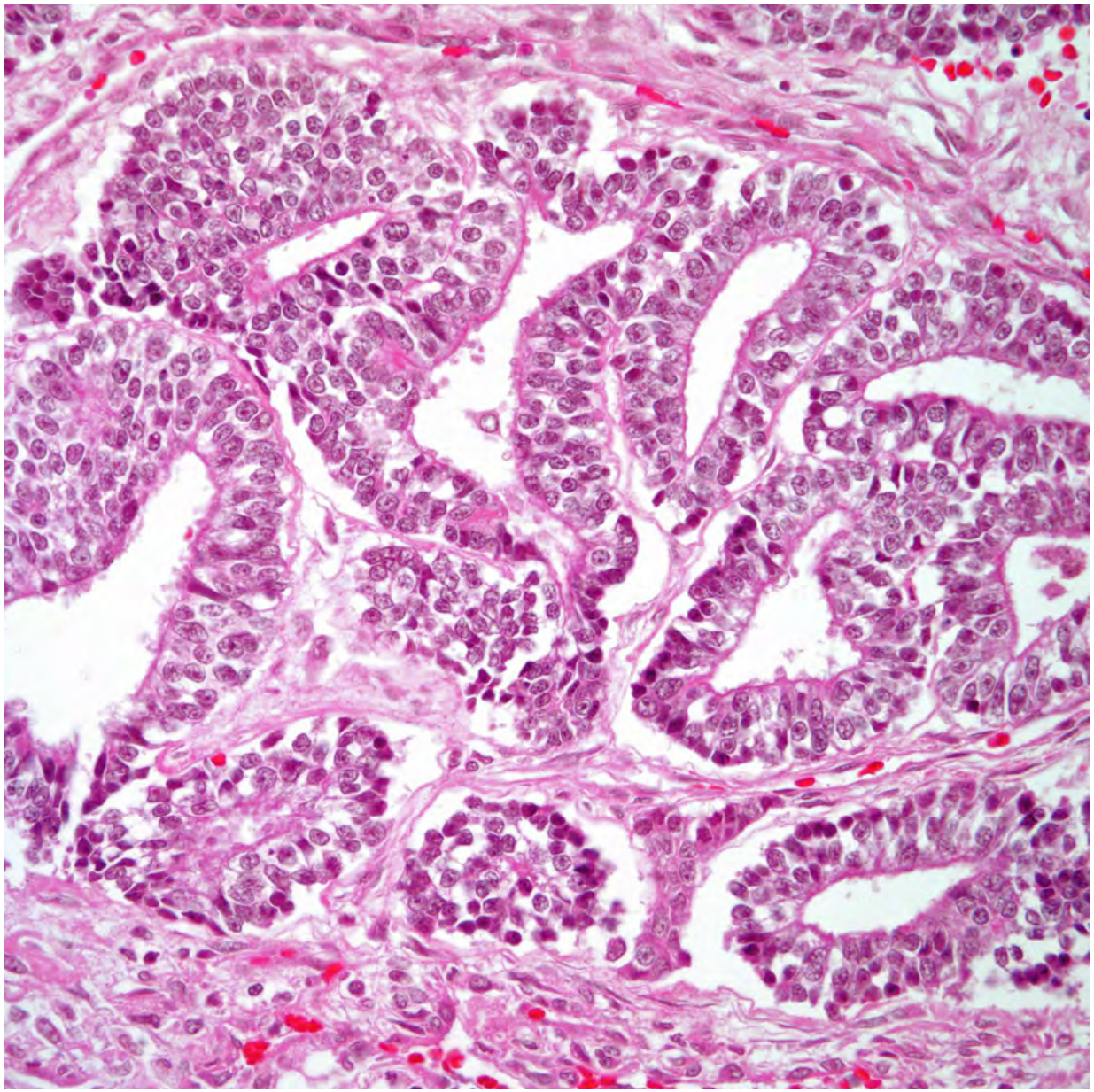


Small biopsies

Adenocarcinoma with fetal features

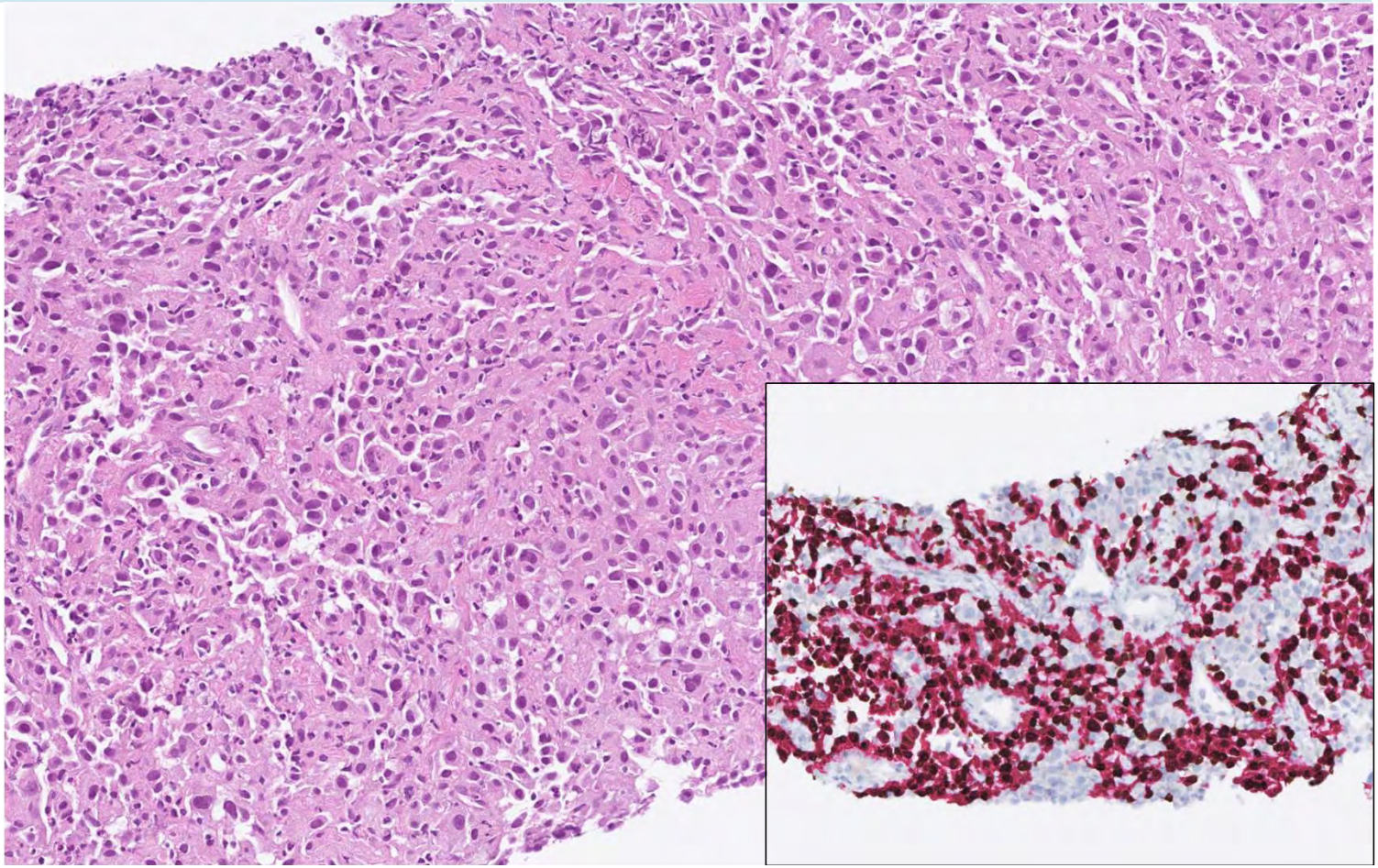
Specimens

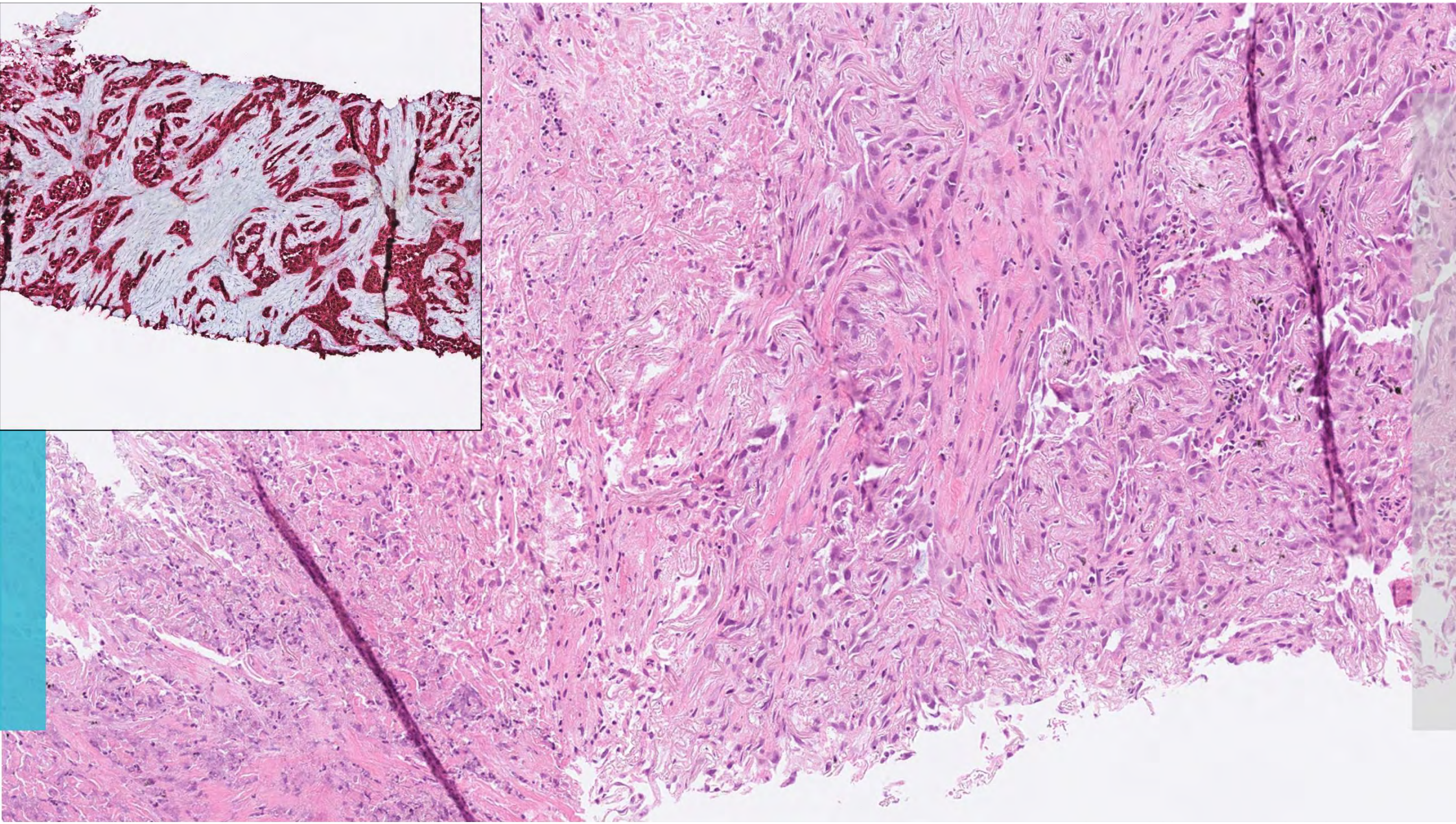
Fetal adenocarcinoma



Terminology in small biopsy and cytology versus resection specimens for Non-small cell cancer

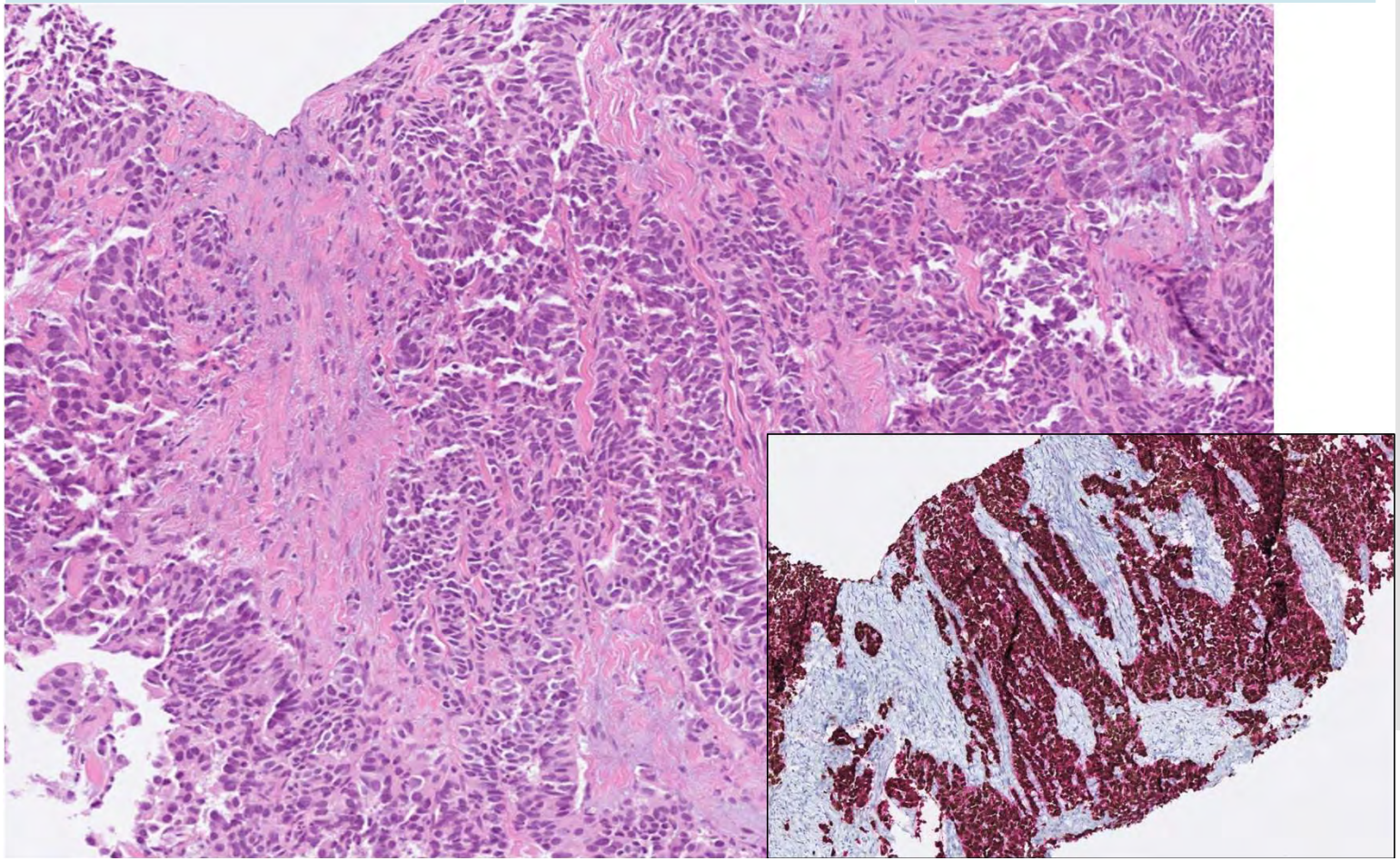
Morphology/stains	Terminology for small biopsies and cytology specimens	Terminology for resection specimens
Morphological squamous cell patterns not present, but supported by stains (i.e. p40+)	Non-small cell carcinoma, favour squamous cell carcinoma	Squamous cell carcinoma (non-keratinizing pattern may be a component of the tumour)





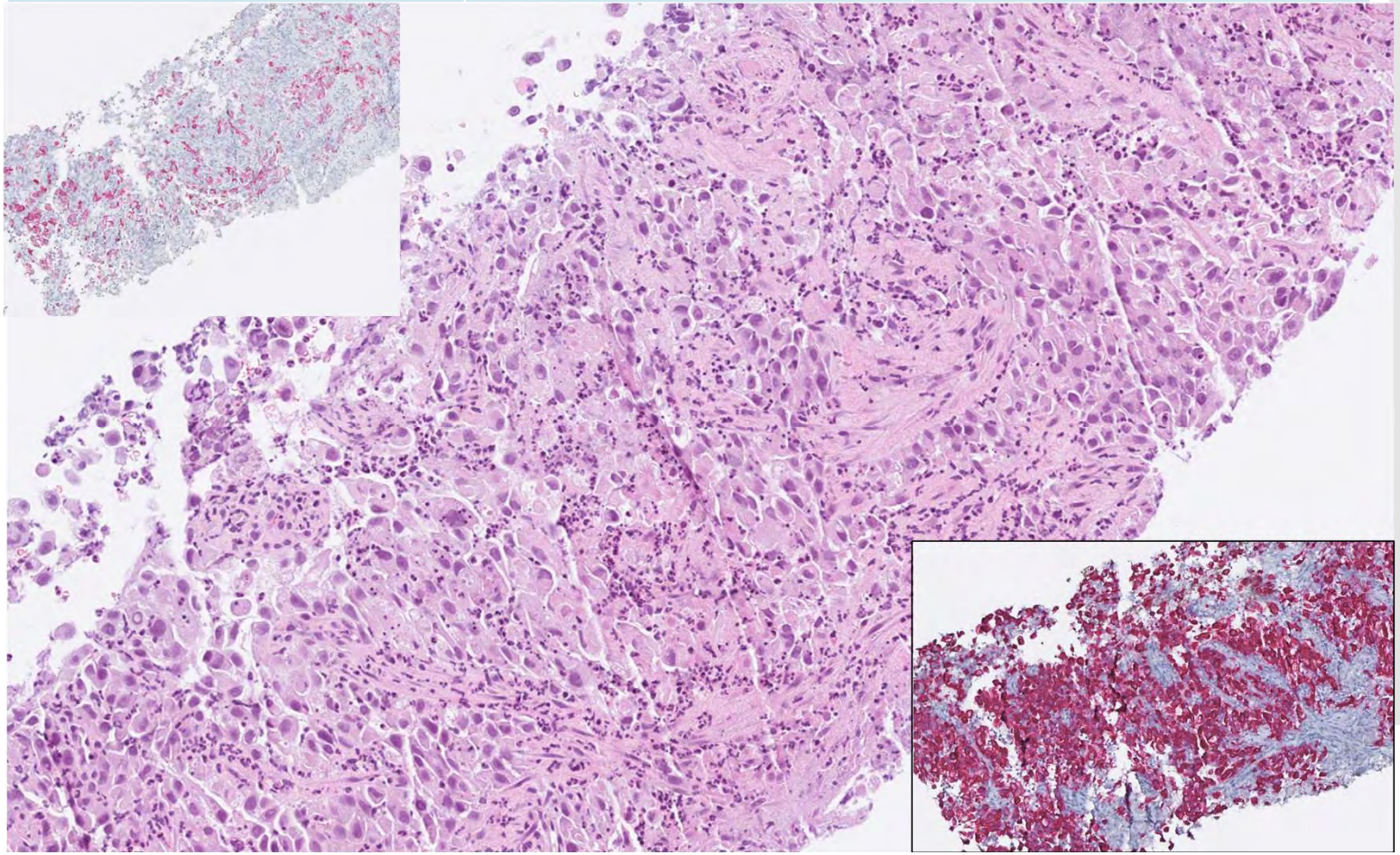
Terminology in small biopsy and cytology versus resection specimens for Non-small cell cancer

Morphology/stains	Terminology for small biopsies and cytology specimens	Terminology for resection specimens
Morphological adenocarcinoma patterns not present, but supported by special stains (i.e. TTF1+)	Non-small cell carcinoma, favour adenocarcinoma	Adenocarcinoma (solid pattern may be just one component of the tumour)



Terminology in small biopsy and cytology versus resection specimens for Non-small cell cancer

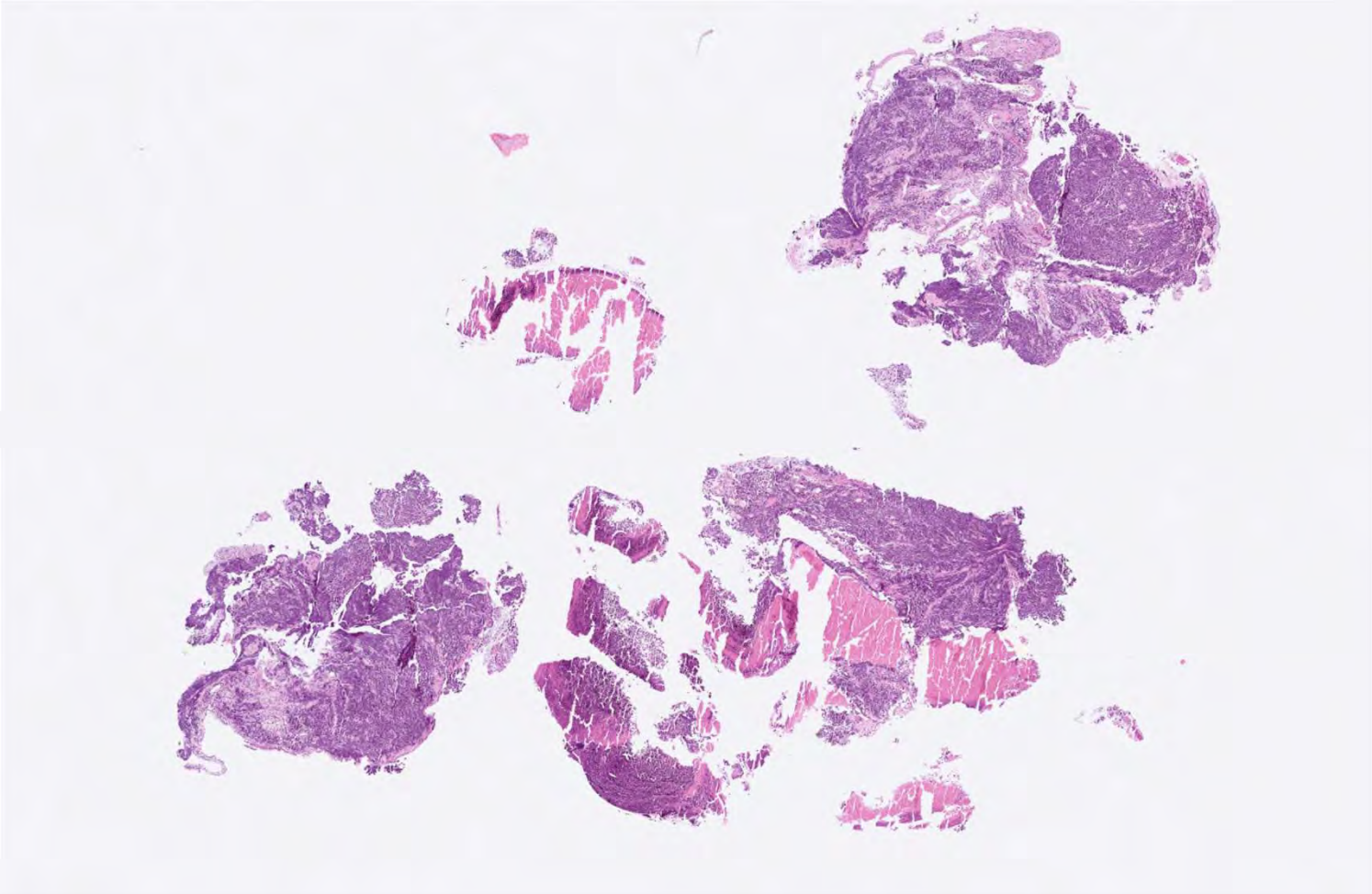
Morphology/stains	Terminology for small biopsies and cytology specimens	Terminology for resection specimens
No clear adenocarcinoma, squamous, or neuroendocrine morphology or staining pattern	Non-small cell carcinoma NOS	Large cell carcinoma

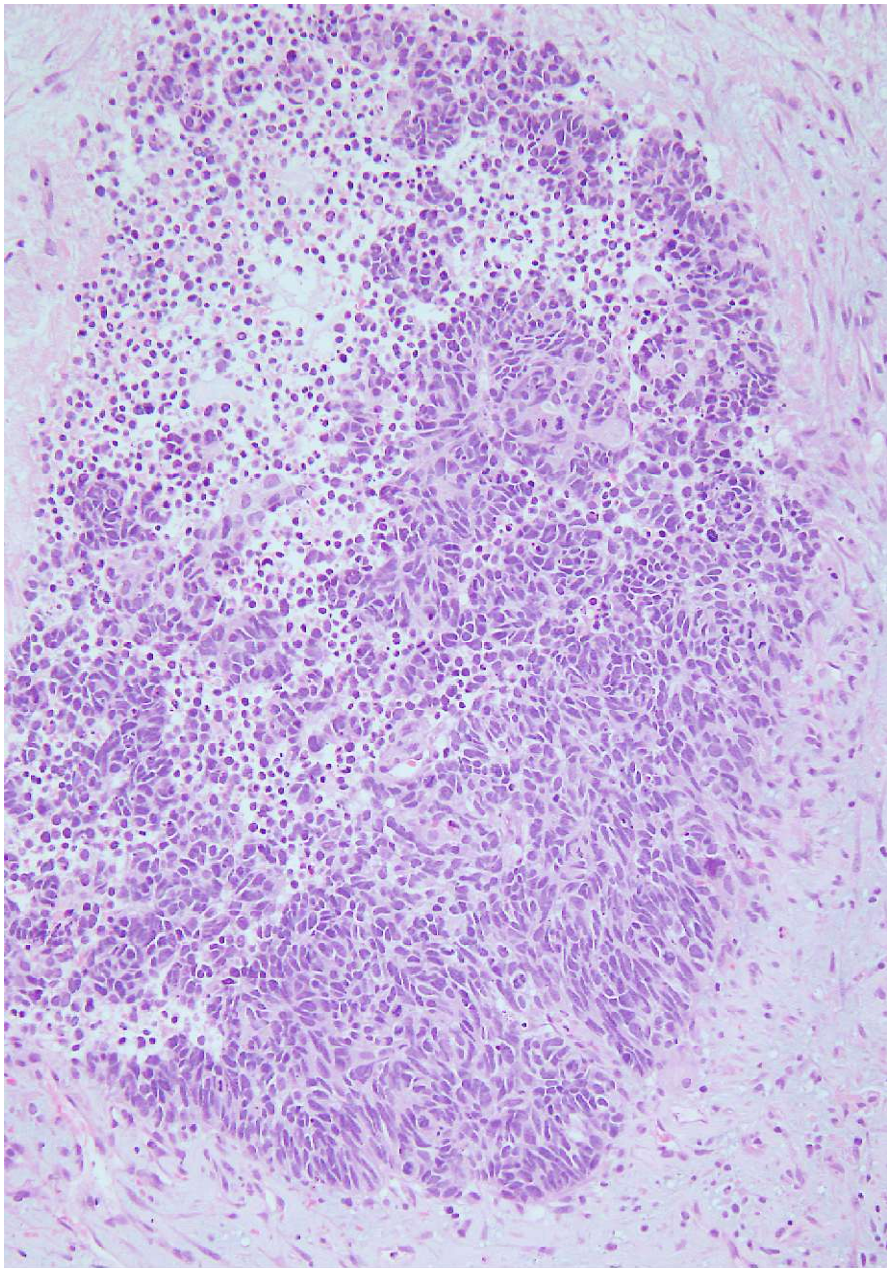


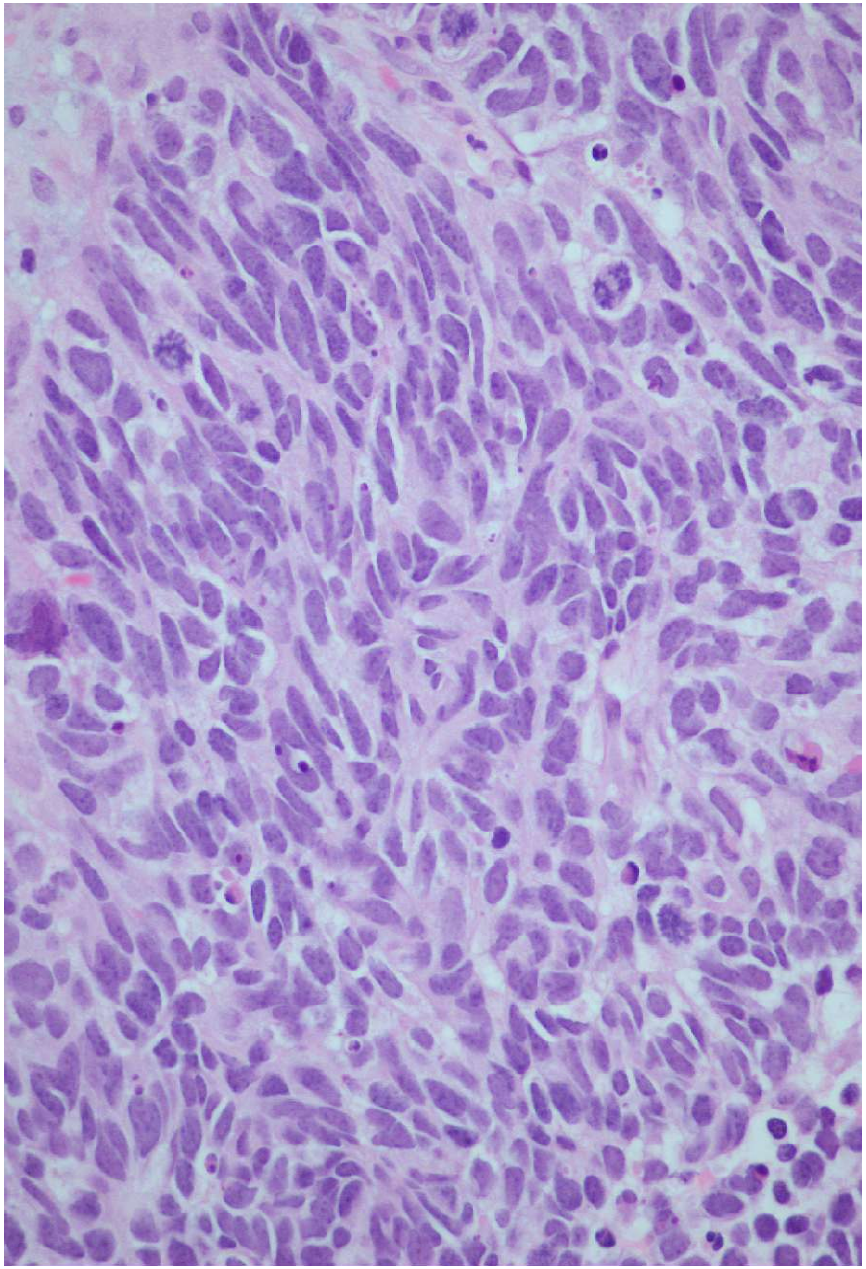
Terminology in small biopsy and cytology versus resection specimens for **Small cell carcinoma**

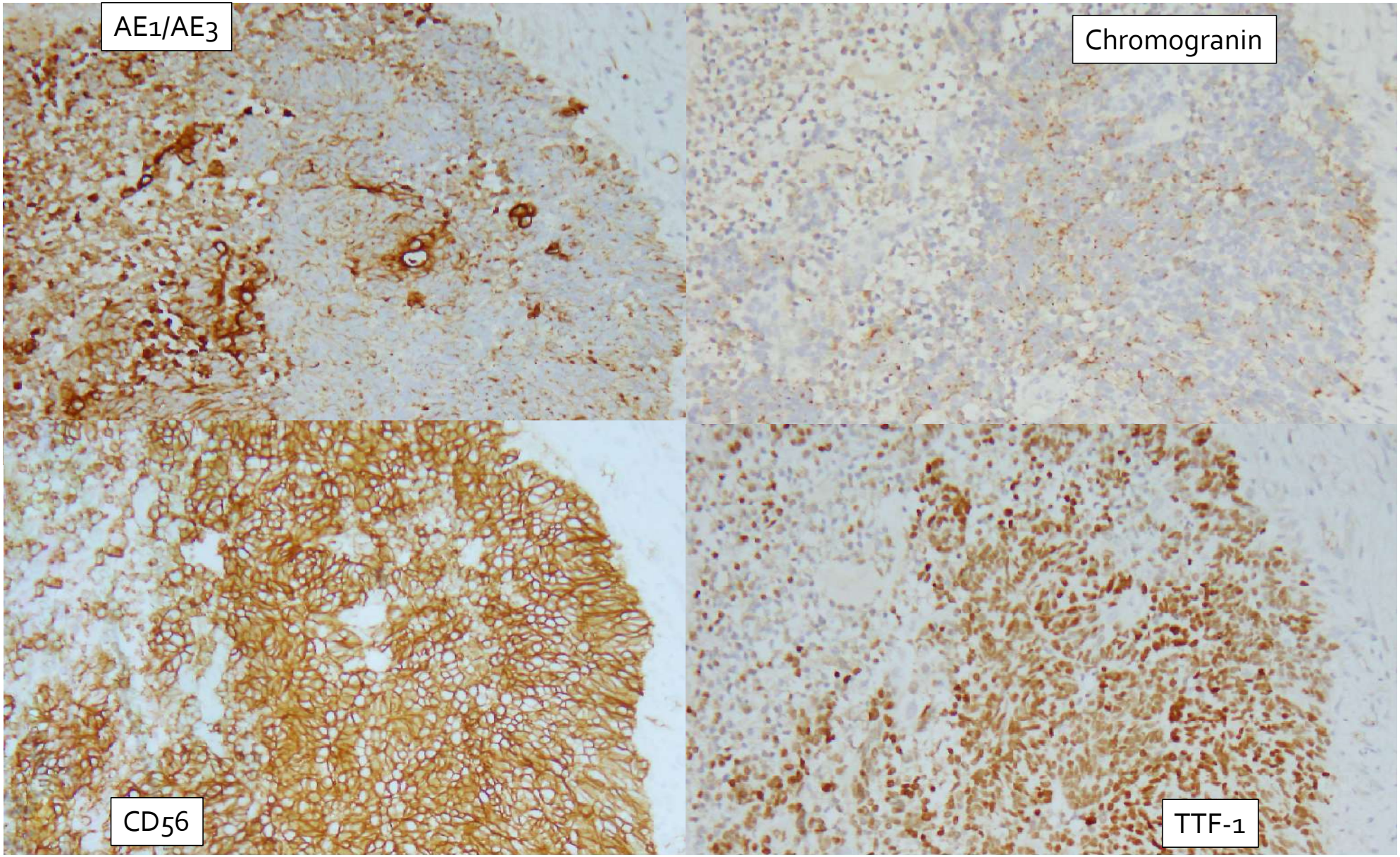
Scant cytoplasm
Poorly defined cell borders
Finely dispersed granular nuclear chromatin
Absent or inconspicuous nucleoli
Combined SCLC

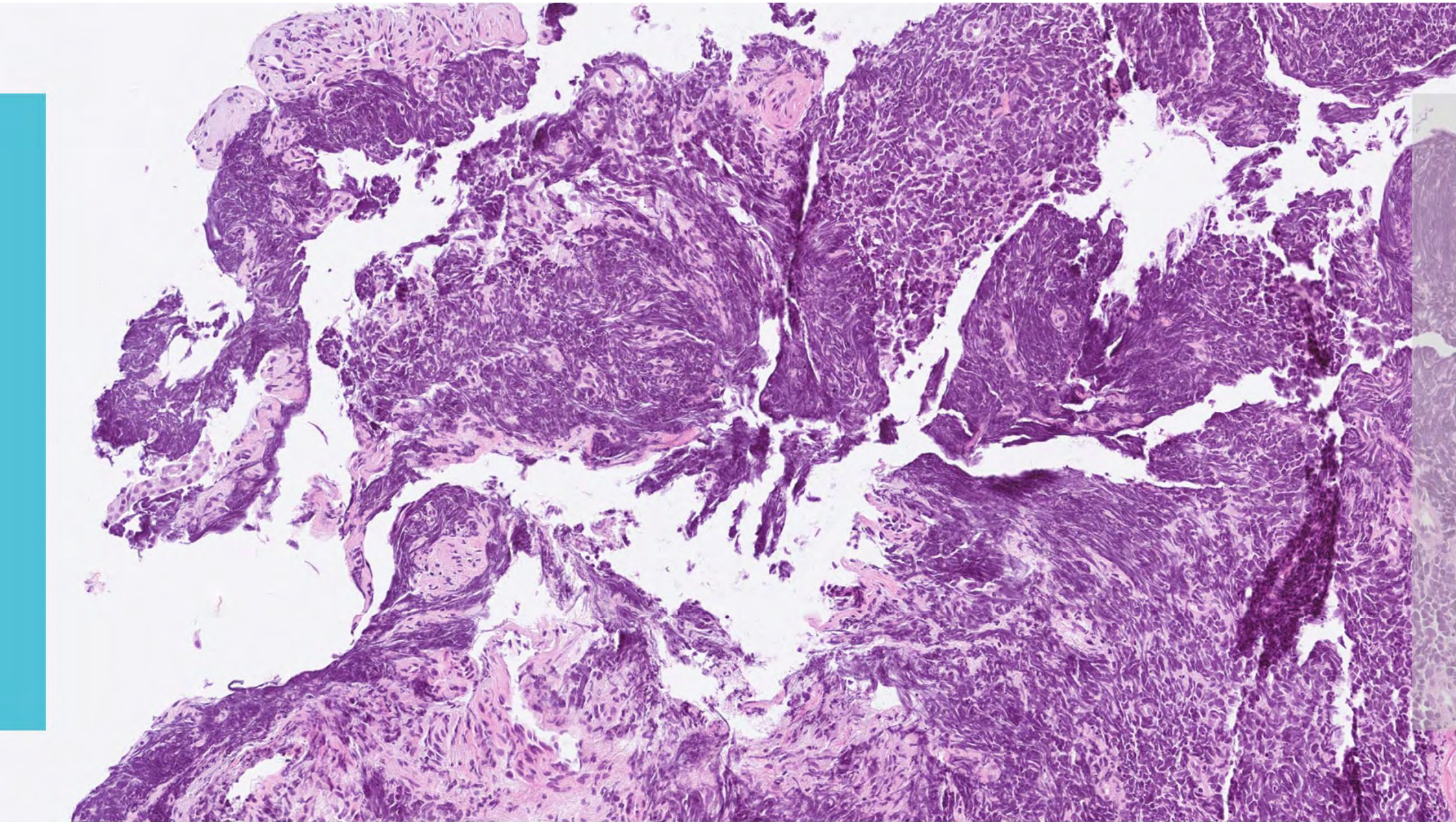
Terminology for small biopsies and cytology specimens	Terminology for resection specimens
Small cell carcinoma	Small cell carcinoma







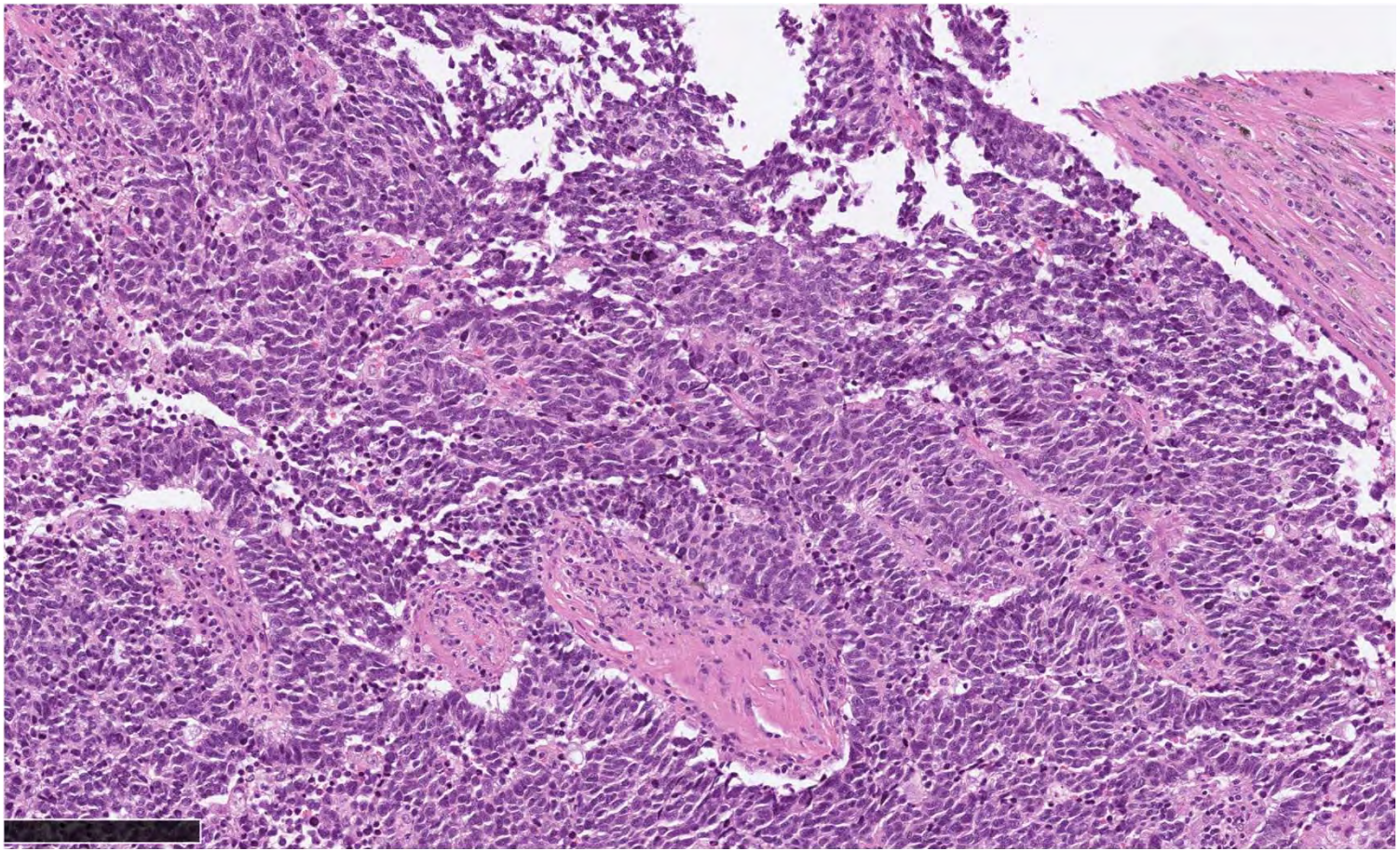

AE1/AE3
Chromogranin
CD56
TTF-1

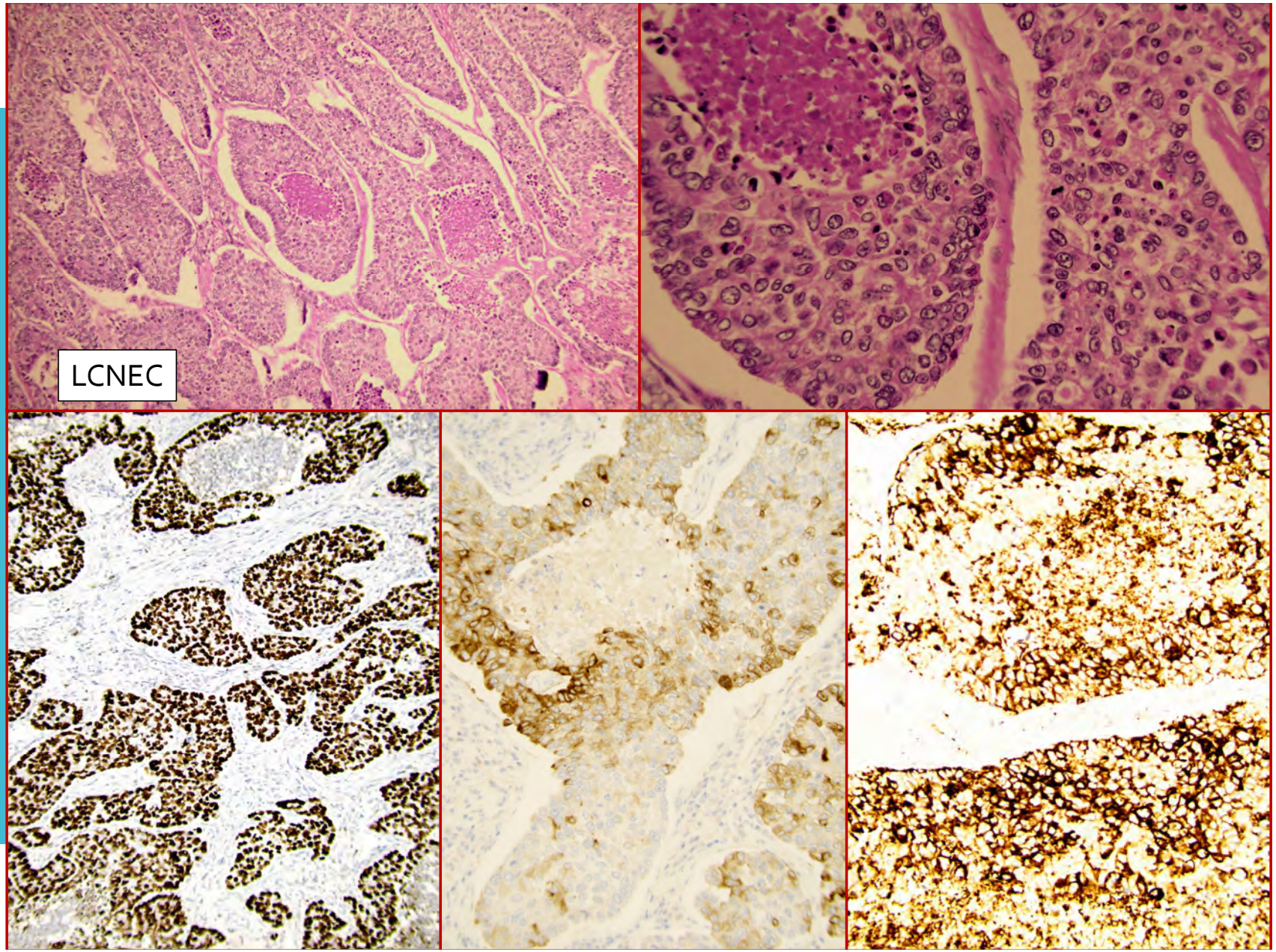


Terminology in small biopsy and cytology versus resection specimens for **Large cell neuroendocrine carcinoma**

NE morphology (rosettes, peripheral palisading)
IHC NE markers
Highly related to smoking
Location: Peripheral, upper lobes 80%, Central 20%

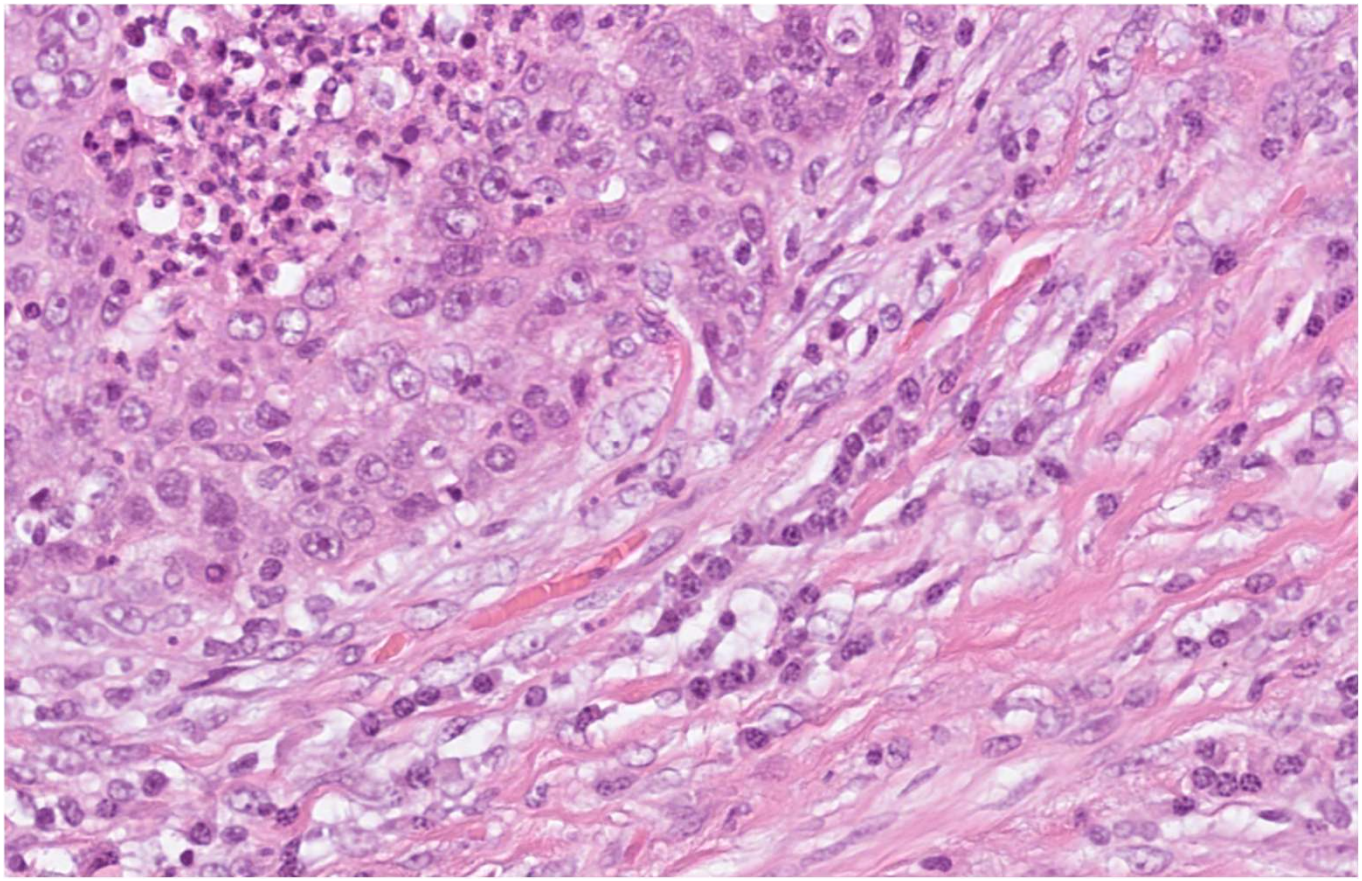
Terminology for small biopsies and cytology specimens	Terminology for resection specimens
Large cell neuroendocrine carcinoma	Non-small cell carcinoma with neuroendocrine morphology and positive neuroendocrine markers, possible large cell neuroendocrine carcinoma




LCNEC

Terminology in small biopsy and cytology versus resection specimens for **Adenosquamous carcinoma**

Terminology for small biopsies and cytology specimens	Terminology for resection specimens
Morphological squamous cell and adenocarcinoma patterns both present: non-small cell carcinoma NOS Comment that adenocarcinoma and squamous components are present, and that this could represent adenosquamous carcinoma	Adenosquamous carcinoma (if both components ≥ 10%)



Terminology in small biopsy and cytology versus resection specimens for Pleomorphic carcinoma

Terminology for small biopsies and cytology specimens	Terminology for resection specimens
Non-small cell carcinoma with spindle cell and/or giant cell carcinoma Mention if adenocarcinoma or squamous carcinoma is present. Comment that this could represent a pleomorphic carcinoma; however, that diagnosis requires a resection specimen.	Pleomorphic, spindle cell, and/or giant cell carcinoma

Poorly diff NSCC that contains at least
- 10% spindle / giant cells or carcinoma that contains only spindle or giant cells

Tobacco smokers

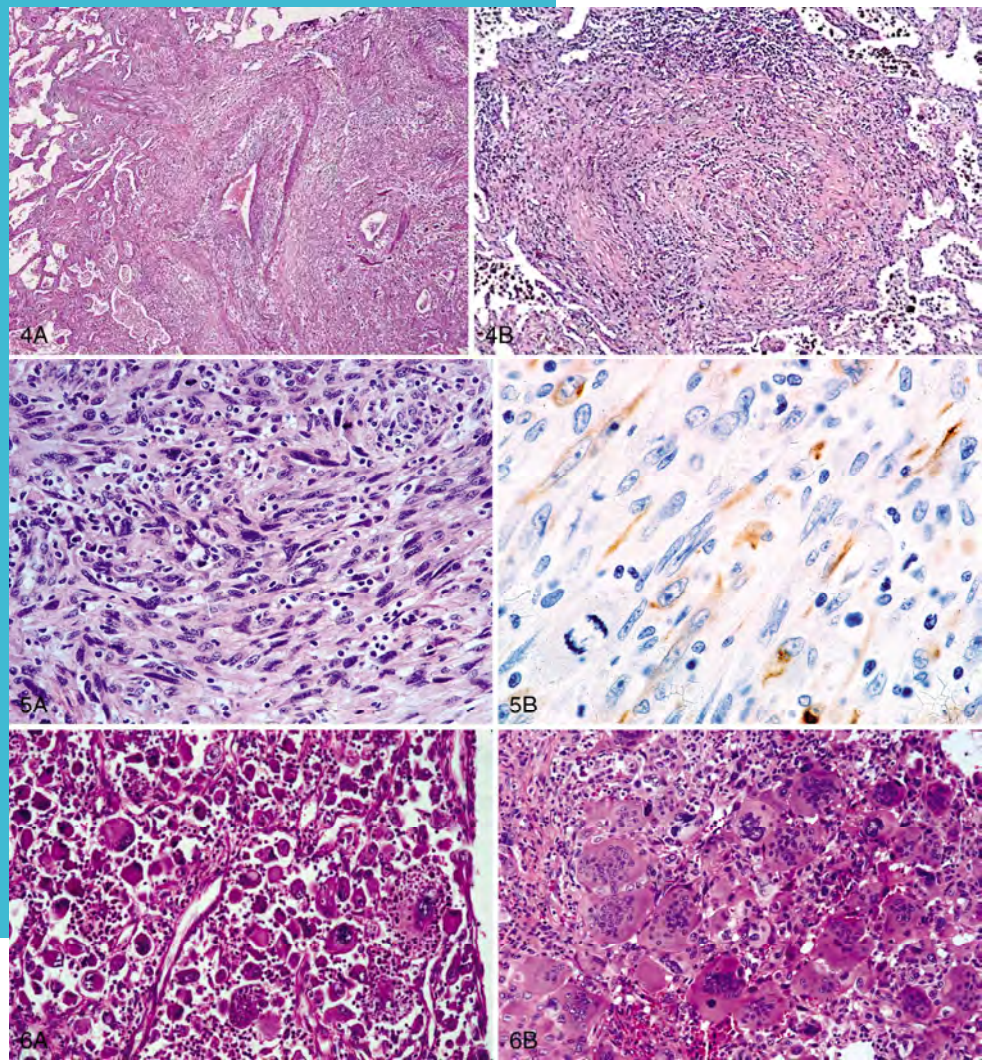
Account for a small percentage of primary lung neoplasms

Large peripheral mass (usually upper lobe)

Aggressive tumours. The small bowel is one of the sites that pleomorphic carcinomas tend to metastasize

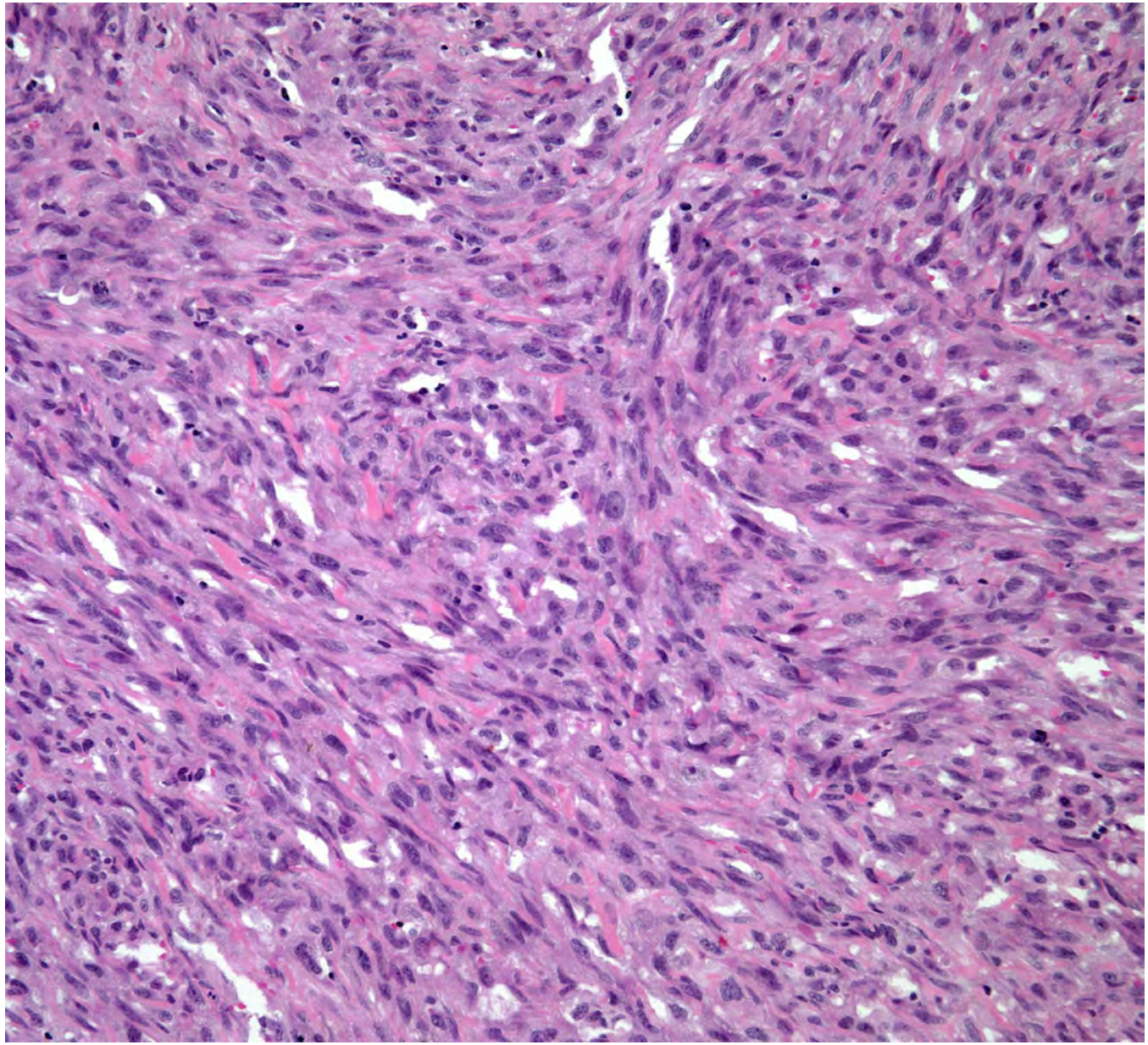
Macroscopy:
- Well circumscribed, grey/tan masses
- Central or peripheral tumours
- > 5 cm
- Necrosis and cavitation

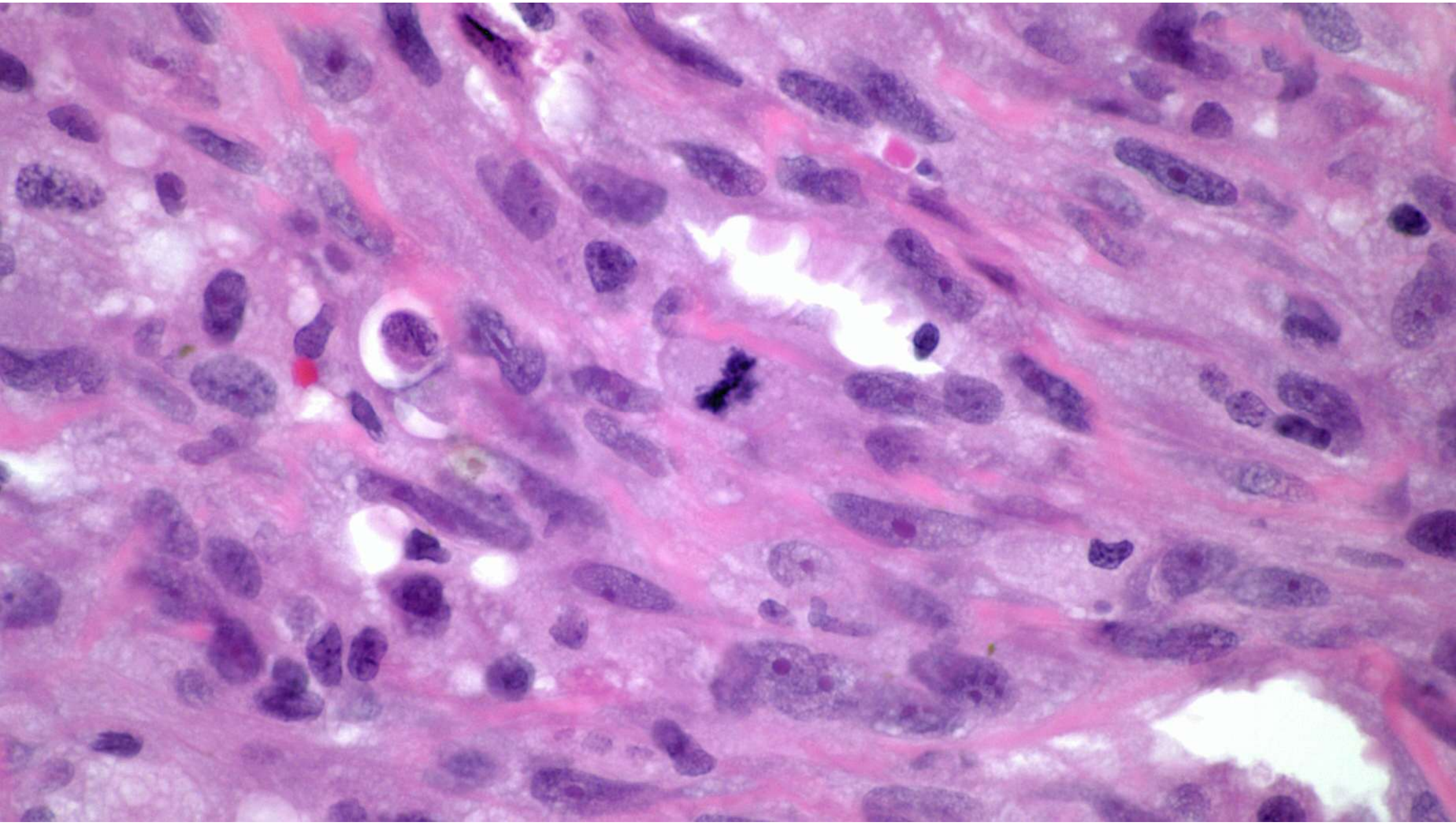
Microscopic features



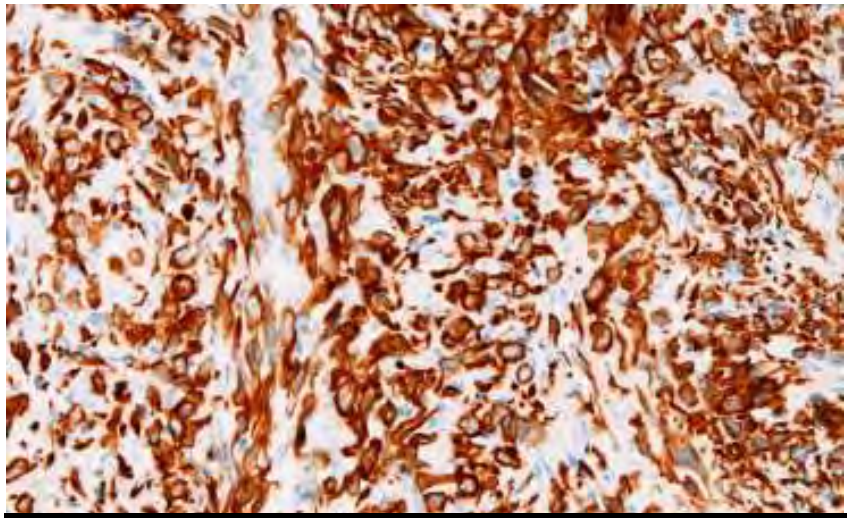
- Pleomorphic carcinoma shows malignant spindle cells or giant cells > 10%.
- Squamous cell carcinoma (SCC)
- Adenocarcinoma (ADC)
- Large cell carcinoma (LCC)
- In small biopsy sarcomatoid elements may be described, but definitive diagnosis is NOT POSSIBLE
  - Predominant sarcomatoid pattern
  - Predominant epithelial cell type

Spindle cell carcinoma consists almost entirely of malignant spindle cells

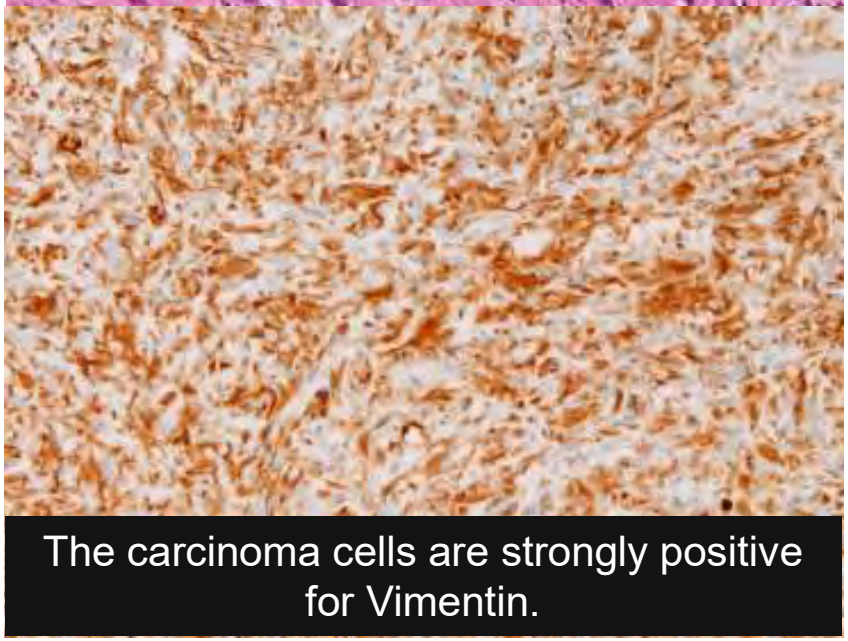




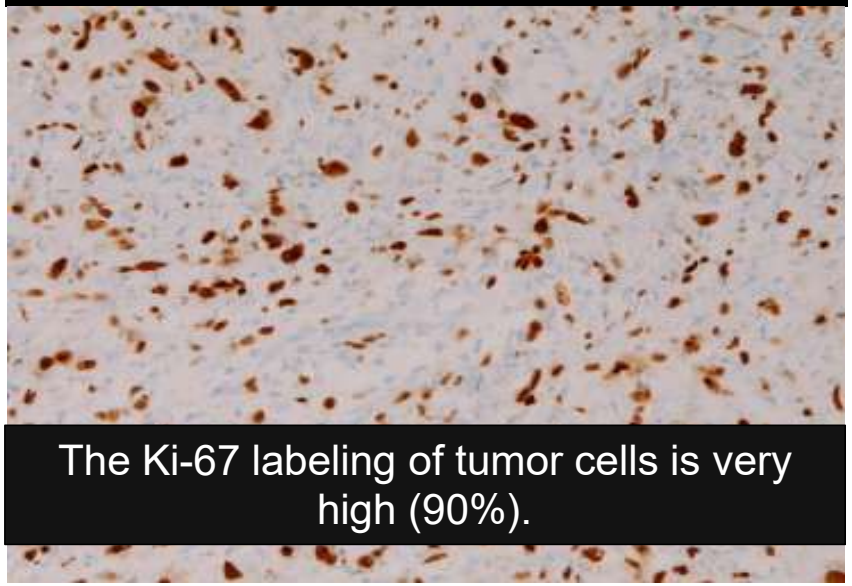
Spindle cell carcinoma



The carcinoma cells are strongly positive for CKAE1/3.



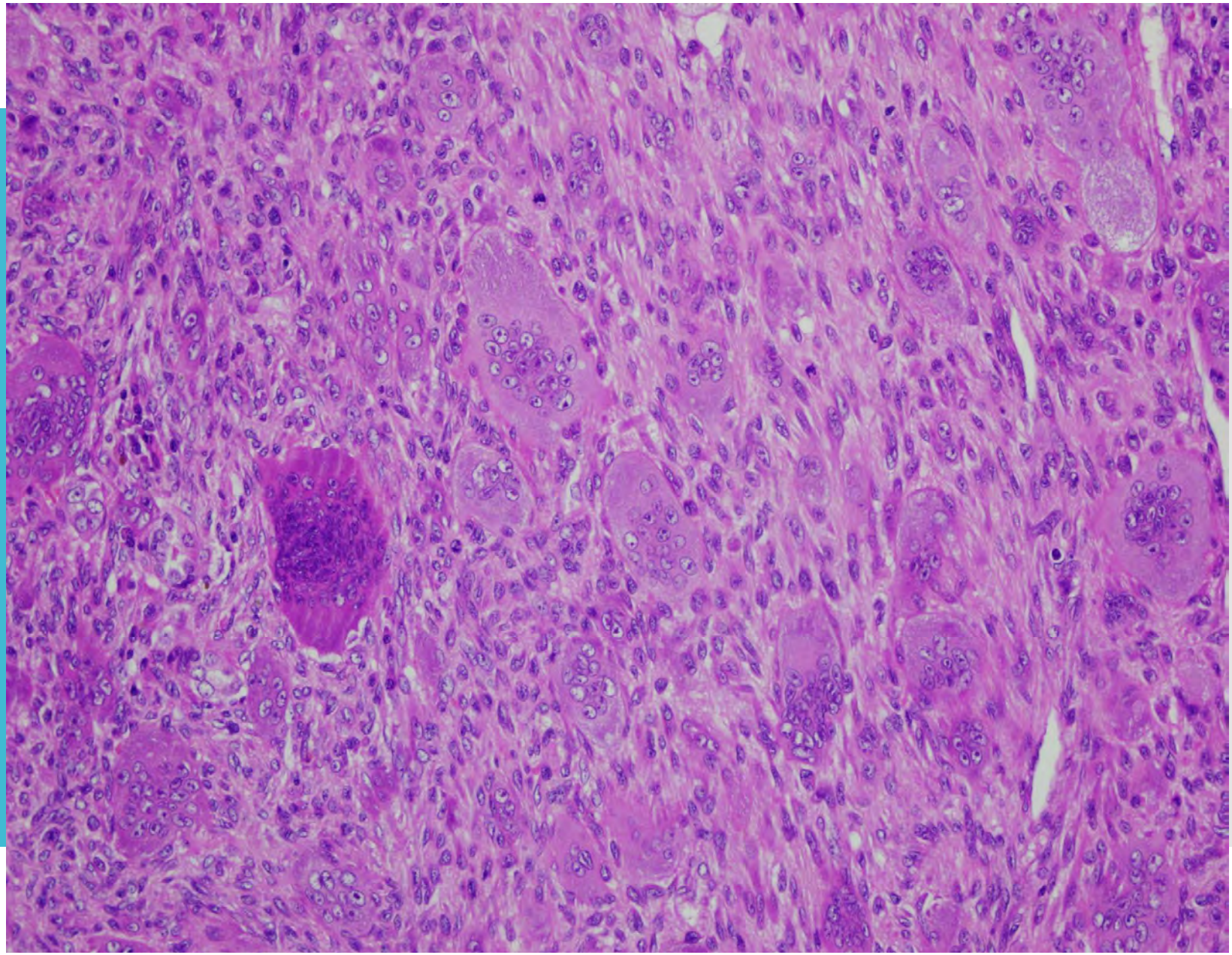
The carcinoma cells are strongly positive for Vimentin.

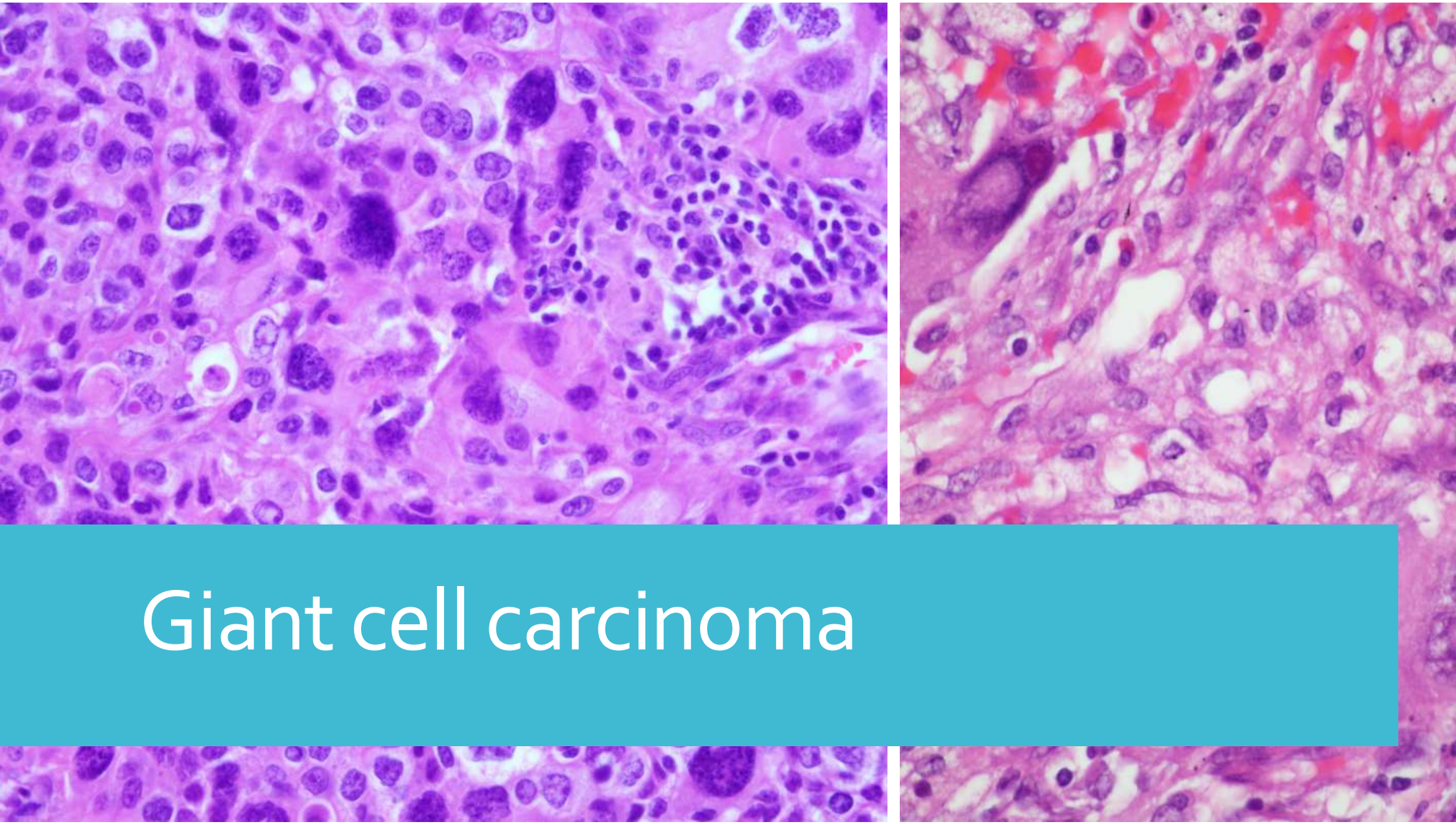


The Ki-67 labeling of tumor cells is very high (90%).

Terada et al. Respiratory Medicine CME, Volume 3, Issue 4, 2010, 241–245

Giant carcinoma cells show eosinophilic-granular cytoplasm, large multinucleated nuclei and vesicular chromatin with prominent nucleoli.





Giant cell carcinoma

Are biopsies the same as surgical samples ?

Immunhistochemistry by Means of Widely Agreed-Upon Markers (Cytokeratins 5/6 and 7, p63, Thyroid Transcription Factor-1, and Vimentin) on Small Biopsies of Non-small Cell Lung Cancer Effectively Parallels the Corresponding Profiling and Eventual Diagnoses on Surgical Specimens

Giuseppe Pelosi, MD, MIAC,† Giulio Rossi, MD,‡ Fabrizio Bianchi, DSc, PhD,§ Patrick Maisonneuve, Eng,‖ Domenico Galetta, MD,¶ Angelica Sonzogni, MD,* Giulia Veronesi, MD,¶ Lorenzo Spaggiari, MD,†¶ Mauro Papotti, MD,# Mattia Barbareschi, MD,** Paolo Graziano, MD,†† Andrea Decensi, MD,‡‡ Alberto Cavazza, MD,§§ and Giuseppe Viale, MD, FRCPath*†*

Journal of Thoracic Oncology • Volume 6, Number 5, May 2011

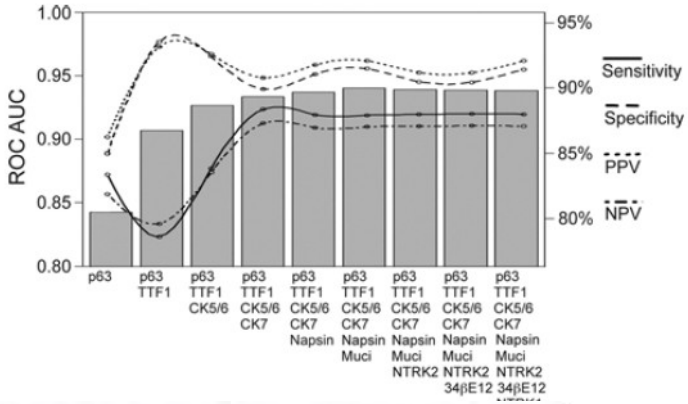
TABLE 3. Comparison of Revised Biopsies with Surgical Specimens by Morphology and Immunohistochemistry

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Diagnostic Accuracy (95% CI)
Revised biopsies vs. surgical specimens					
Squamous cell carcinoma	30/30	29/33	30/34	29/29	59/63
	100 (80–100)	88 (72–97)	88 (73–97)	100 (88–100)	94 (85–98)
Adenocarcinoma	22/22	35/41	22/28	35/35	57/63
	100 (85–100)	85 (71–94)	79 (59–92)	100 (90–100)	90 (80–96)
Sarcomatoid carcinoma	1/8	55/55	1/1	55/62	56/63
	13 (3–53)	100 (94–100)	100 (2–100)	89 (78–95)	94 (85–98)
Immunostained biopsies vs. surgical specimens					
Squamous cell carcinoma	30/30	30/31	30/31	30/30	62/63
	100 (88–100)	97 (83–100)	97 (83–100)	100 (88–100)	98 (91–100)
Adenocarcinoma	22/22	40/41	22/25	40/40	62/63
	100 (85–100)	98 (87–100)	88 (69–97)	100 (92–100)	98 (91–100)
Sarcomatoid carcinoma	5/8	55/55	5/5[a]	55/58	60/63
	63 (24–91)	100 (94–100)	100 (48–100)	95 (86–99)	95 (87–99)
Adenosquamous carcinoma	2/2	61/61	2/2	61/61	63/63
	100 (16–100)	100 (94–100)	100 (16–100)	100 (94–100)	100 (94–100)

Optimal Immunohistochemical Markers For Distinguishing Lung Adenocarcinomas From Squamous Cell Carcinomas in Small Tumor Samples

Jefferson Terry, MD, PhD, Samuel Leung, MSc,* Janessa Laskin, MD,†*
*Kevin O. Leslie, MD,‡ Allen M. Gown, MD,§ and Diana N. Ionescu, MD**

(*Am J Surg Pathol* 2010;34:1805–1811)



200 AD & 225 SQC on TMA
9 markers (TTF1, CK7, napsin, mucin
SCC: p63, CK5/6, 34βE12, NTRK1 & NTRK2)

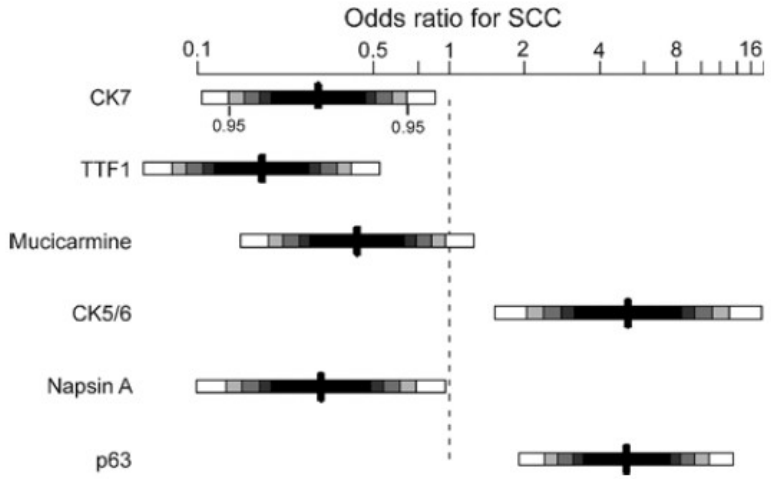
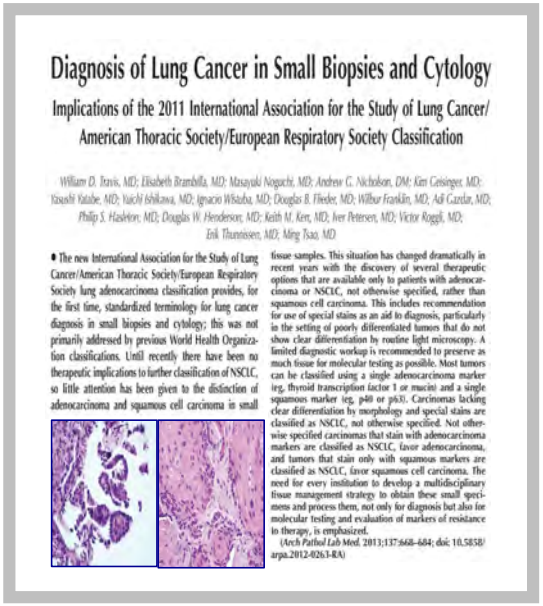


TABLE 4. Sensitivity, Specificity, PPV and NPV of Markers Used in This Study

Marker	Subtype	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CK5/6	SCC	66 (60-72)	95 (92-98)	94 (90-97)	72 (66-77)
CK7	ADC	93 (90-97)	63 (57-70)	70 (64-75)	91 (86-95)
HMWCK	SCC	69 (63-75)	83 (78-88)	82 (76-87)	71 (65-76)
Muci	ADC	30 (24-36)	91 (87-94)	74 (65-84)	59 (54-64)
Napsin A	ADC	59 (52-65)	94 (91-97)	90 (84-95)	72 (67-77)
NTRK1	SCC	70 (64-76)	91 (86-94)	89 (84-94)	73 (68-78)
NTRK2	SCC	53 (46-59)	97 (94-99)	94 (90-98)	65 (59-70)
p63	SCC	84 (79-88)	85 (80-90)	86 (81-91)	82 (77-87)
TTF1	ADC	62 (56-69)	92 (89-96)	88 (83-93)	73 (68-78)

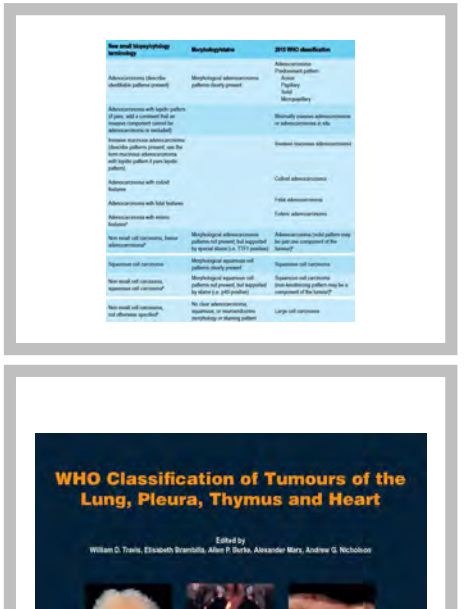
….2015

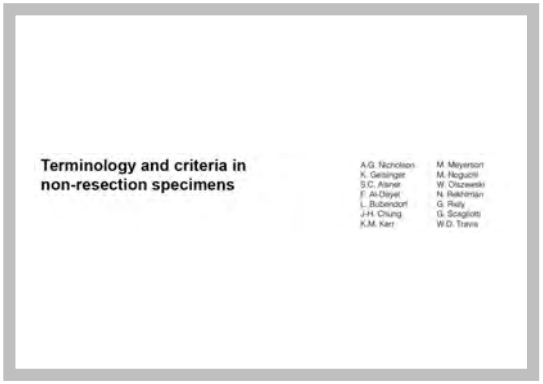
…recommended one marker for squamous (P40), one marker for adenocarcinoma (TTF1)



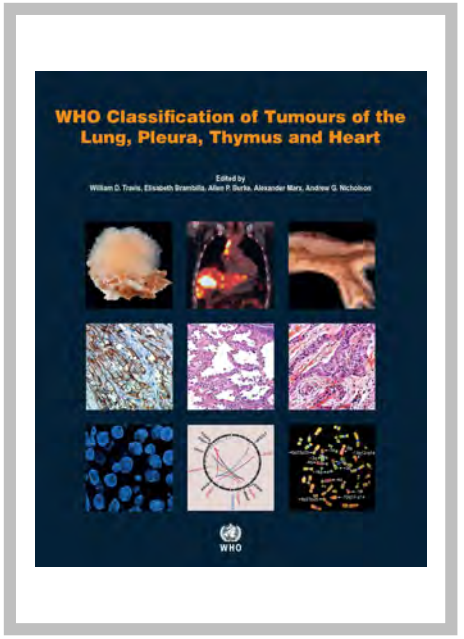
Diagnosis of Lung Cancer in Small Biopsies and Cytology

Implications of the 2011 International Association for the Study of Lung Cancer/ American Thoracic Society/European Respiratory Society Classification





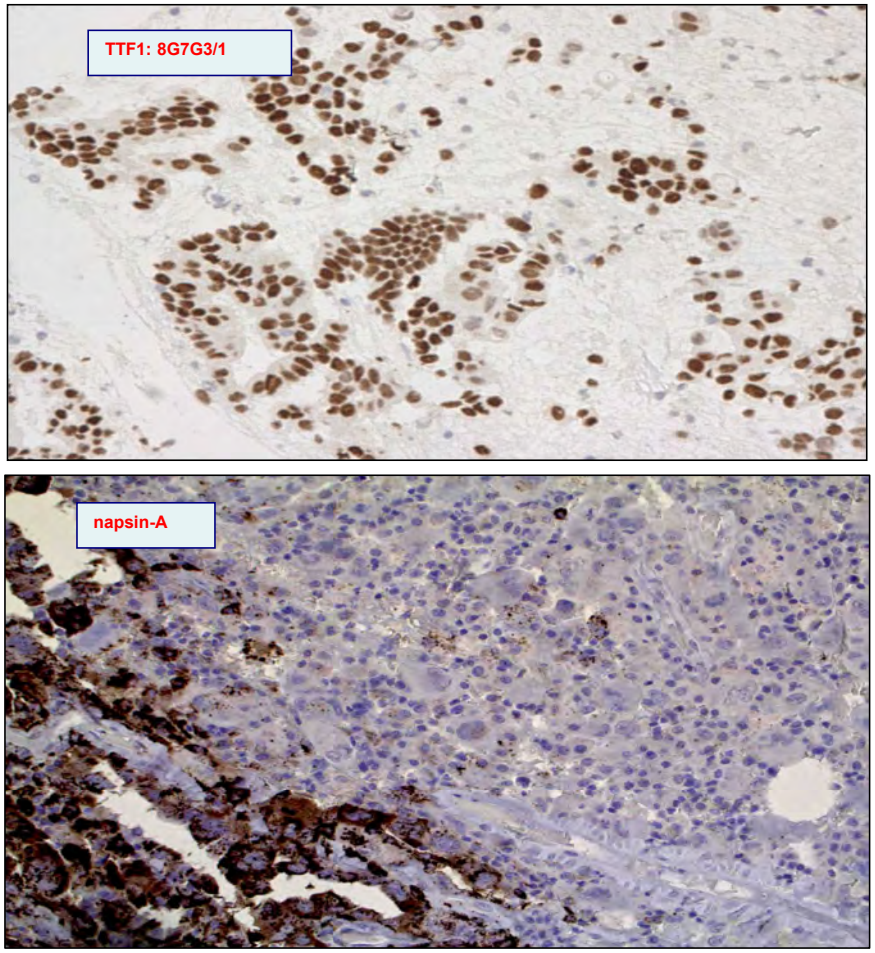
Terminology and criteria in non-resection specimens



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

TTF1 and Napsin: first of all the right clone

- TTF-1 works well for lung adenocarcinoma and NE tumors with clone **8G7G3/1** (dilution > 1:500-1000)
- SPT24, A22-A clones are less specific
- MoAb to napsin-A is more specific than polyclonal antiserum

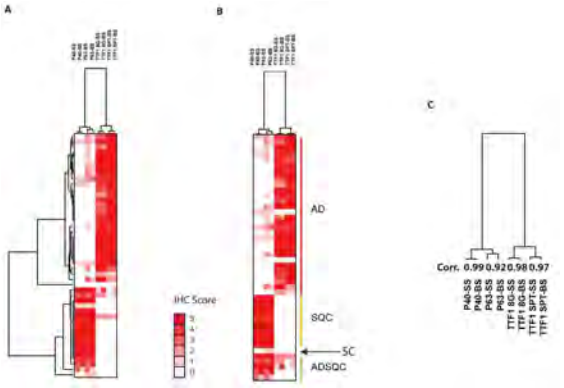


ΔNp63 (p40) and Thyroid Transcription Factor-1 Immunoreactivity on Small Biopsies or Cellblocks for Typing Non-small Cell Lung Cancer

A Novel Two-Hit, Sparing-Material Approach

Giuseppe Pelosi, MD, MIAC,† Alessandra Fabbri, MD,* Fabrizio Bianchi, DSc, PhD,‡ Patrick Maisonneuve, Eng,§ Giulio Rossi, MD,‖ Mattia Barbareschi, MD,¶ Paolo Graziano, MD,# Alberto Cavazza, MD,** Natasha Rekhtman, MD, PhD,†† Ugo Pastorino, MD,‡‡ Paolo Scanagatta, MD,‡‡ and Mauro Papotti, MD§§*

(*J Thorac Oncol.* 2012;7: 281–290)

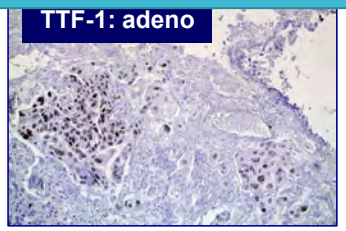


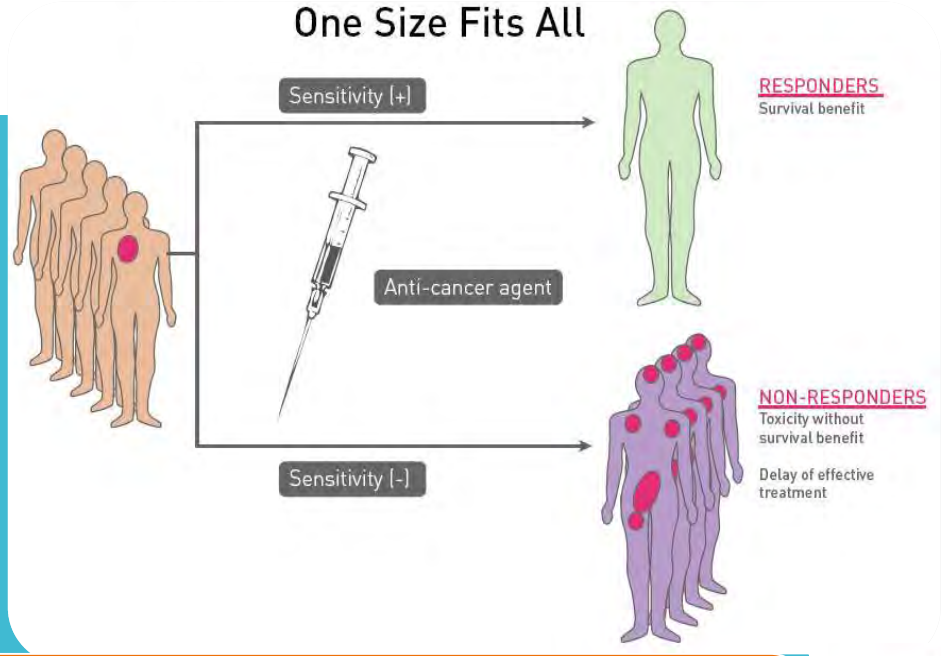
P63 may be positive in 20-30% of adenocarcinomas

Immunoreactivity for Thyroid Transcription Factor-1 in Stage I Non–Small Cell Carcinomas of the Lung

The American Journal of Surgical Pathology *25(3): 363–372, 2001*

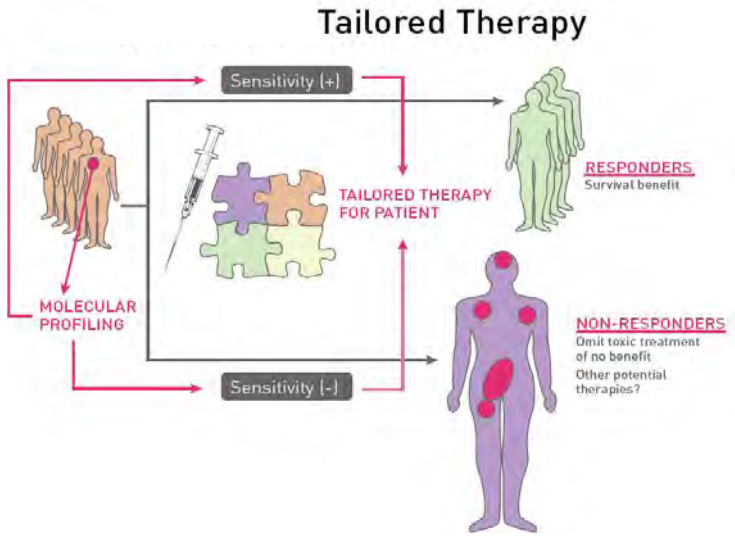
Giuseppe Pelosi, M.D., M.I.A.C., Filippo Fraggetta, M.D.,
Felice Pasini, M.D., Patrick Maisonneuve, ING., Angelica Sonzogni, M.D.,
Antonio Iannucci, M.D., Alberto Terzi, M.D.,
Enrica Bresaola, C.T.(I.A.C.), C.M.I.A.C., Francesco Valduga, M.D.,
Carmelo Lupo, LAB. TECH., and Giuseppe Viale, M.D., F.R.C.Path.

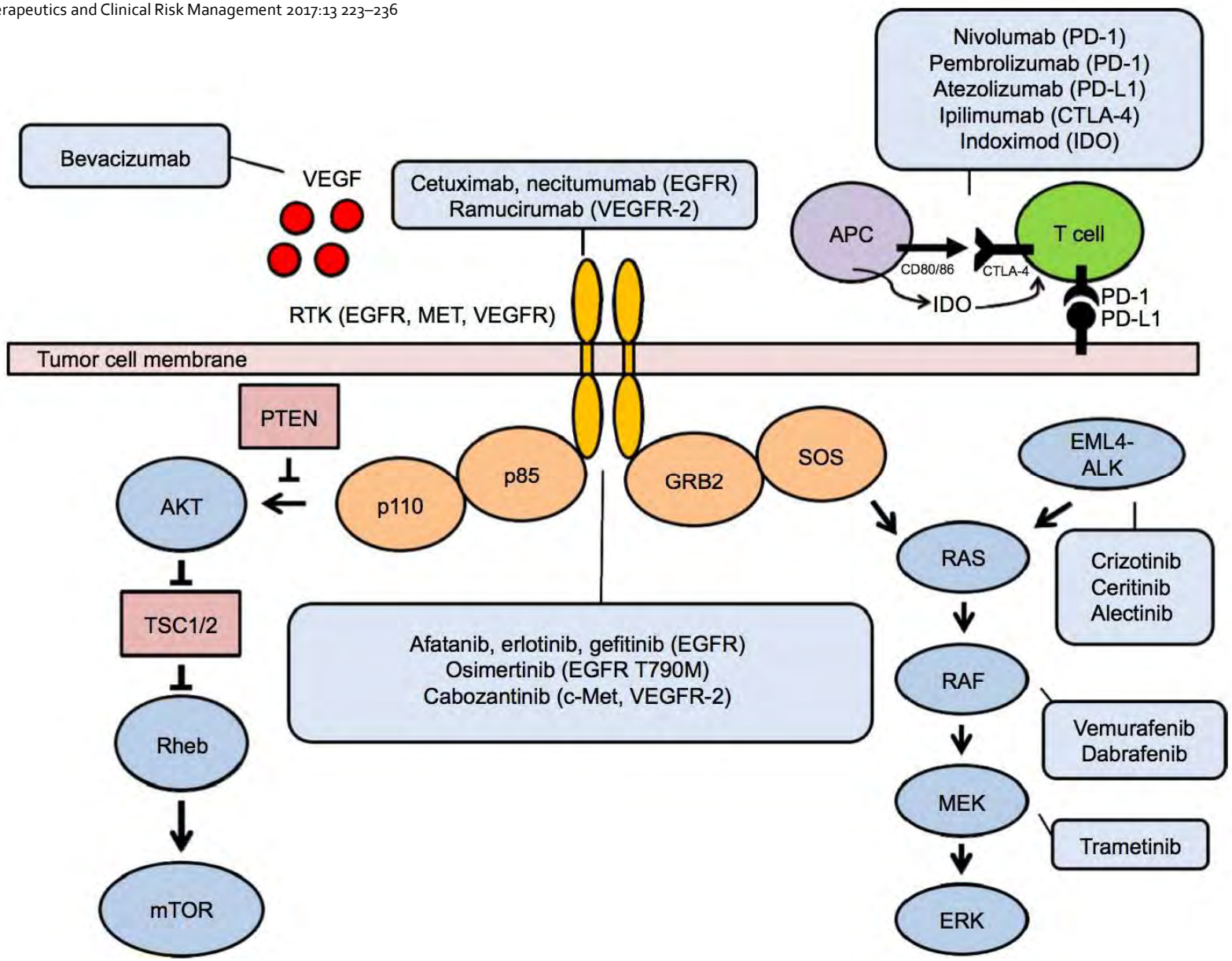




A **predictive marker** is a particular protein or gene that indicates sensitivity or resistance to a specific therapy

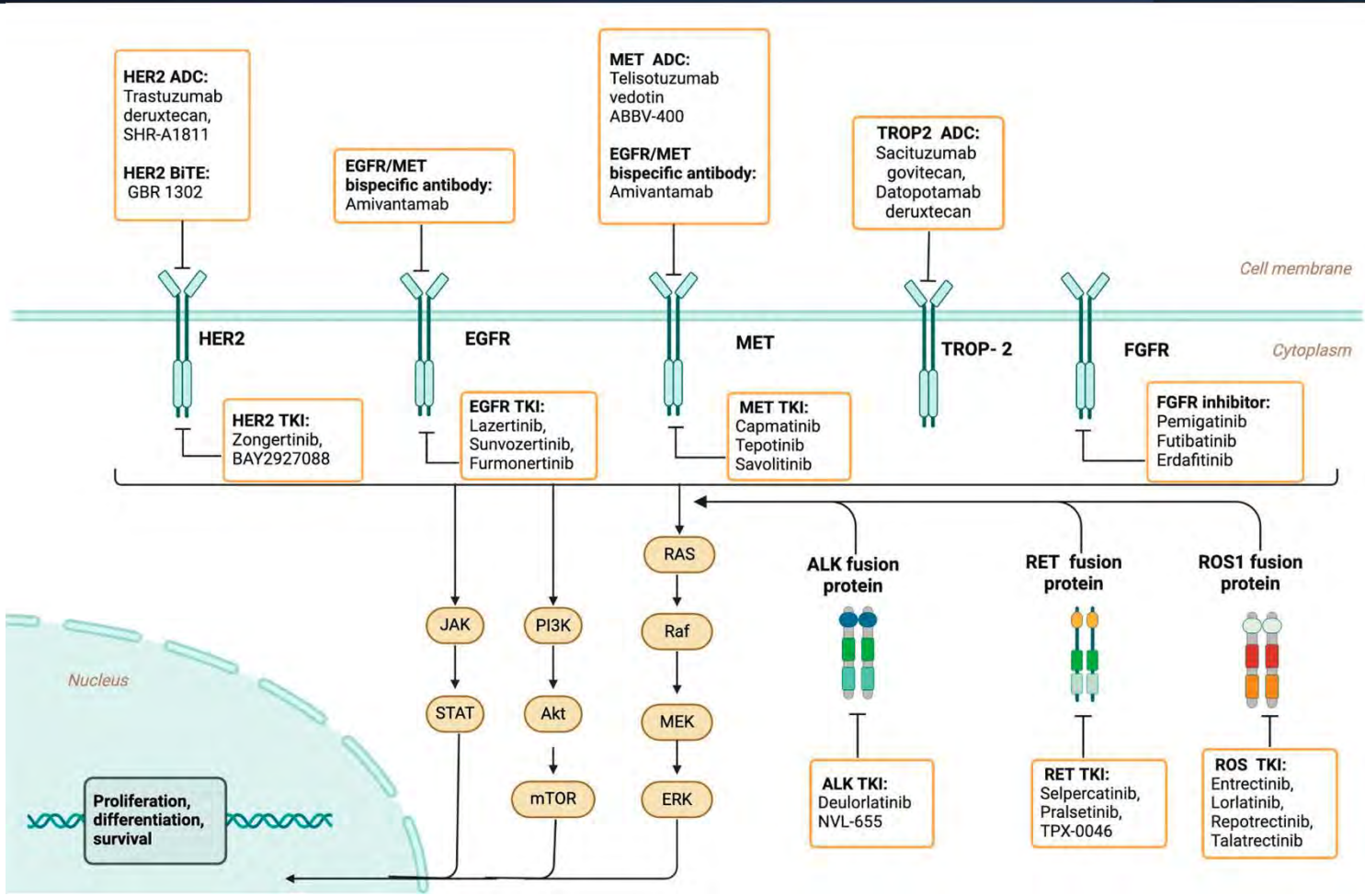
Prognostic markers are biomarkers used to measure the progress of a disease in the patient sample




Nivolumab (PD-1)
Pembrolizumab (PD-1)
Atezolizumab (PD-L1)
Ipilimumab (CTLA-4)
Indoximod (IDO)
Bevacizumab
VEGF
Cetuximab, necitumumab (EGFR)
Ramucirumab (VEGFR-2)
APC
T cell
CD80/86
CTLA-4
IDO
PD-1
PD-L1
RTK (EGFR, MET, VEGFR)
Tumor cell membrane
PTEN
AKT
TSC1/2
Rheb
mTOR
p110
p85
GRB2
SOS
EML4-
ALK
RAS
Crizotinib
Ceritinib
Alectinib
RAF
Vemurafenib
Dabrafenib
MEK
Trametinib
ERK
Afatanib, erlotinib, gefitinib (EGFR)
Osimertinib (EGFR T790M)
Cabozantinib (c-Met, VEGFR-2)

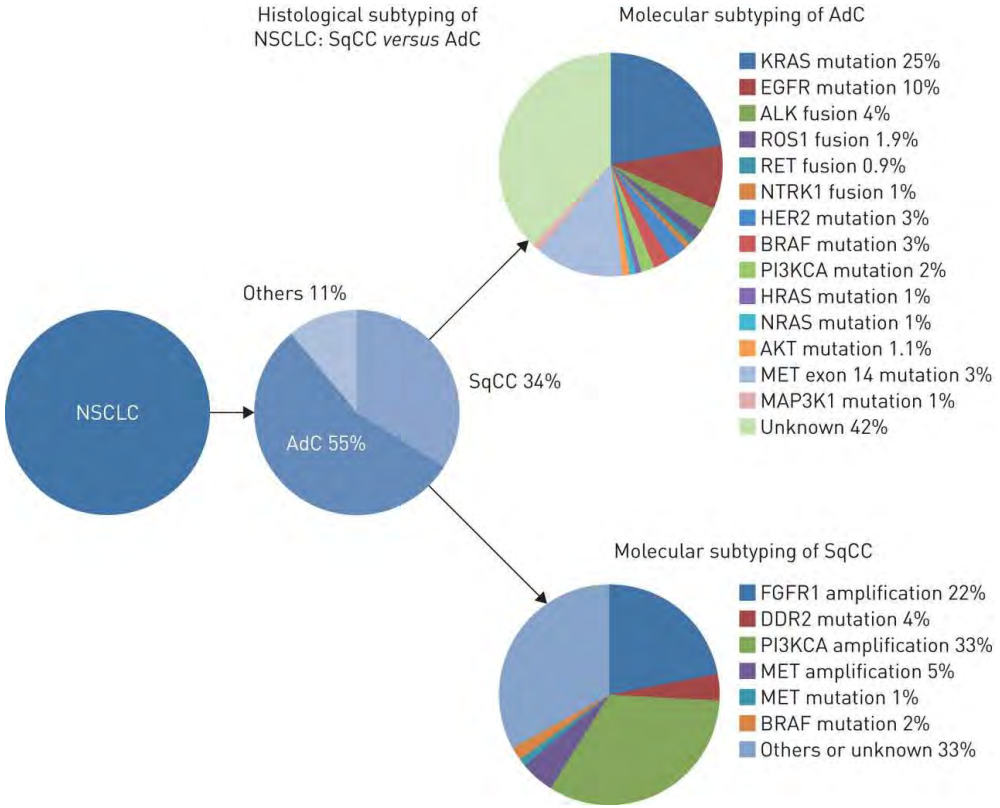
Emerging Targeted Therapies in Non-Small-Cell Lung Cancer (NSCLC)

Cancers 2025, 17, 353



Guideline recommendation

Lung cancer 154 (2021) 161-175



Eur Respir Rev 2017;26:170007

Emerging biomarkers	ESMO guidelines	NCCN guidelines	CAP/IASLC/AMP guidelines	ASCO guidelines	Pan-Asian guidelines
KRAS	●	●	▨	●	●
MET	●	●	●	●	●
RET	●	●	●	●	●
ERBB2/HER2	●	●	●	●	●
TMB	●	●	●	●	●

● Testing recommended ● Expanded panel testing recommended ▨ Single gene or expanded panel testing recommended ● No guideline recommendations to date

Predictive biomarkers	ESMO guidelines	NCCN guidelines	CAP/IASLC/AMP guidelines	ASCO guidelines	Pan-Asian guidelines
EGFR	●	●	●	●	●
ALK	●	●	●	●	●
ROS1	●	●	●	●	●
BRAF	●	●	●	●	●
PD-L1	●	●	●	●	●
NTRK	●	●	●	●	●

Genetic alterations in NSCLC and respective targeted therapies

Genetic Alteration	Adenocarcinoma	Squamous	Other	Targeted Therapeutics
KRAS				
G12C mutation	13–17%	2–4%	Rare	sotorasib, adagrasib divarasib, opunarasib olomorasib, garsorasib
G12D mutation	14–18%	1–1.5%	Rare	MRTX-1133, HRS-4642, LY3962673 RMC-9805, ASP3082
EGFR				
Ex19del	21%	3–5%	Rare	EGFR TKIs (1st–3rd generation)
Exon 21 L858R	29%	1–3%	Rare	EGFR TKIs (1st–3rd generation)
Exon20ins	2–3%	<1%	Rare	amivantamab, furmonertinib sunvozertinib
Amplification	10–15%	15–20%	Rare	EGFR TKIs (1st–3rd generation)
Overexpression	40–60%	60–89%	Rare	Not predictive of response to TKI

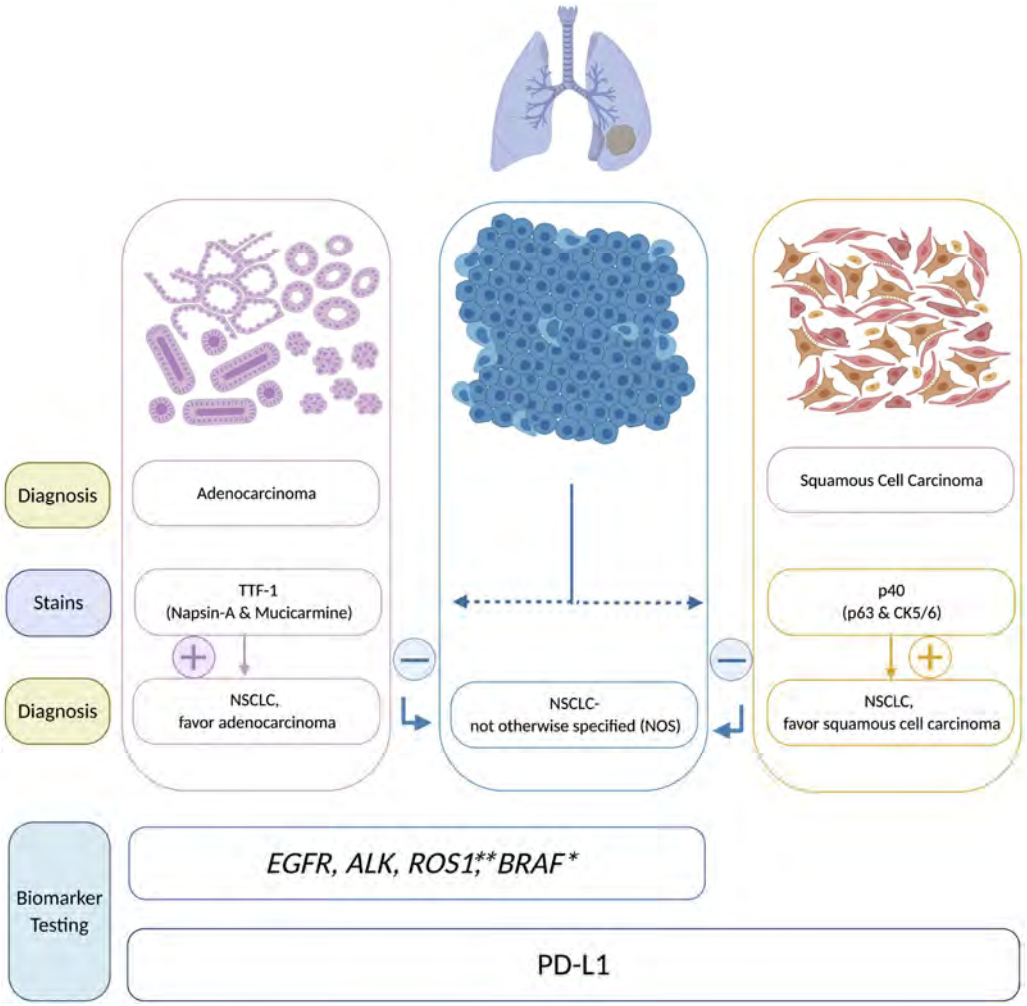
Genetic alterations in NSCLC and respective targeted therapies

HER 2				
Mutation exons 18–21	1–4%	<1%	Rare	HER2 TKIs (zongertinib, BAY2927088), ADC (TDXd SHR-A1811), monoclonocal antibody and BiTEs
Amplification	2–5%	1–2%	Rare	ADC (TDXd SHR-A1811), monoclonocal antibody and BiTEs
Overexpression	6–30%	2–10%	Rare	ADC (TDXd SHR-A1811), monoclonocal antibody and BiTEs
MET				
METex14 skipping mutations	3–4%	1–2%	15–20% in sarcomatoid	TKIs crizotinib, cabozantinib, capmatinib, tepotinib) and MET-ADC (Teliso-V, REGN5093-M114)
Amplification	1–5% (High-level <1%)	2–5%	~1–2%	TKIs crizotinib, cabozantinib, capmatinib, tepotinib) and MET-ADC (Teliso-V, REGN5093-M114)
Overexpression	15–50%	20–60%	Rare	MET-ADC (Teliso-V, REGN5093-M114)

Genetic alterations in NSCLC and respective targeted therapies

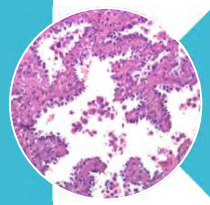
RET				
Fusion	1–2%	<0.1%	Rare	RET-targeting inhibitors like selpercatinib and pralsetinib.
FGFR				
Fusion	1–2%	<0.1%		FGFR inhibitors, such as erdafitinib, pemigatinib, and infigratinib.
TROP2				
Overexpression	20–30%	10–20%	Rare	Therapeutic antibodies (e.g., sacituzumab govitecan

Non-small cell carcinoma (NSCLC) algorithm for classification, immunohistochemistry, and biomarker testing.

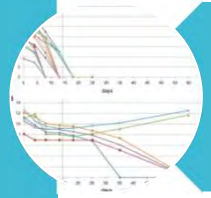


- Journal of the American Society of Cytopathology
- Volume 9, Issue 5, September–October 2020, Pages 332-345

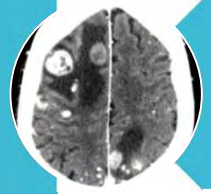
EGFR Mutations in NSCLC.



Seen primarily in *non-smoking women* with peripheral *non-mucinous ADC*/well to moderately differentiated adenocarcinoma with prominent *lepidic growth.*

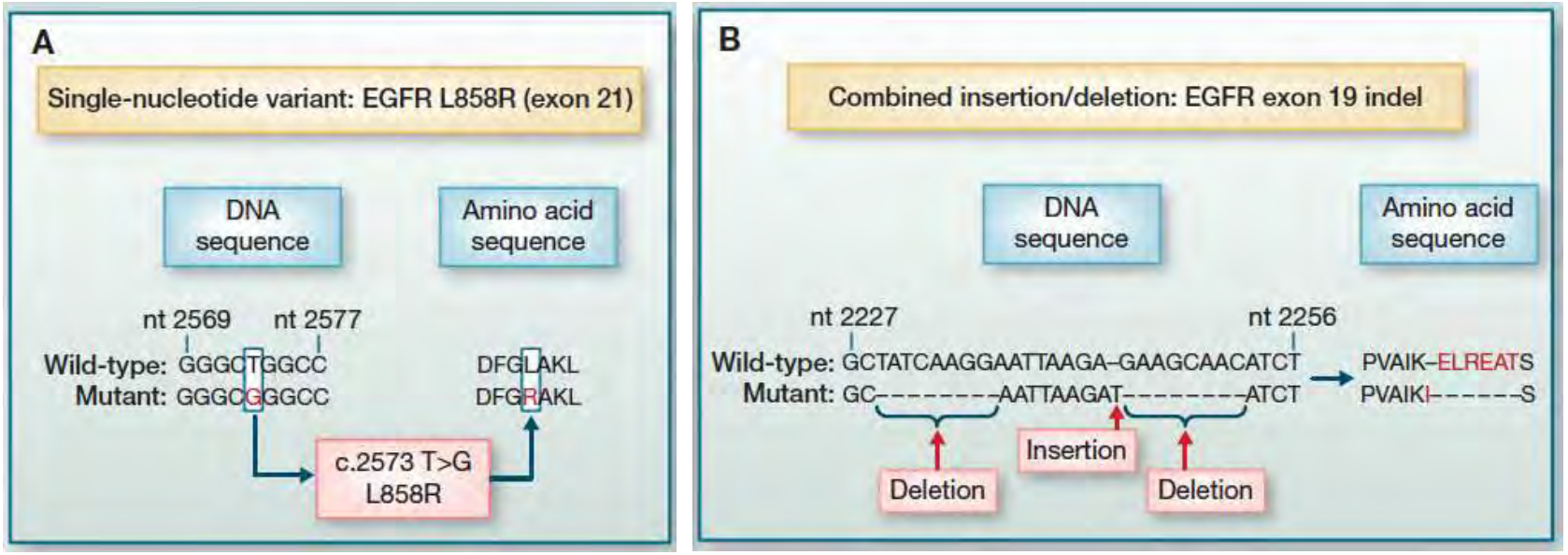


EGFR *mutations at exon #19 and 21* are tied to clinical responsiveness to TKIs.



Poor response tied to poorly differentiated, TTFI negative tumors; K-ras mutations; progression of EGFR mutations.

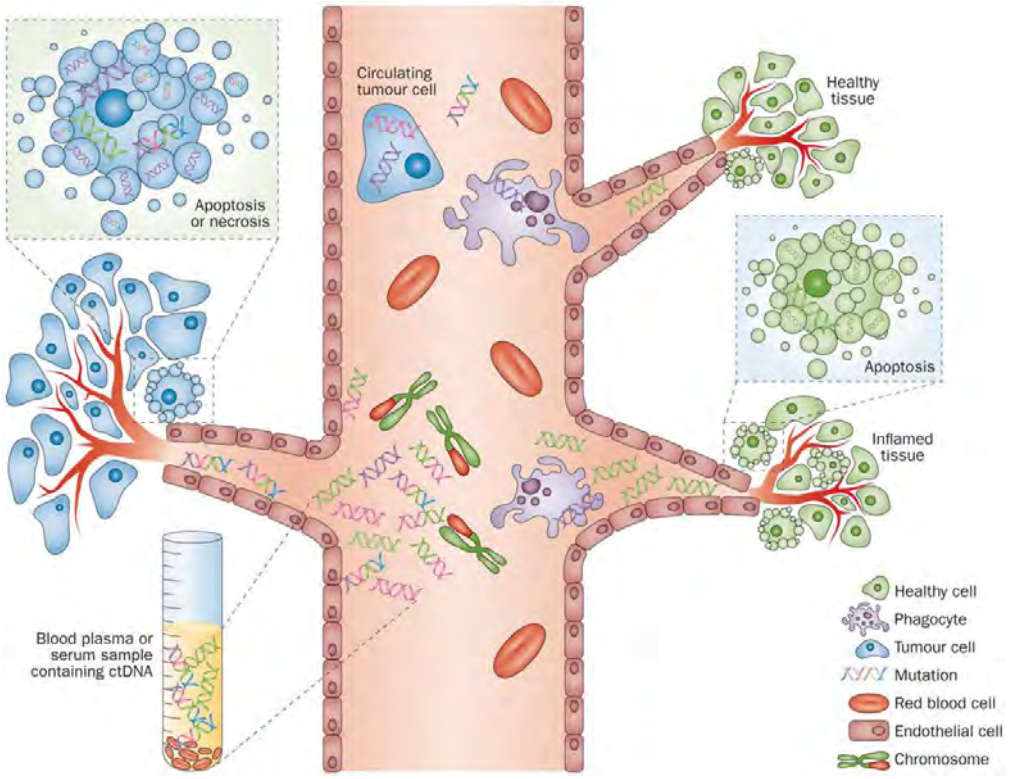
The most common mutations in EGFR in lung cancer



SNV in exon 21 of EGFR (c.2573 T>G) encoding a substitution of leucine to arginine at codon 858 (L858R).

Combined insertion/deletion (indel) in exon 19 of EGFR confer sensitivity to EGFR TKIs. Red, a nucleotide or amino acid that has been altered in the mutant form.

The liquid biopsy concept
- circulating cell-free DNA and circulating tumor cells -



"liquid biopsy"

Applications of liquid biopsy
• Early detection
• Assessment of molecular heterogeneity of overall disease
• Monitoring of tumor dynamics
• Evaluation of early treatment response
• Monitoring of minimal residual disease
• Assessement of evolution of therapy resistance in real time

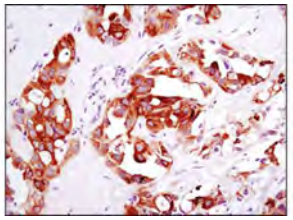
J Clin Oncol 2014; 32:579-586

- the half-life of cfDNA in the circulation as between 16 minutes and 2.5 hours

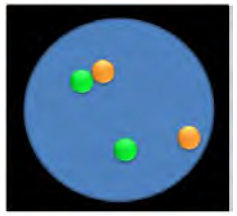
ALK gene rearrangement

Methods for testing ALK chromosomal rearrangement in tumors

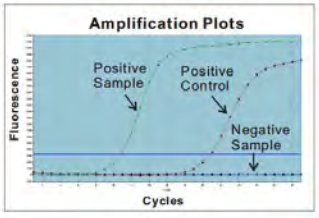
IHC
D5F3
rabbit mAb
Ventana, Roche



FISH
Break apart
probe
Abott Molecular



RT-PCR
ALK gene
fusions
detection kit

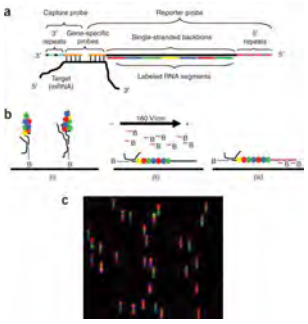


Real-time PCR output

NGS
RNA-fusion
transcript
Ion Torrent

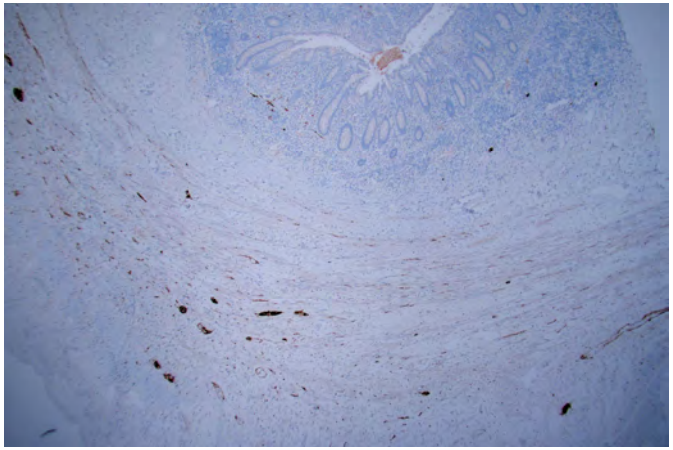
Digital RNA Counting

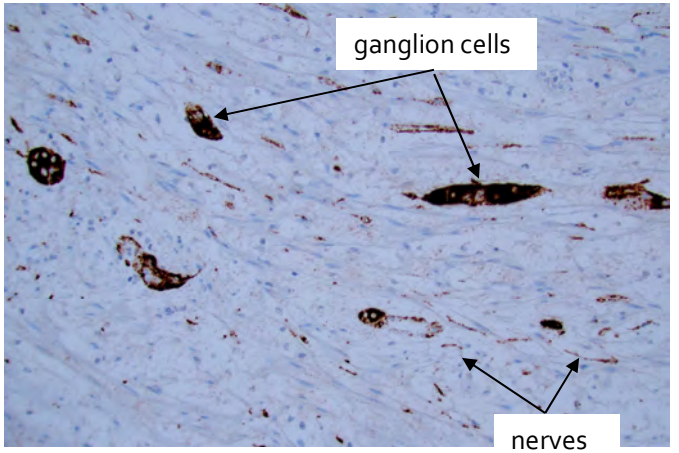
Nanostring



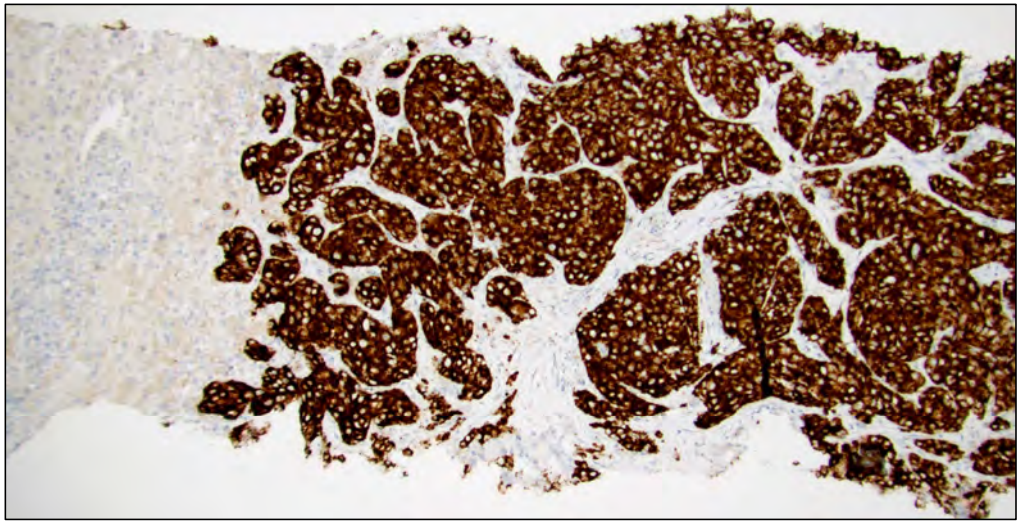
Ventana ALK (D5F3) CDx assay- normal appendix as external IHC control

appendix



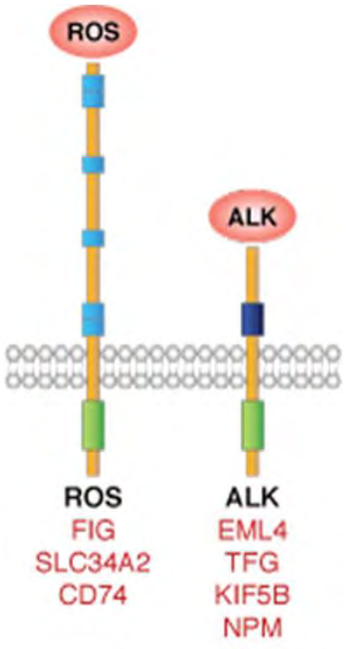


Liver metastasis of an ALK-positive adenocarcinoma



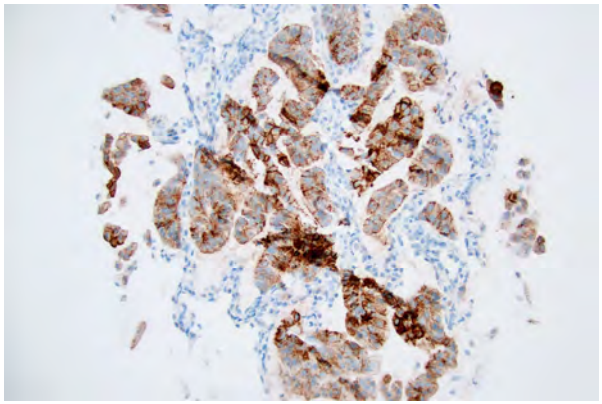
ROS1 gene rearrangement

ROS1 translocation in NSCLC



- The official name of this gene is "c-ros oncogene 1 , receptor tyrosine kinase."
  ROS1 is the gene's official symbol.
- ROS1 is a receptor tyrosine kinase of the insulin receptor family.
- Orphan receptor tyrosine kinase (RTK) that plays a role in epithelial cell differentiation and regionalization of the proximal epididymal epithelium.
- Chromosomal rearrangements involving the ROS1 gene were originally described in glioblastomas, where ROS1 (chromosome 6q22) is fused to the FIG gene (chromosome 6q22 immediately adjacent to ROS1)
- identified in 1–2% of unselected patients with NSCLCs

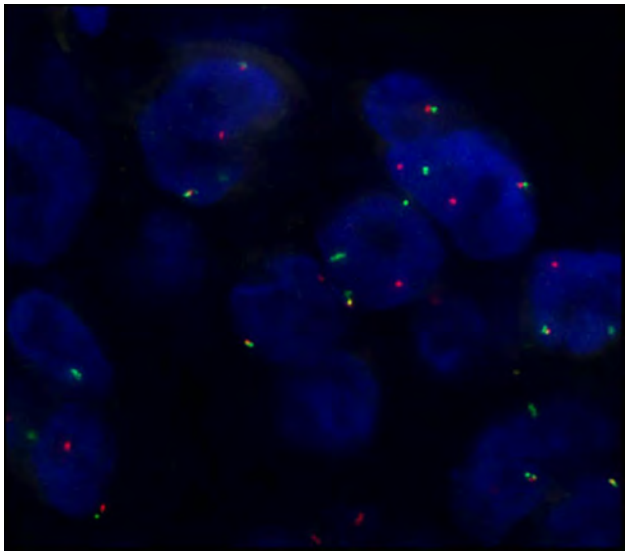
ROS1 IHC
(D4D6 rmAb, Cell Signaling)



confirmation by

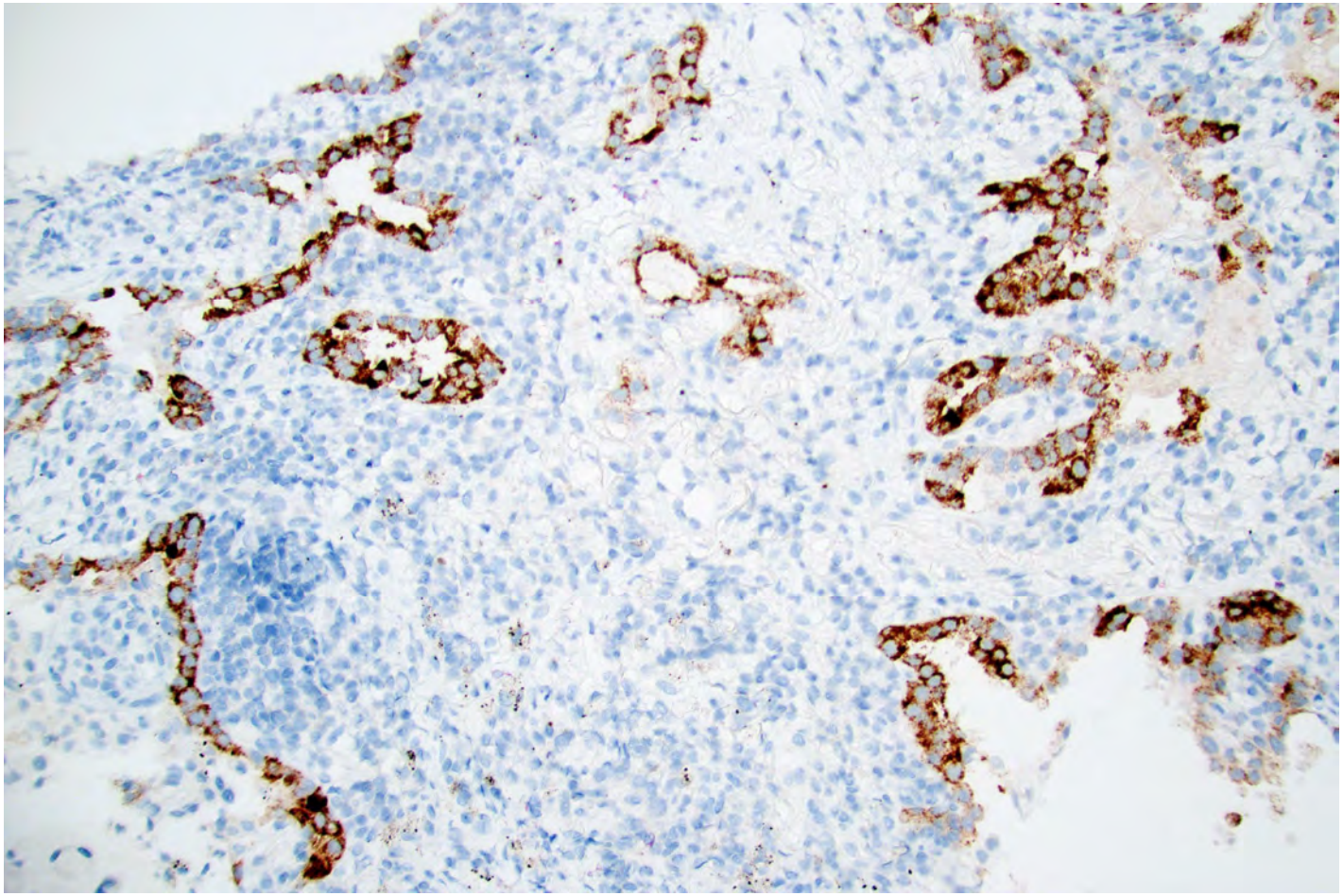
FISH

ROS1 FISH with break apart probe



Expert Consensus Opinion.—ROS1 IHC may be used as a screening test in advanced-stage lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method.

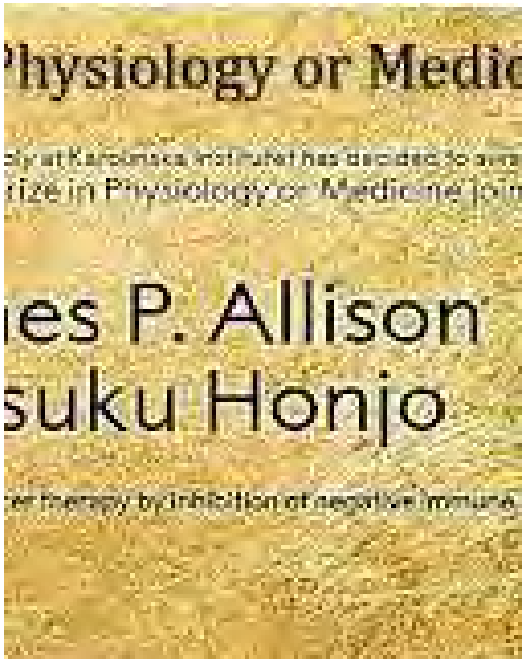
ROS1 IHC



Reactive pneumocytes are positive!!!

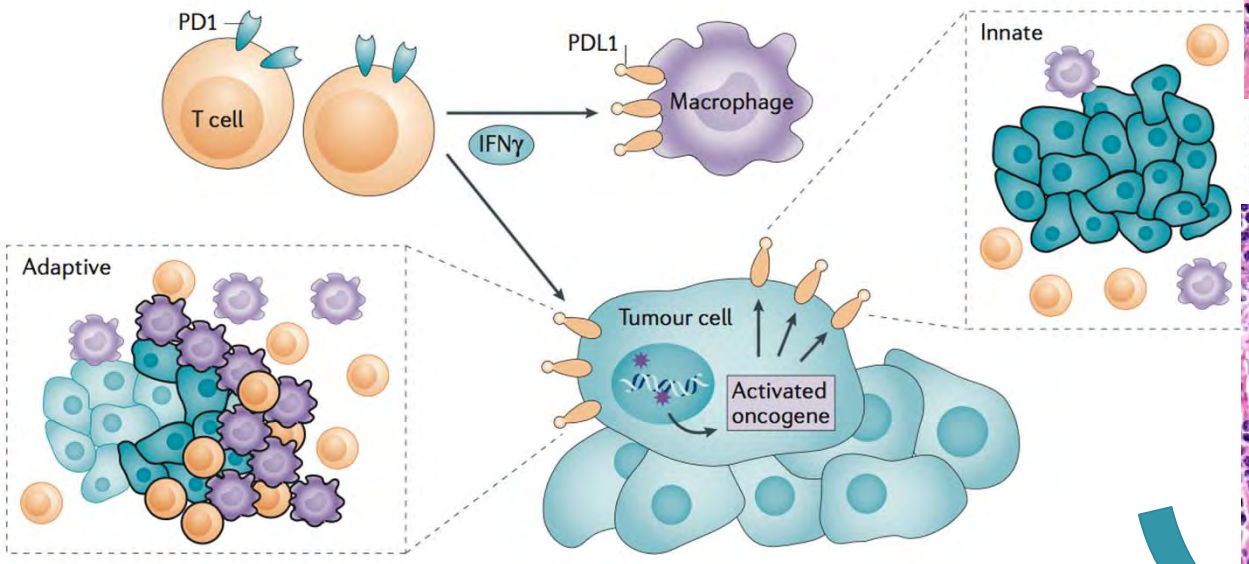

nature
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Antitumour immunity enhanced by inhibiting PD-L1/PD-1 and identifying mutant neo-antigens
IMMUNE-CHECKPOINT BLOCKADE IN
CANCER
ACCEPT YOUR OWN PAPER
THE CASE FOR AIMING HIGHER
'NIGHT-TIME' COOLING BY DAY

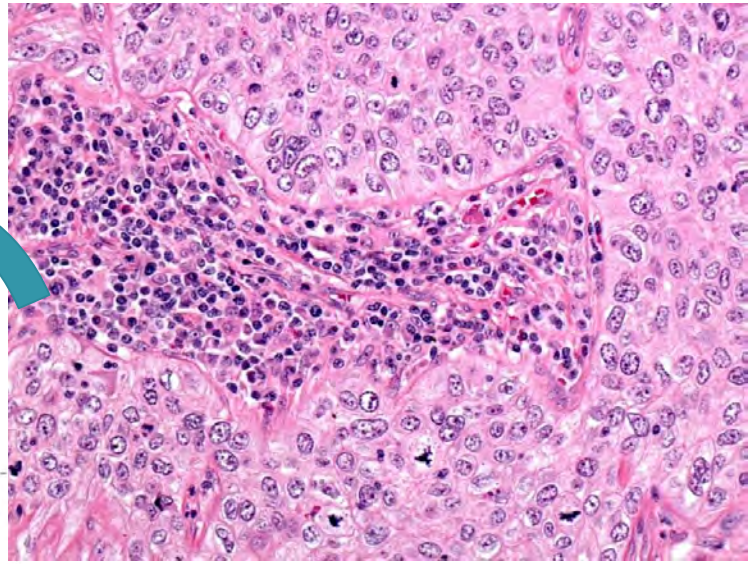

Science
Breakthrough of the Year
Cancer Immunotherapy

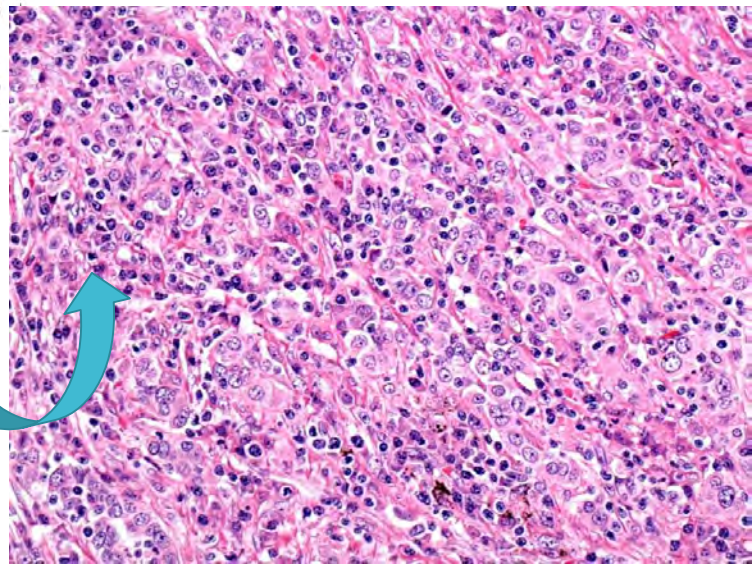

Physiology or Medic
es P. Allison
suku Honjo

PD-L1

Intratumoural PD-L1 expression

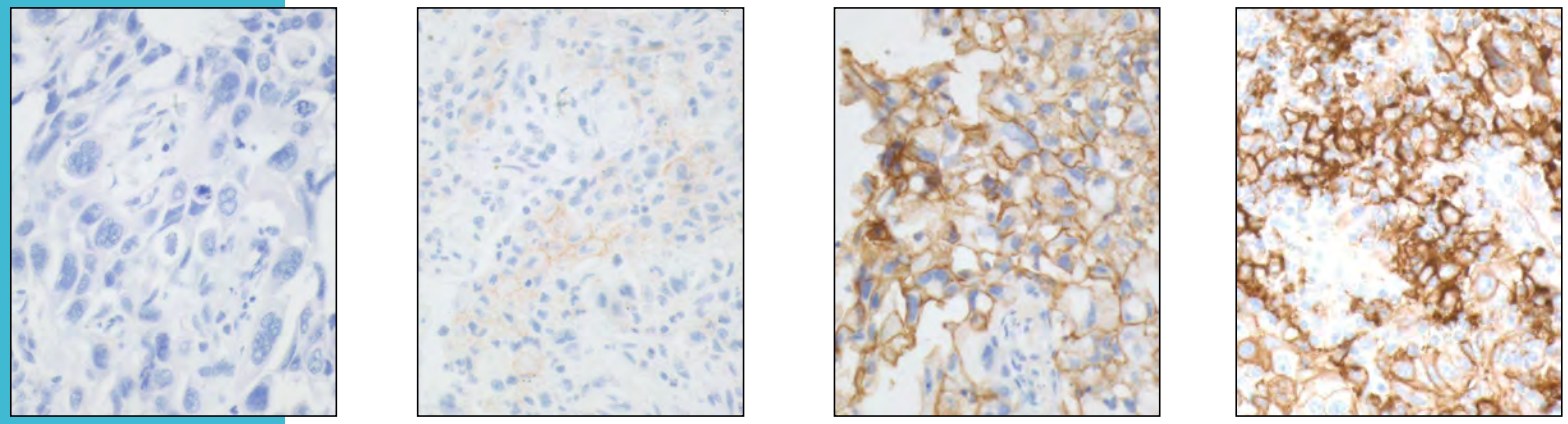






Block PD1 or PDL1
Immune damage to tumour

PD-L1 Biomarker for Immunotherapy



PD-L1 Negative

PD-L1 Positive (predictive of response)

Less response

More response

1%

5%

10%

50% cell positive

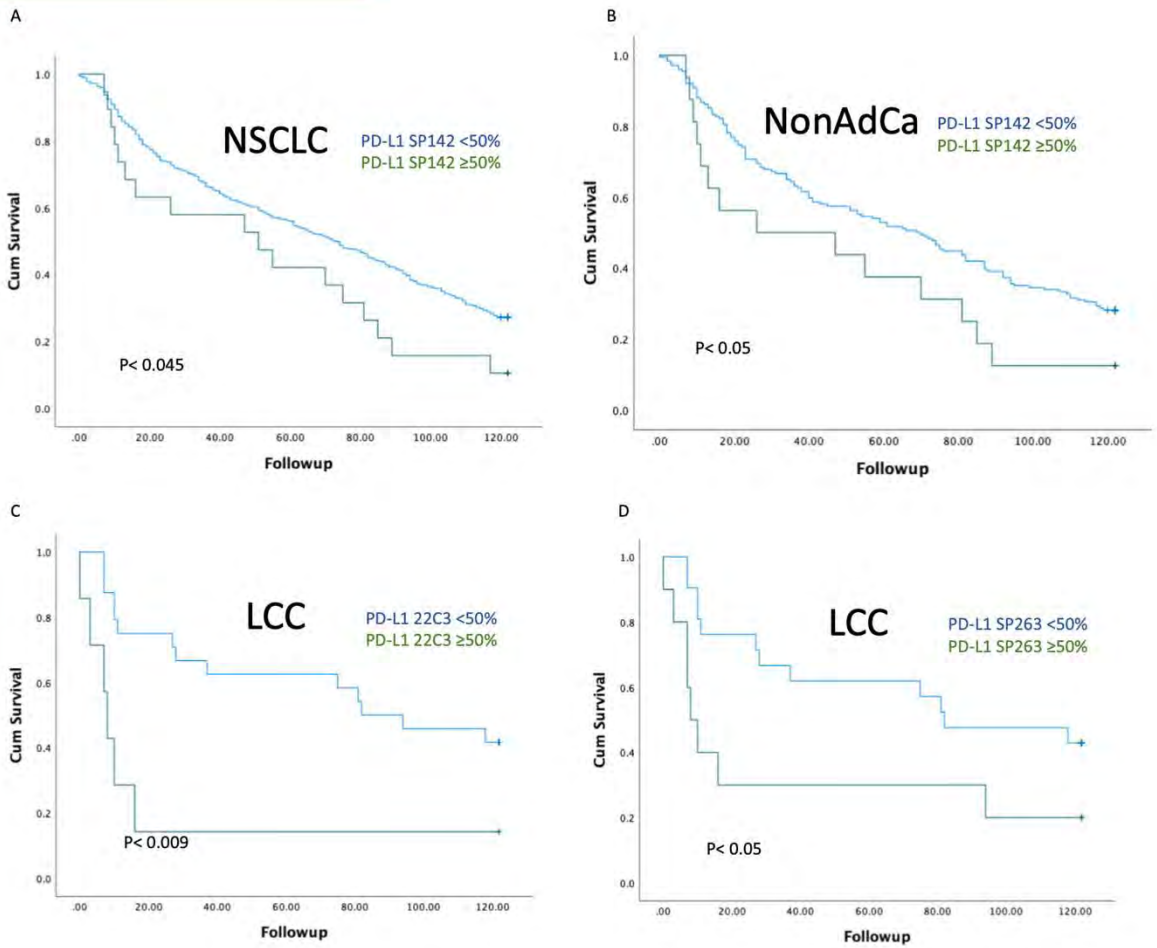
Table 1. Five-Drug PD-L1 Assay Trial-Validated Combinations

Drug	Company	PD-L1 Diagnostic Ab Clone	Staining Platform	Clinically Relevant Cutoffs[a]
Nivolumab	Bristol-Myers Squibb	28-8 (Dako)	Dako Link 48	TC ≥1%, 5%, and 10%
Pembrolizumab	Merck/Merck Sharp and Dohme	22C3 (Dako)	Dako Link 48	TC ≥ 1% and 50%
Atezolizumab	Genentech/Roche	SP142 (Ventana)	Ventana BenchMark ULTRA	TC ≥ 1%, 10%, and 50% IC ≥ 1%, 5%, and 10%
Durvalumab	AstraZeneca	SP263 (Ventana)	Ventana Benchmark	TC ≥ 25%
Avelumab	Pfizer/Merck Serono	73-10 (Dako)	Dako Link 48	TC ≥ 1%, 50%, and 80%

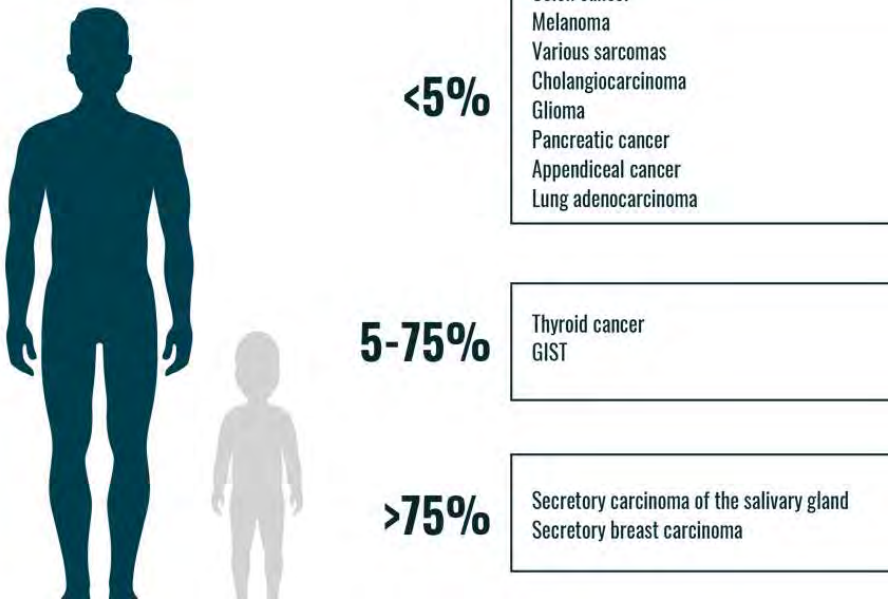
[a]Variable according to trials and line of therapy.
Modified with permission from Tsao et al.[9]
PD-L1, programmed death ligand 1; Ab, antibody; TC, tumor cells by percentage staining for PD-L1; IC, percentage of tumor area infiltrated by PD-L1-positive immune cells.

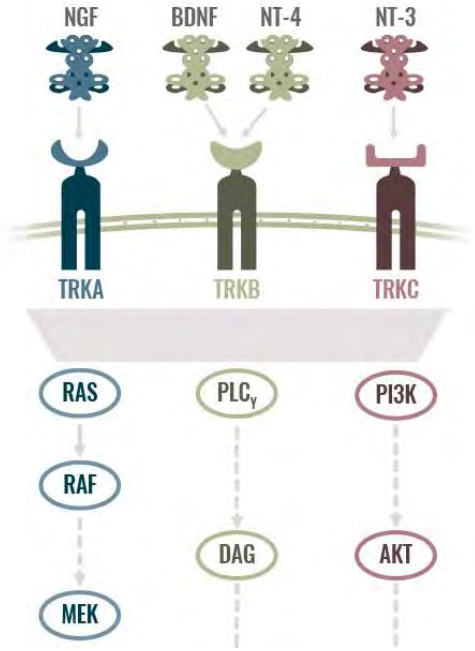
Gandhi L, et al. AACR 2014. Abstract CT105.

Clinicopathological significance of the expression of PD-L1 in non-small cell lung cancer



https://doi.org/10.1016/j.anndiagpath.2021.151701


<5%
Colon cancer
Melanoma
Various sarcomas
Cholangiocarcinoma
Glioma
Pancreatic cancer
Appendiceal cancer
Lung adenocarcinoma
5-75%
Thyroid cancer
GIST
>75%
Secretory carcinoma of the salivary gland
Secretory breast carcinoma


NGF
BDNF
NT-4
NT-3
TRKA
TRKB
TRKC
RAS
RAF
MEK
PLCγ
DAG
PI3K
AKT

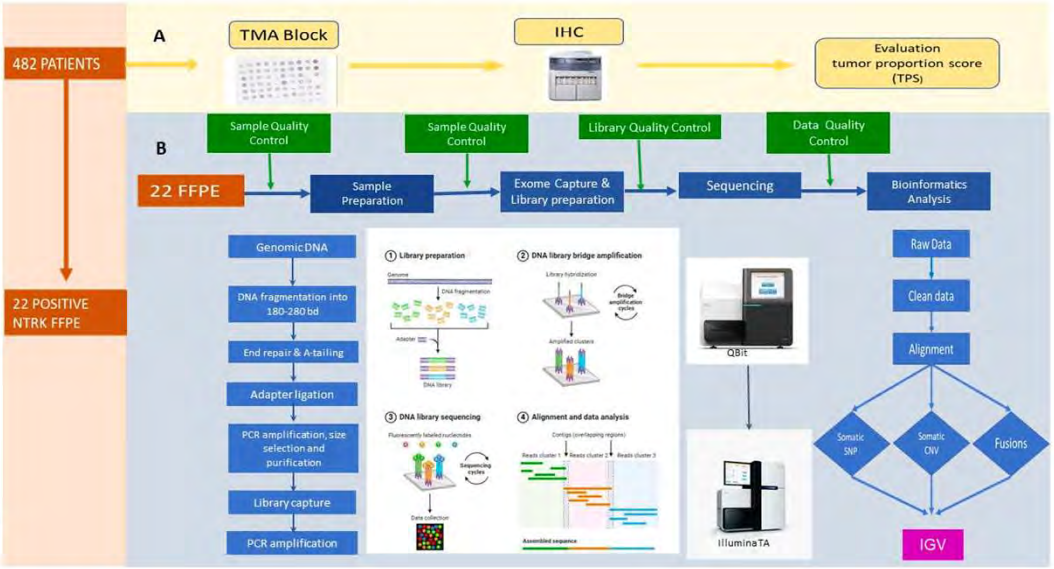
NTRK

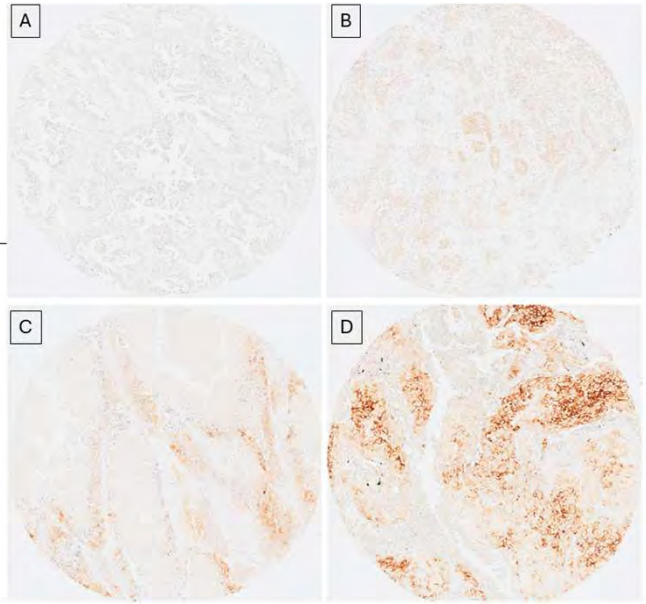
Journal of *Respiration*

Article

NTRK Gene Expression in Non-Small-Cell Lung Cancer

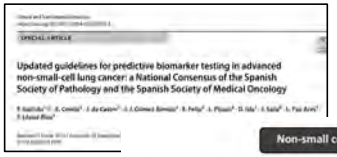
Jair Gutierrez-Herrera [1], M. Angeles Montero-Fernandez [2], Georgia Kokaraki [3], Luigi De Petris [3,4], Raul Maia Falcão [5], Manuel Molina-Centelles [1], Ricardo Guijarro [3,6], Simon Ekman [3,4] and Cristian Ortiz-Villalón [3,7,*]

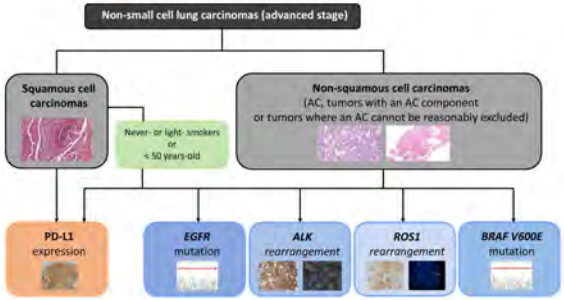




Cases	Fusion Partner	Partner 1	Partner 2	Histology	NTRK-IHC	Other Somatic Mutations
Sample 1	*AFAP1–NTRK2*	Chr4	Chr9	Adca	+1	*TP53 *, PIK3CA*
Sample 3	*ALK–KCNQ5*	Chr2	Chr6	SqCC	-	
Sample 4	*ALK–CTLC*	Chr2	Chr17	Adca	-	
Sample 5	*ALK–HIP1*	Chr2	Chr7	SqCC	-	*PTEN*
Sample 6	*ALK–HIP1*	Chr2	Chr7	SqCC	-	
Sample 6	*ALK–KCNQ5*	Chr2	Chr6	SqCC	-	
Sample 7	*TPM3–NTRK1*	Chr1	Chr1	SqCC	+2	
Sample 7	*ALK–VCL*	Chr2	Chr10	SqCC	-	
Sample 8	*ALK–HIP1*	Chr2	Chr7	SqCC	-	*APC*
Sample 8	*ROS1–CEP85L*	Chr6	Chr6	SqCC	-	
Sample 9	*PCM1–NRG1*	Chr8	Chr8	Adca	-	
Sample 10	*RASEF–NTRK2*	Chr9	Chr9	SqCC	+3	
Sample 10	*MET–KIF5B*	Chr7	Chr10	SqCC	-	
Sample 12	*RET–SPECC1L*	Chr10	Chr22	SqCC	-	*NTRK1, RET*
Sample 13	*ROS1–GOPC*	Chr6	Chr6	LCC	-	*BRAF*
Sample 13	*MET–CAPZA2*	Chr7	Chr7	LCC	-	

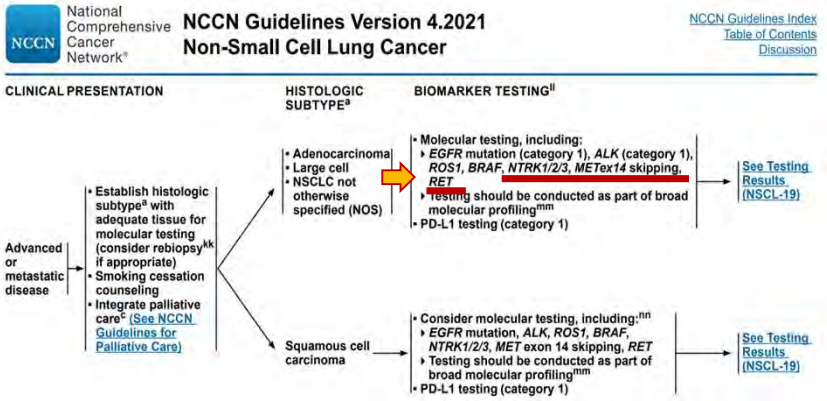
2018-2019


Updated guidelines for predictive biomarker testing in advanced non-small-cell lung cancer: a National Consensus of the Spanish Society of Pathology and the Spanish Society of Medical Oncology



NCCN Guidelines Version 4,2021, Kohno T et al, Transl Lung Cancer Res. 2015 Apr; 4(2): 156–164.

2025



Sensitizing EGFR Mutations
ALK Rearrangement
ROS1 Rearrangement
BRAF V600E Point Mutation
RET Rearrangements
NTRK Gene Fusions
MET Exon 14 Skipping Mutations
PD-L1 Expression*

KRAS Mutations
MET Amplifications
ERBB2 (HER2) Alterations

Table 1 Essential biomarkers in NSCLC patients

Gene/protein	Predictive alteration	Methodology (in tissue)
EGFR	Mutation	PCR: sanger, real-time PCR and NGS
ALK	Rearrangement	IHC, FISH and NGS
ROS1	Rearrangement	IHC (screening), FISH and NGS
BRAF V600	Mutation	PCR: sanger, real-time PCR and NGS
PD-L1	Overexpression	IHC

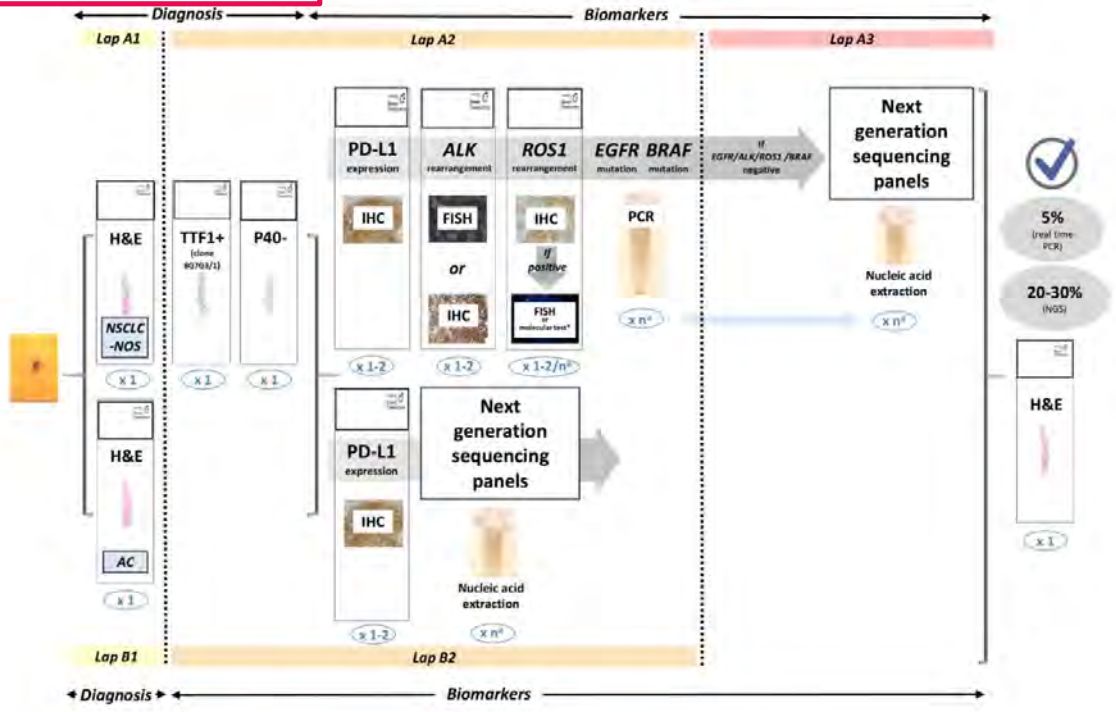
EGFR epidermal growth factor receptor, *FISH* fluorescence in situ hybridisation, *H&E* haematoxylin/eosin, *IHC* immunohistochemistry, *NGS* next-generation sequencing, *NSCLC* non-small-cell lung cancer, *PCR* polymerase chain reaction, *PD-L1* programmed death ligand-1

Table 2 Other biomarkers of interest in NSCLC patients

	Gene	Predictive alteration	Methodology (in tissue)
3%	*HER2*	Mutation	PCR: sanger, real-time PCR and NGS
		Amplification	FISH, NGS, real-time PCR
3-7%	*MET*	Mutation	NGS
		Amplification	FISH, NGS, real-time PCR
1-2%	*RET*	Rearrangement	FISH and NGS
<1%	*NTRK*	Rearrangement	IHC (screening) and NGS
	TMB	Mutations*	NGS

FISH fluorescence in situ hybridisation, *IHC* immunohistochemistry, *NGS* next-generation sequencing, *NSCLC* non-small-cell lung cancer, *PCR* polymerase chain reaction, *TMB* tumour mutation burden

*Measurement of somatic mutations present in tumour cells



Garrido P et al. Clin Transl Oncol. 2020 Jul;22(7):989-1003; Timar J. Cancer Metastasis Rev (2020) 39:1029–1038

TAKE HOME MESSAGE

“Evaluation of tissue by a pathologist and molecular testing remains the gold standard”

“We have a lot to learn about what liquid biopsy tells us, and for that reason, we should not lose sight of the fact that tissue testing is the basis for nearly all of what we know about targeted therapy.”

Clinicians should be aware of the technique used in the lab and the tissue characteristics for the requested parameters.

Pathologists should:
Obtain an informative request form
Preserve precious tissue for molecular marker analyses(in situ or not)/immune check-point inhibitors
Be a full member of the MDD team (taking active part in the diagnostic workup, receiving feedback on treatment results)

THANK YOU!