Recent Advances in Lung Neuroendocrine Neoplasm Classification

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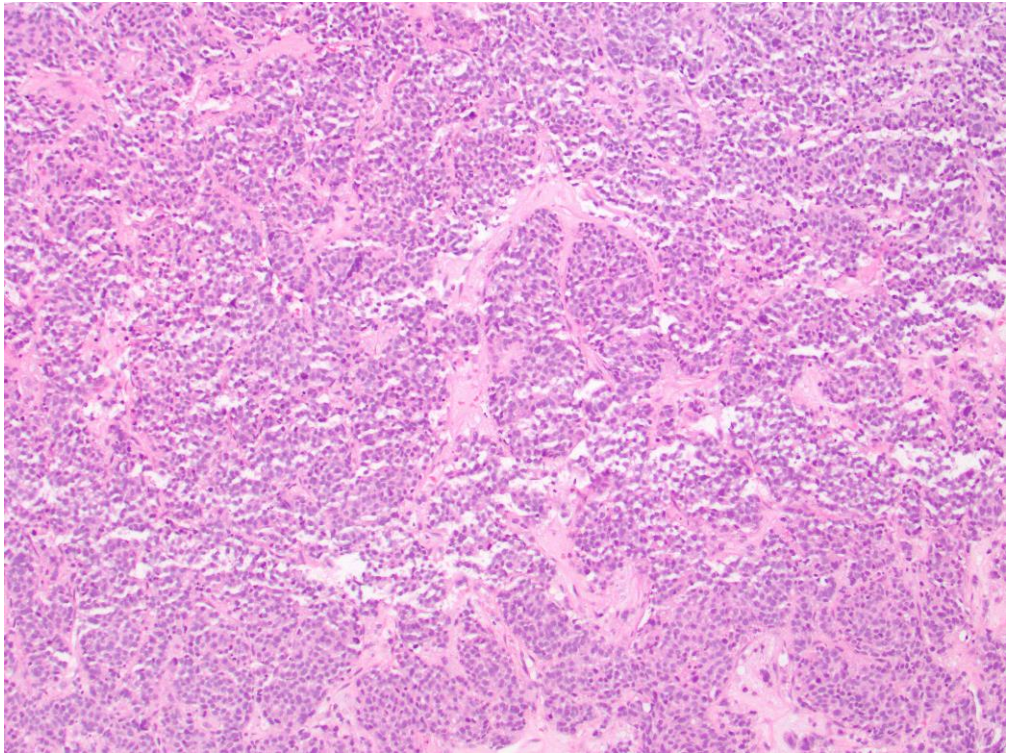
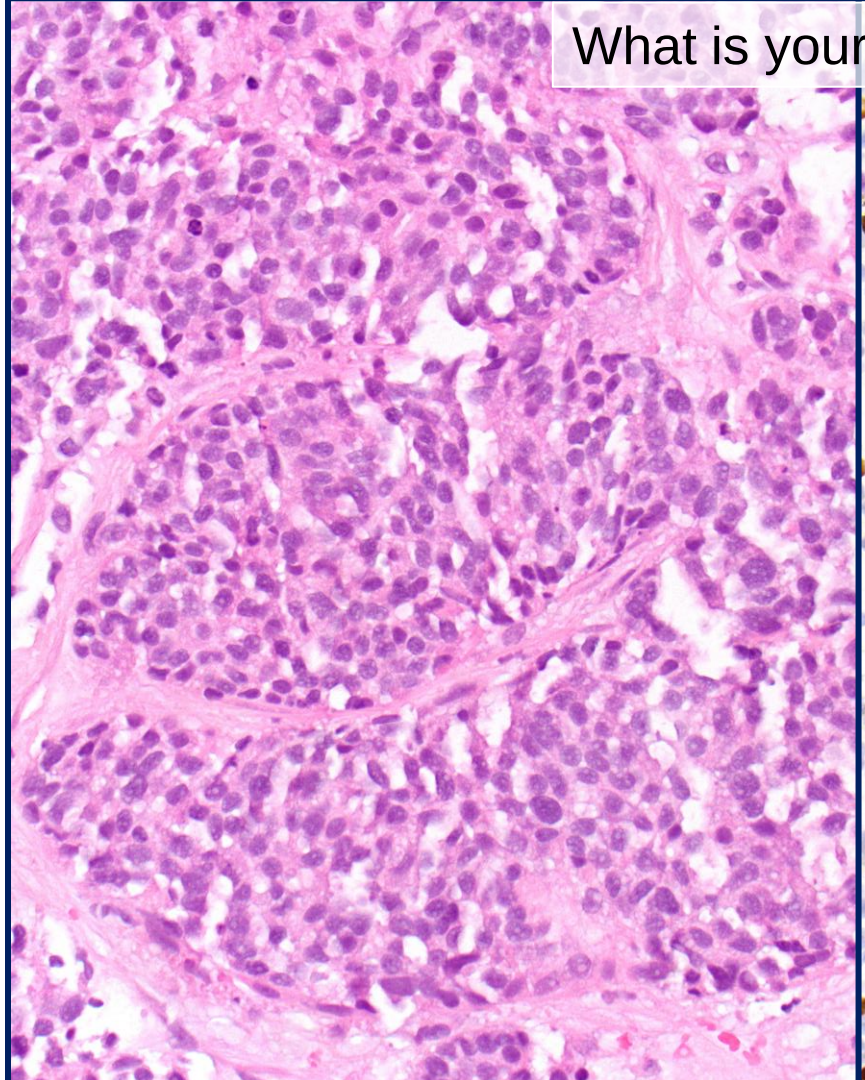
2021 WHO Classification of Lung Neuroendocrine Tumors

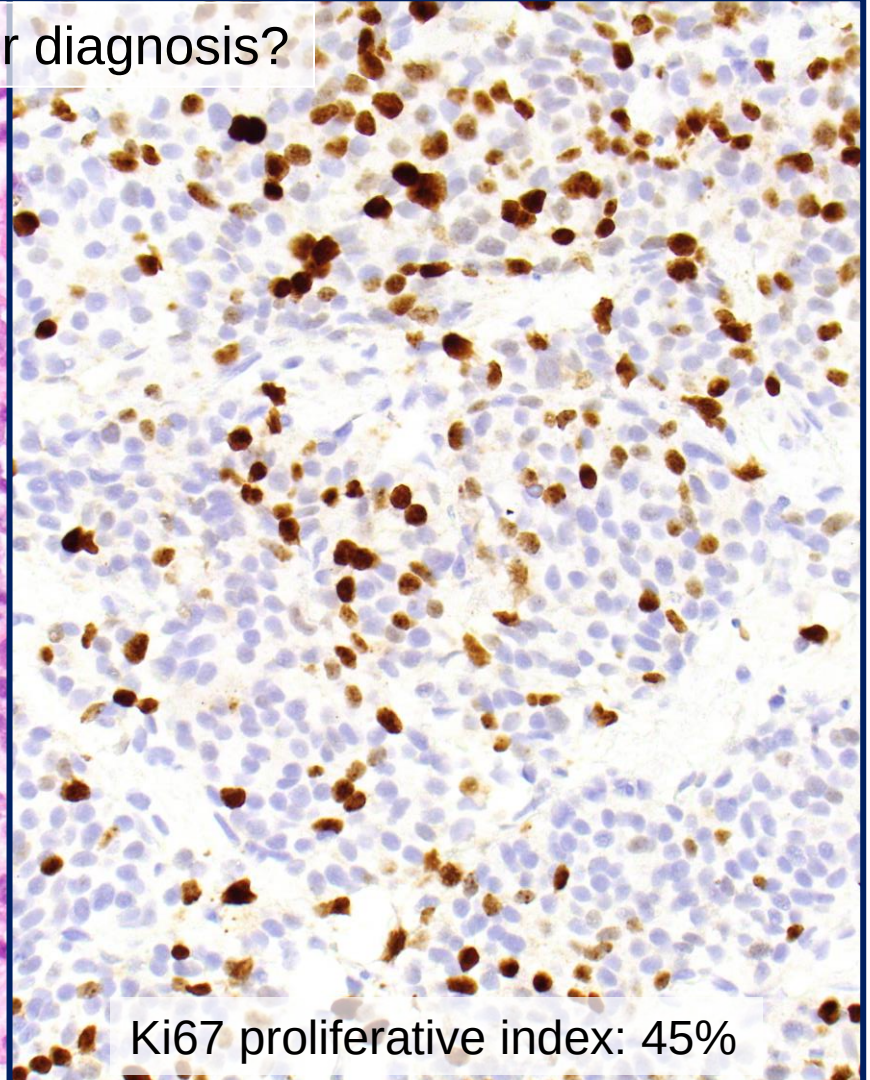
- Precursor lesion
  - Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
- Neuroendocrine tumors (NET) of the lung
  - Carcinoid tumor, NOS - for small bx, metastases or limited sampling
  - Typical carcinoid
  - Atypical carcinoid
- Neuroendocrine carcinomas
  - Small cell lung carcinoma
    - Combined small cell carcinoma
  - Large cell neuroendocrine carcinoma (LCNEC) of the lung
    - Combined LCNEC

Liver biopsy from a patient with a lung nodule, mediastinal lymphadenopathy, and multiple liver lesions



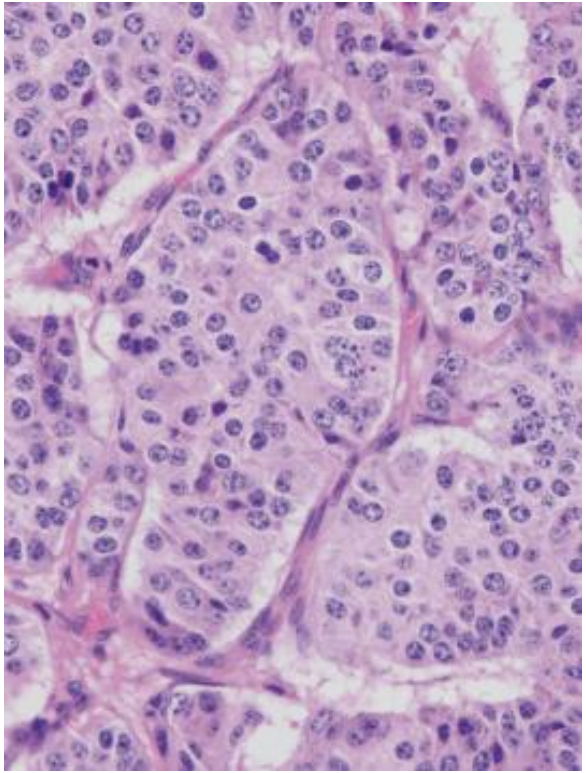
What is your diagnosis?



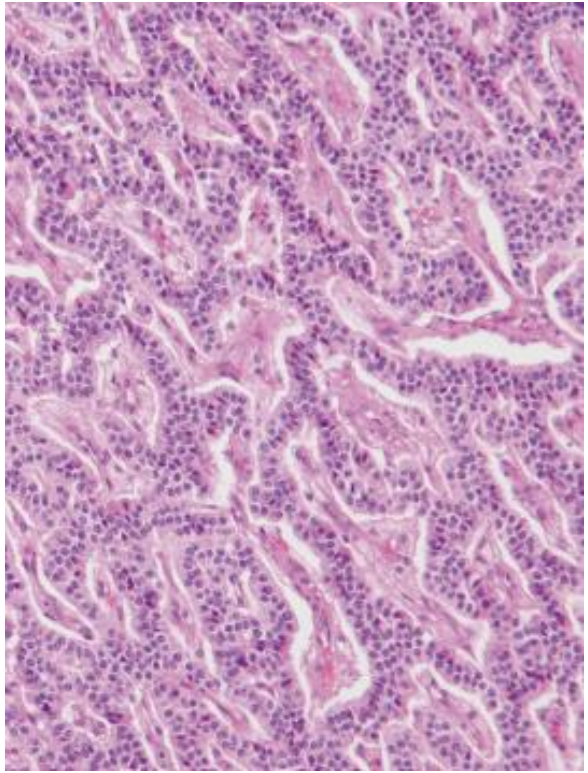


Ki67 proliferative index: 45%

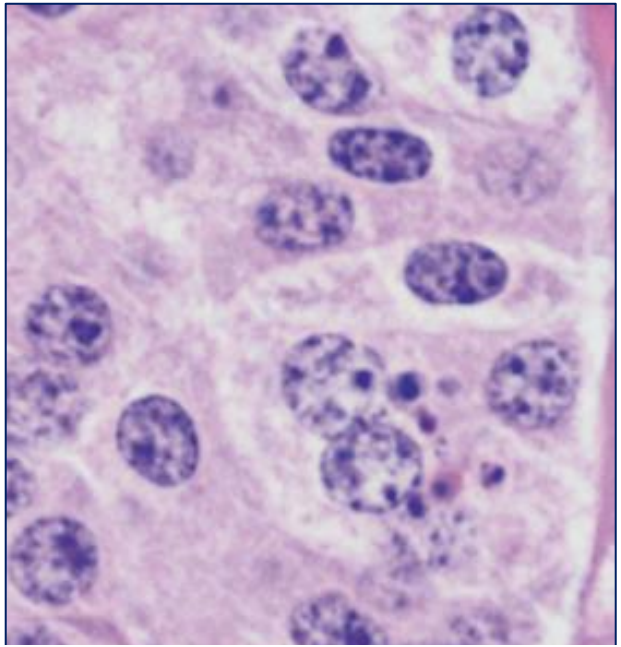
Lung Carcinoids: Classic Features



Organoid (nested) pattern

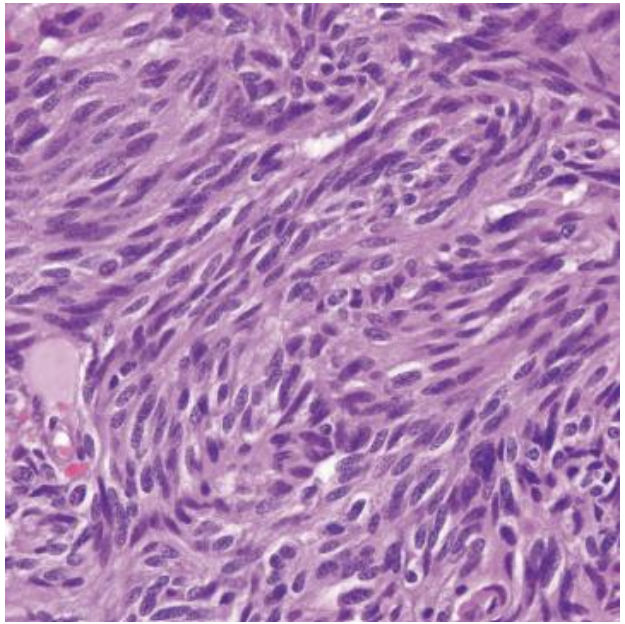


Trabecular pattern

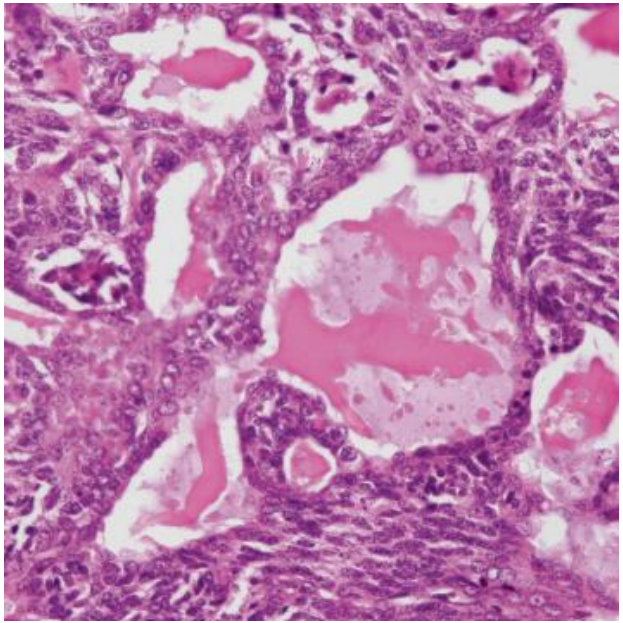


“Salt & pepper” chromatin pattern with a uniform population of small, round nuclei containing scattered clumps of nuclear chromatin of various sizes

Lung Carcinoids: Variant Morphology



Spindle cell



Pseudoglandular

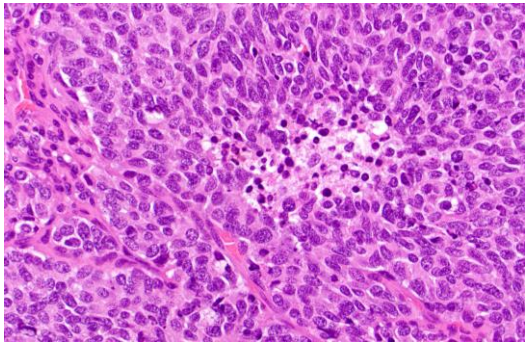
Other patterns

- Oncocytic
- Solid
- Sieve-like
- Adenoma-like
- Basaloid

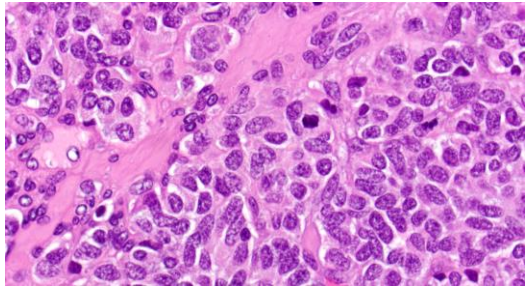
Typical vs. Atypical Carcinoid: WHO 2021 Criteria

	Mitoses / 2 mm^2	Necrosis
Typical	0-1	No
Atypical	2-10	Yes (often punctate)

- Either necrosis or increased mitoses classify the tumor as atypical carcinoid
- Counting mitoses is per 2 mm^2, not per 10 HPFs
- When mitotic counts are close to cut-off, WHO recommends that at least 3 sets of 2 mm^2 be counted and the mean be used for determining the mitotic rate, instead of the single highest rate



Punctate necrosis



Increased mitoses

Adjustments of Mitotic Counts in Accordance with Field of View of Microscope

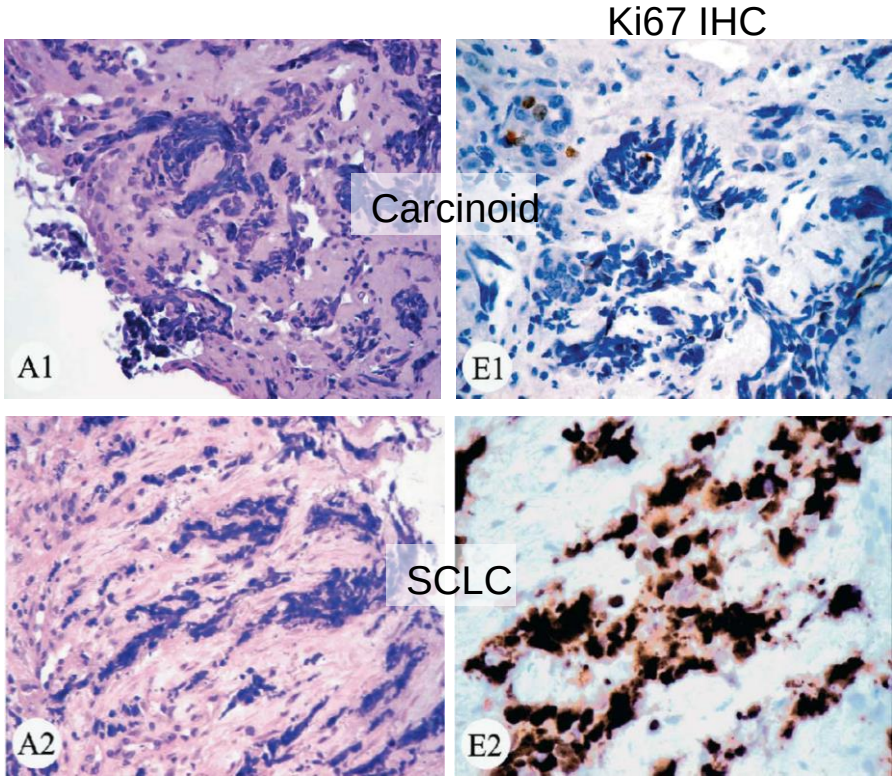
Microscope manufacturer and model	Standard eyepiece		Wide field eyepiece		Standard eyepiece (No. of HPF needed to view 2 mm^2 area)	Wide field eyepiece (No. of HPF needed to view mm^2 area)
	Field of view	Area (mm^2)	Field of view	Area (mm^2)		
OLYMPUS (BH2)	20	0.2	26.5	0.34	10	5.7
OLYMPUS (BX40)	22	0.24	26.5	0.34	8.3	5.7
Nikon	22	0.24	25	0.3	8.3	6.3
Zeiss	20	0.2	25	0.3	10	6.3
Leica	20	0.2	25	0.3	10	6.3

HPF, high-power field.

Travis WD, et al. Am J Surg Pathol 1998;22:934-44
Cree IA, et al. Modern Pathol 2021;34:1651-7

Established Role of Ki-67 in NE Tumors of the Lung

- Differentiating carcinoid tumors from NE carcinomas in small biopsies, in particular, those affected by crush artifacts
- Caveat:
  - Carcinoids in the metastatic setting may exhibit elevated proliferation
  - CytoLyt fixation in cytology specimens may reduce immunoreactivity to MIB1 clone
    - Clone 30-9 is recommended in this context



Pelosi G, et al. J Thorac Oncol 2014;9:273-84; Buonocore D, et al. Cancer Cytopathol 2019;127:643-9

Grading Systems of NE Tumors

	Digestive WHO 2019		Thoracic WHO 2021		
Terminology			**Terminology**	**Essential**	**Desirable**
	Mitotic counts per 2 mm²	**Ki-67**		**Mitotic counts per 2 mm²**	**Ki-67**
NET, grade 1	<2	< 3%	Typical carcinoid	< 2	< 5%
NET, grade 2	2-20	3-20%	Atypical carcinoid	2-10 (or necrosis)	5-30% (typically <20%)
NET, grade 3	> 20	> 20%			
Small cell or large cell NEC	> 20	> 20%	Small cell or large cell NEC	> 10	30-100%*

NET: neuroendocrine tumor, NEC: neuroendocrine carcinoma ***Typical ranges: 65-100%for SCLC & 40-80% for LCNEC; high Ki-67 index is a desirable diagnostic criterion only for LCNEC**

- The 2021 WHO recommended term in the lung remains carcinoid
- Ki-67 is not part of the essential diagnostic criteria, but now recommended by WHO as a “desirable” feature to include in the reporting of lung carcinoids and LCNEC

Klimstra DS Seminar in Oncol 2013;40:23-36, modified; Pelosi G, et al. J Thorac Oncol 2014;9:273-84

Hyper-proliferative Carcinoids
- new concept in WHO 2021 -

- Tumors with atypical carcinoid morphology with mitotic counts of >10 mitoses/2 mm^2 (usually < 20 mitoses/2 mm^2)
- Ki-67 proliferation index is often > 30% (usually < 60%)
- Rare at primary site, but very common at metastatic sites
- Equivalent to grade 3 NETs of the pancreas and GI tract
- Currently, these tumors are classified as LCNEC
- Limited genetic data have shown alterations similar to those of carcinoid tumors
- Their prognosis appears to be better than that of LCNEC

Rekhtman N, et al. Modern Pathol 2019;32:1106-22

Hyper-proliferative Carcinoids
- new concept in WHO 2021 -

Thoracic WHO 2021		
Terminology	**Mitotic counts per 2 mm^2**	**Ki-67**
Typical carcinoid	< 2	< 5%
Atypical carcinoid	2-10 (or necrosis)	5-30% (typically <20%)
To be determined*	> 10 (usually < 20)	> 20 - 30% (typically < 60%)
Small cell or large cell NEC	> 10	30-100%

NET: neuroendocrine tumor, NEC: neuroendocrine carcinoma, SCLC: small cell carcinoma of the lung, LCNEC: large cell neuroendocrine carcinoma

*Until further defined, the diagnosis of LCNEC with a note stating the presence of histological features of a carcinoid tumor with the mitotic counts and, if available, the Ki-67 index is suggested

Stage IV Lung Carcinoids

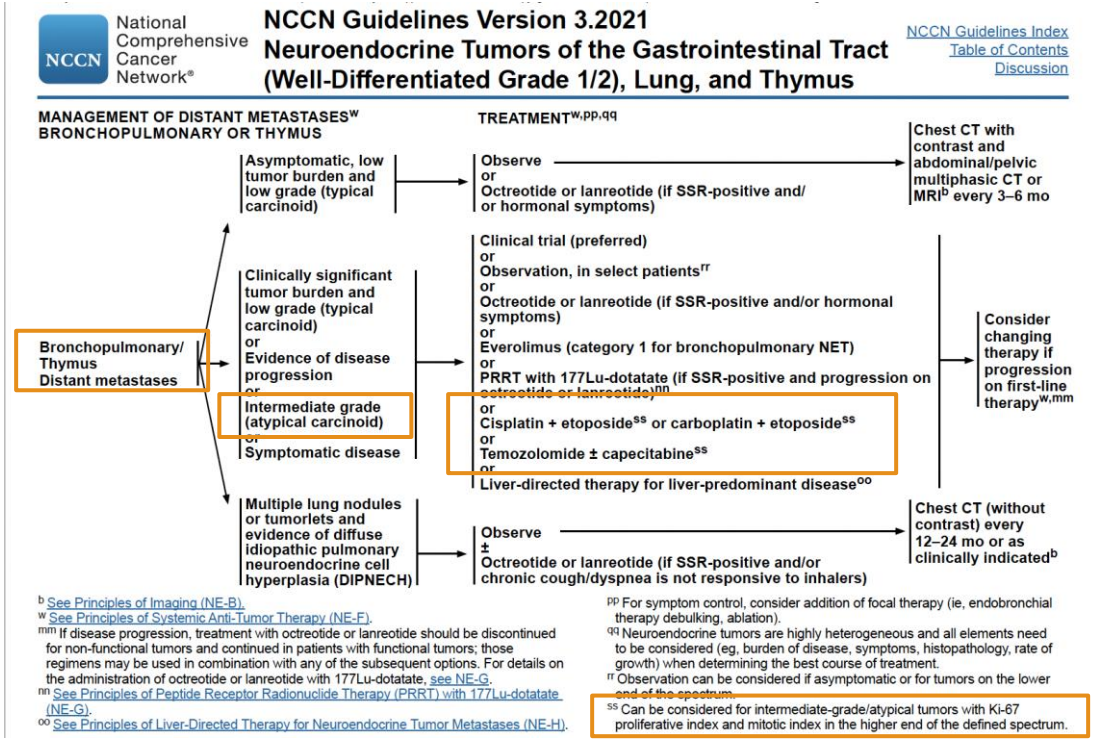
- Mitoses and/or Ki-67 proliferative index may exceed the standard criteria in 30%, primarily in specimens from metastatic sites
- They are still part of the spectrum of carcinoids:
  - Well-differentiated morphology
  - Conventional proliferation rates in other samples from same patient
  - Genetic alterations characteristic of high-grade neuroendocrine carcinomas are lacking
  - Median overall survival of 2.7 years compared to < 1 year survival of

Stage IV carcinoids should be classified separately from high-grade neuroendocrine carcinomas

Rekhtman N, at al. Modern Pathol 2019;32:1106-1122

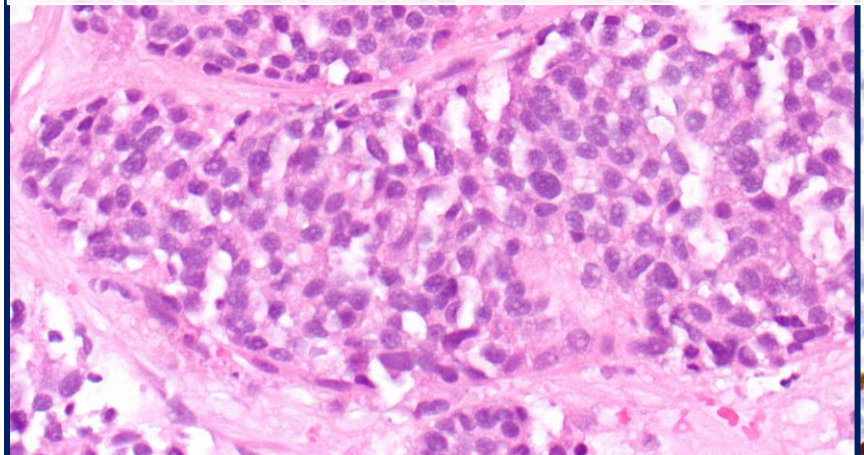
Stage IV carcinoids

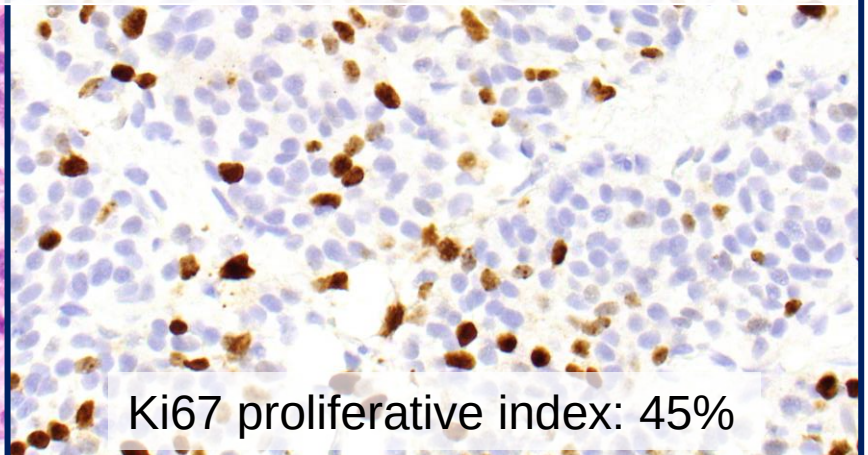
- Treated similarly to GI NETs
- Atypical carcinoid with increased proliferation may be treated with chemotherapy for SCLC
- Ki-67 index likely requested by the oncologist for treatment decision making



Liver Biopsy:
Metastatic neuroendocrine neoplasm with increased proliferation (see note).

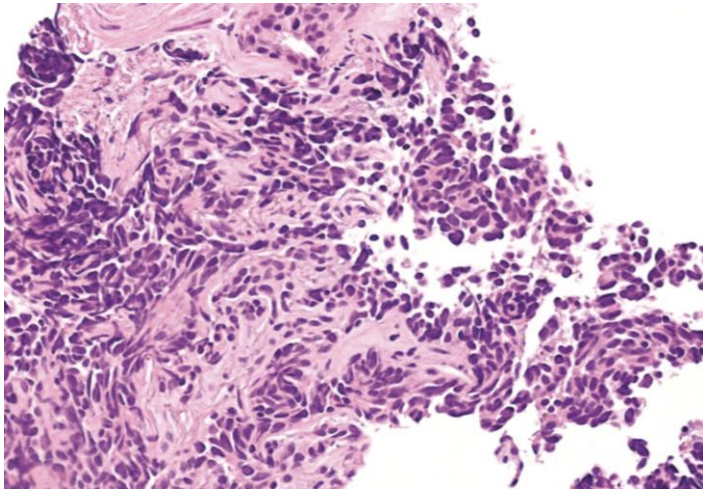
Note: The tumor exhibits histological features of a carcinoid tumor with up to 18 mitoses/2 mm^2 and Ki-67 index of 45%. In accordance with the current WHO criteria, the tumor is classified as LCNEC, but its biology may be similar to that of atypical carcinoid.





Ki67 proliferative index: 45%

New in WHO 2021: Carcinoid Tumor, NOS for Biopsies



- Mitosis-based criteria for typical vs. atypical carcinoids were developed for resected primary tumors to predict the risk of recurrence
- Mitotic criteria may not be accurately applied to small biopsies
- The recommended term “carcinoid tumour, NOS”
- With a note including
  - Presence or absence of necrosis (including extent – punctate or extensive)
  - The best estimated mitotic counts / 2 mm^2
  - Ki-67 index (if available)

WHO 2021 Criteria for Small Cell Lung Carcinoma

Essential:

- Composed of small cells (usually < the size of 3 resting lymphocytes) with scant cytoplasm, oval to spindle shape, and high mitotic count (> 10 mitoses/2 mm^2 but usually higher, ~60 mitoses/2 mm^2), often with necrosis
- Finely granular nuclear chromatin
- Absent or inconspicuous nucleoli

Desirable:

- Positive immunohistochemistry for low-molecular-weight cytokeratin
- Frequent expression of neuroendocrine markers (90-95% of cases)
- Lack of diffuse p40 expression, unless in areas of squamous cell carcinoma in a combined SCLC

WHO 2021 Criteria for Small Cell Lung Carcinoma

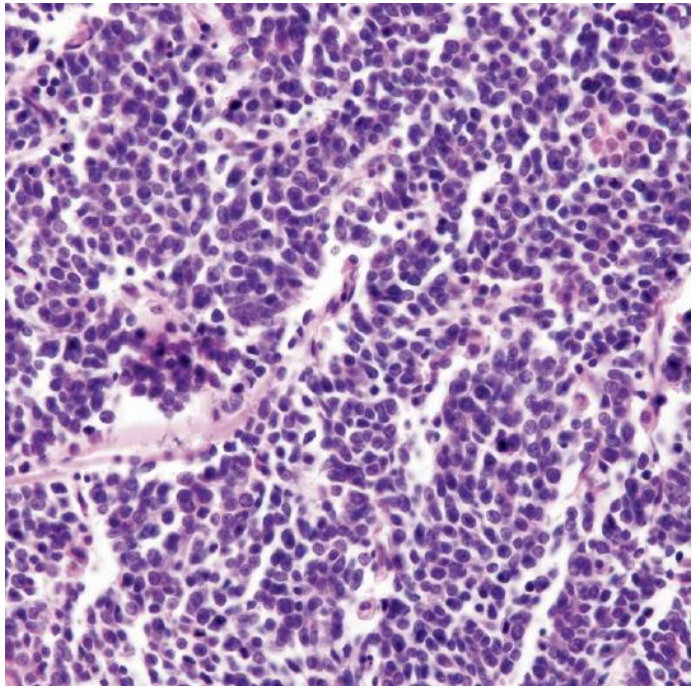
Essential for combined small cell lung carcinoma:

- Features of SCLC but with a component of a non-small cell lung carcinoma (LCC, LCNEC, adenocarcinoma, SCC, or less commonly spindle and/or giant cell carcinoma)
- In the case of SCLC combined with LCNEC or LCC, but not the other histological types, the second component should make up ≥ 10% of the tumor

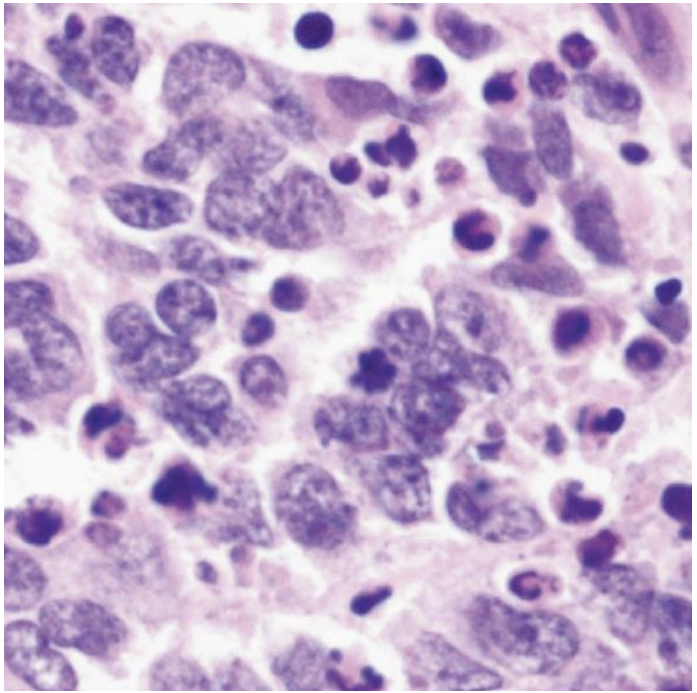
Desirable:

- Positive immunohistochemistry for low-molecular-weight cytokeratin
- Frequent expression of neuroendocrine markers (90-95% of cases)
- Lack of diffuse p40 expression, unless in areas of squamous cell carcinoma in a combined SCLC

Small Cell Carcinoma

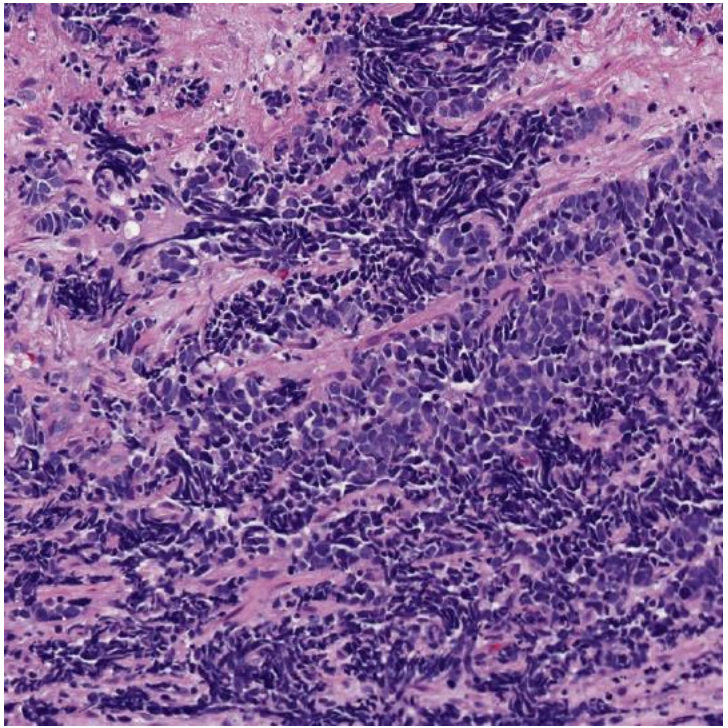


Sheets of small blue cells

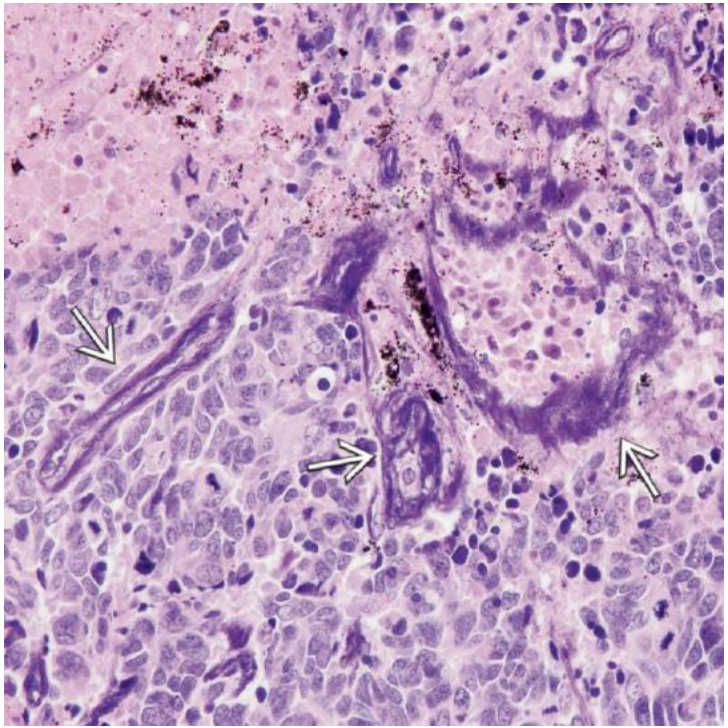


Fine granular nuclear chromatin and numerous mitoses

Small Cell Carcinoma



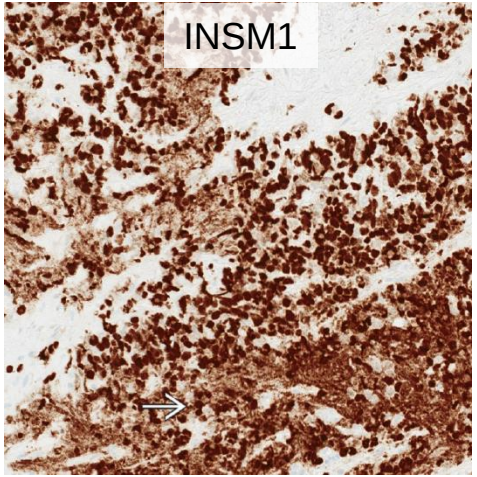
Crush artefact



Azzopardi effect

IHC for Small Cell Carcinoma

- Not required for the diagnosis, but commonly used to exclude an alternative diagnosis
- Positive for cytokeratins (except HMW cytokeratins) often with a perinuclear dot-like pattern
- At least one NE marker positive in 90-95%
- Sole expression of CD56 may not be reliable
- INSM1 is a constantly reliable marker
- TTF-1 expression in 90-95%
- p63 and p40 are generally negative
- High Ki67 proliferative index (65%-100%)

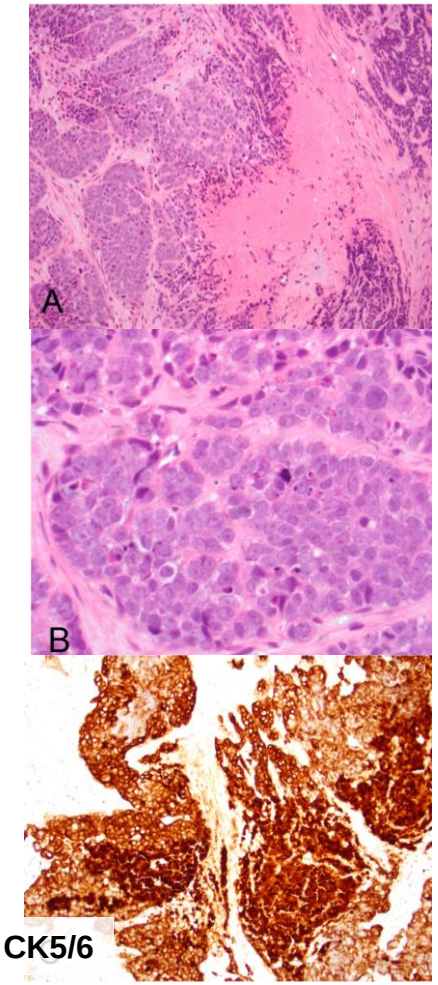


Diagnostic Molecular Pathology

- No established role for molecular testing in the diagnosis of SCLC
- The vast majority harbors biallelic alteration of both *TP53* and *RB1*
- p53 and Rb IHC can be used as surrogates – null or overexpression of p53 and loss of Rb expression are supportive of a small cell carcinoma diagnosis

Differential Diagnosis of SCLC

Smokers	
LCNEC	• Non-small cell cytomorphology: moderate-abundant cytoplasm and prominent nucleoli • A subset of LCNEC are weakly/focally Napsin A positive
Basaloid squamous cell carcinoma	• p40, p63, CK5/6+ • Caution: CD56 and synaptophysin (typically focal) can be positive
Thoracic SMARCA4-deficient undifferentiated tumor	• Loss of SMARCA4 (BRG1) and SMARCA2 (BRM) expression • TTF-1, keratins and claudin 4: -ve to weak • Caution: synaptophysin +ve in up to 70%, but other NE markers are usually -ve

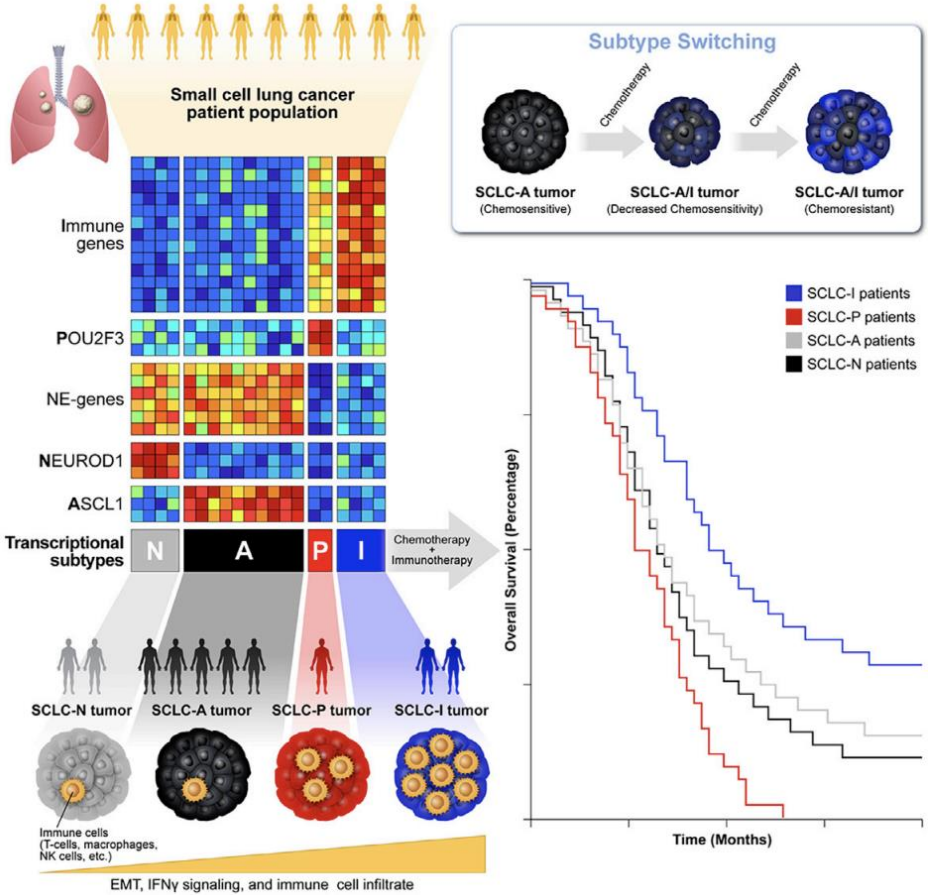


Basaloid SCC

Differential Diagnosis of SCLC

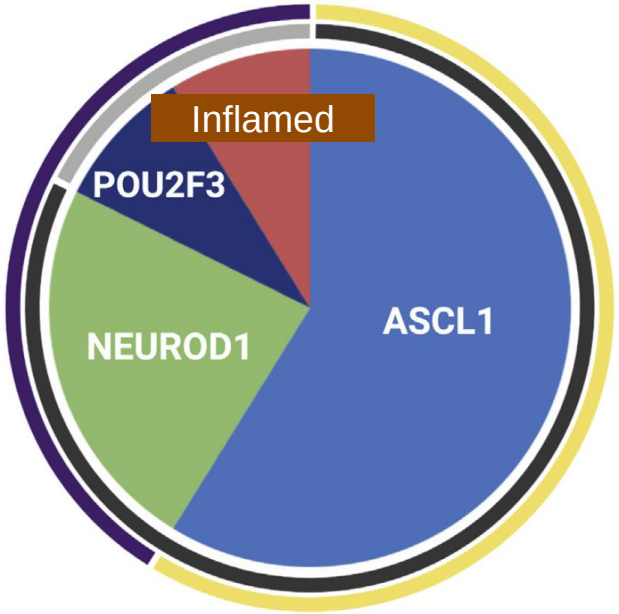
Non-smokers	
Lymphoma	• More discohesive morphology • Lymphoid markers + • Keratin -
(Crushed) carcinoid tumor	• Low Ki-67 • Rb and p53 IHC may be helpful
Merkel cell carcinoma	• History of skin lesion, albeit regression can rarely occur • CK20+, TTF-1-, Markel polyoma virus + (majority)
NUT carcinoma	• p63+ (p40+ in 2/3) • Abrupt keratinization in 1/3 (may be missing in small biopsy) • Caution: expression of NE markers can be seen
Small round cell sarcomas including Ewing and Ewing-like	• Clinical features (young age, significant involvement of chest wall, history of extrathoracic disease, etc.) • IHC: CD99+, NKX2.2+, keratin- for Ewing • NGS fusion assay or FISH to confirm the presence of translocation

Lineage-Defining Transcription Factor Subsets



Gay et al. Cancer Cell 2021

Implications of Transcription Factor Subtyping in SCLC



NE Character
- Neuroendocrine
- Non-Neuroendocrine*

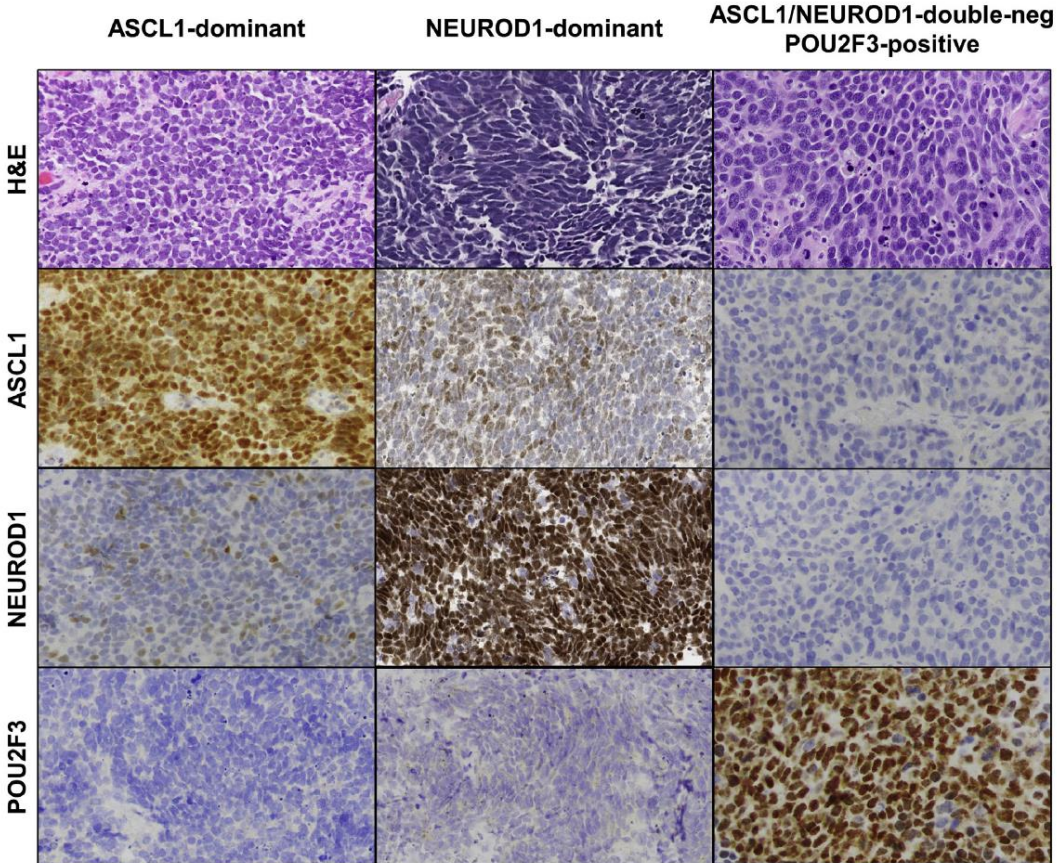
MYC Status
- MYC
- MYCL

*Negative or low expression of NE markers

	ASCL1	NEUROD1	POU2F3	Inflamed
Targeted Therapies	BCL2 CREBBP DLL3 LSD1	Arginine Deprivation AURKA/B CHK1 IMPDH LSD1	Arginine Deprivation AURKA/B CHK1 IGF-R1 IMPDH	Arginine Deprivation AURKA/B CHK1 IMPDH IO

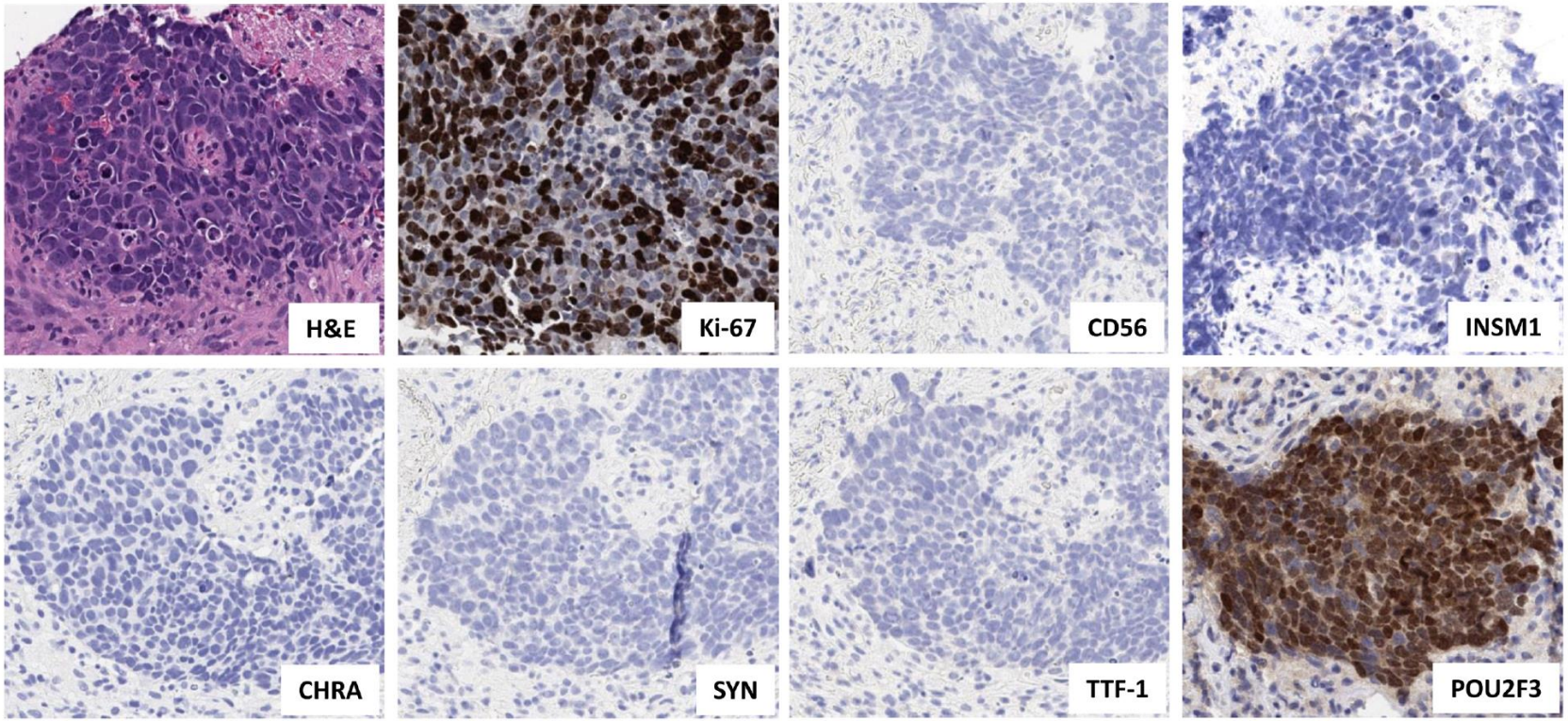
Poirier et al. J Thorac Oncol 2020

SCLC subtypes: NeuroD1, ASCL1, POU2F3, Null (Inflamed)



Baine et al. J Thorac Oncol 2020

POU2F3 (tuft cell marker) as Marker for NE-Low SCLC



Baine et al. JTO 2022

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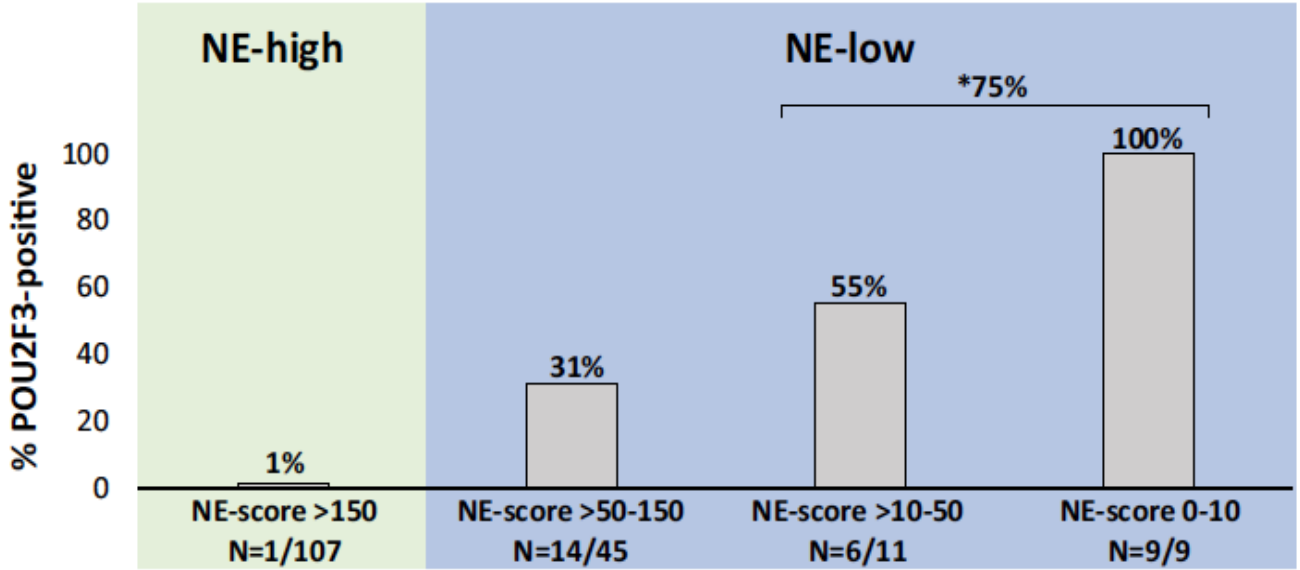
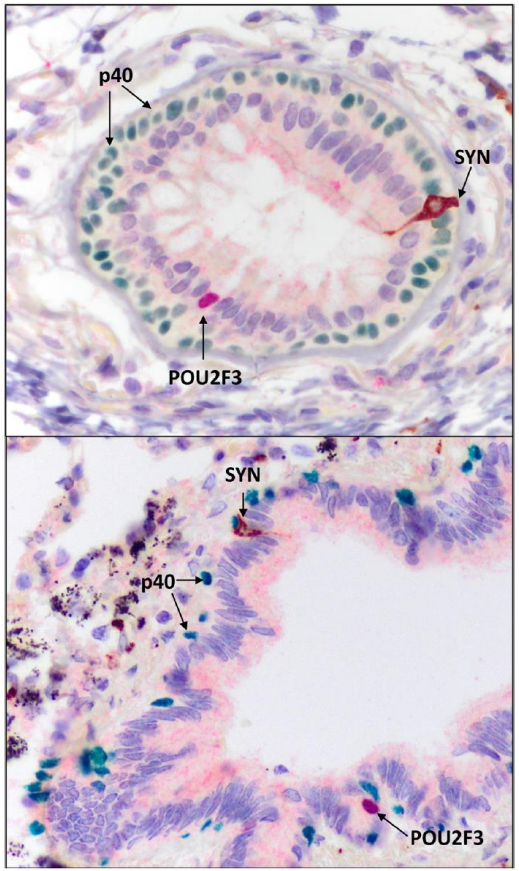


*NE-score: an average H-score of the 4 NE markers (synaptophysin, chromogranin, CD56 and INSM1)

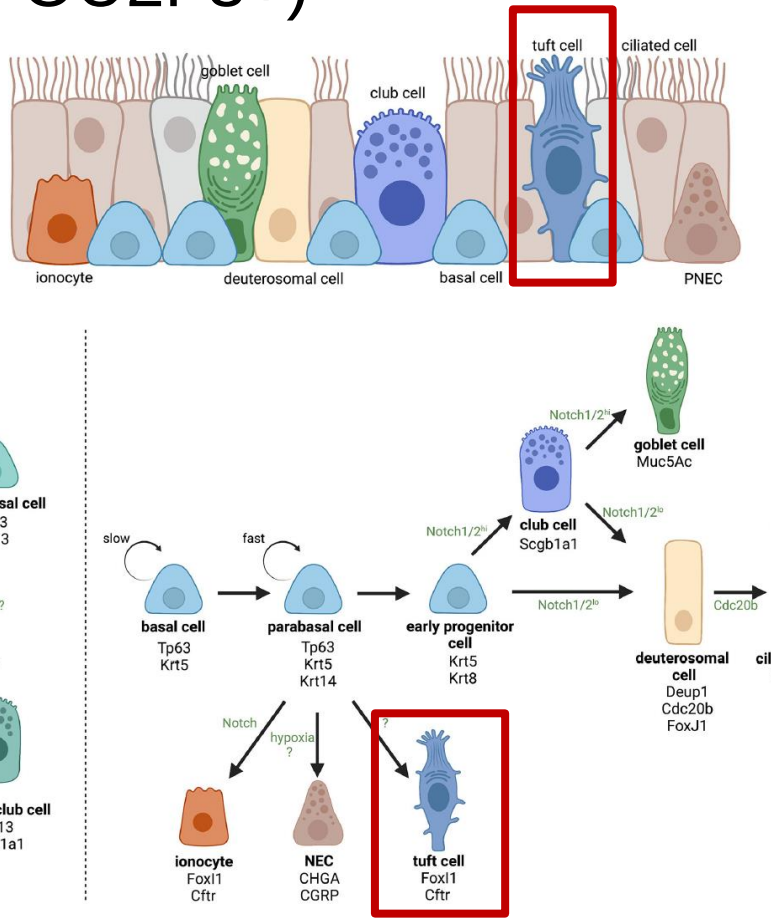
- The majority of NE-low SCLC (NE-score 0-50) express POU2F3
- POU2F3 may be a useful marker to support the diagnosis of SCLC when the expression of standard NE markers are negative or extremely low

Baine et al. JTO 2022

Tuft Cell (POU2F3+)



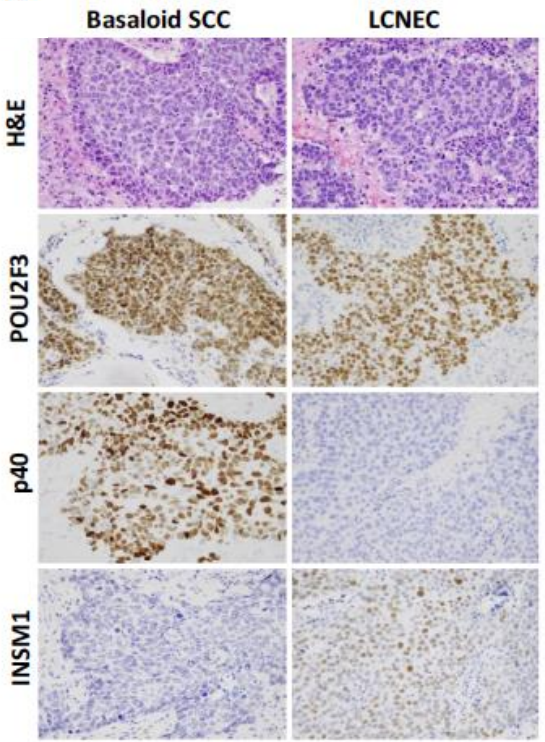
Baine et al. JTO 2022



Ruysseveldt et al. Front Allergy 2021

Of Somber Note

	Total tested	POU2F3+ N (%)
Lung tumors:		
Adenocarcinoma	100	0
SCC, NOS	63	0
SCC, basaloid	32	7 (22%)*
LCNEC	52	6 (12%)**
Carcinoids Typical Atypical	 136 31	 0 0
SMARCA4-UT	19	0
Other tumors that can mimic SCLC#:		
Merkel cell carcinoma	49	0
Melanoma	25	0
Lymphoma	29	0
Round cell sarcoma	20	0

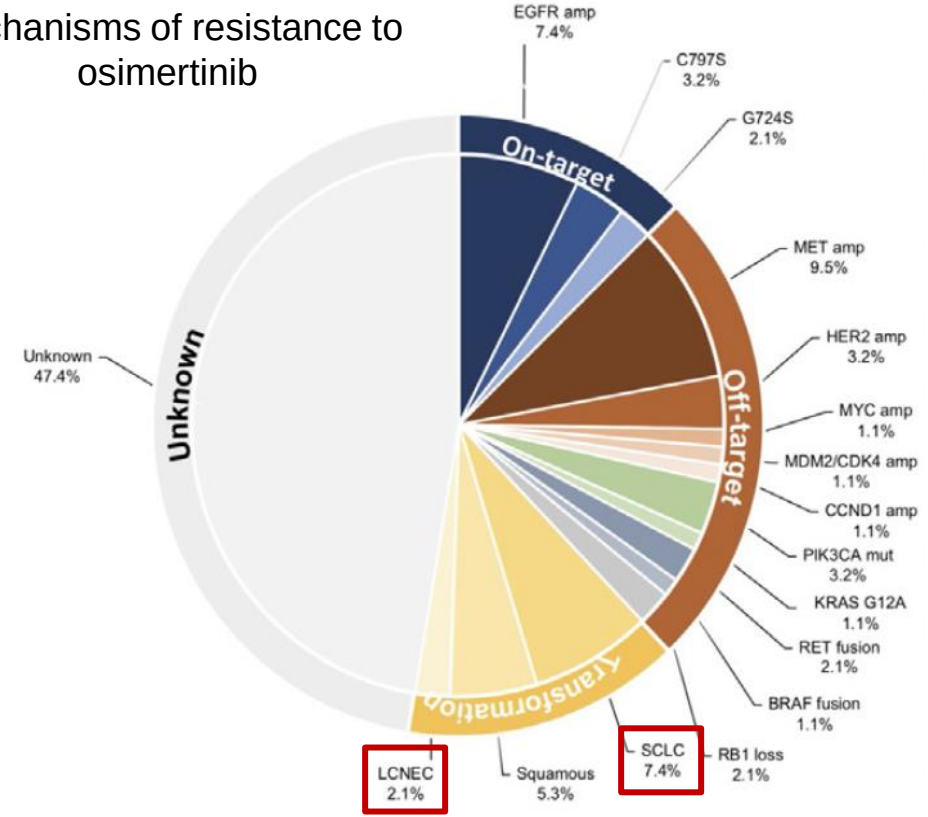


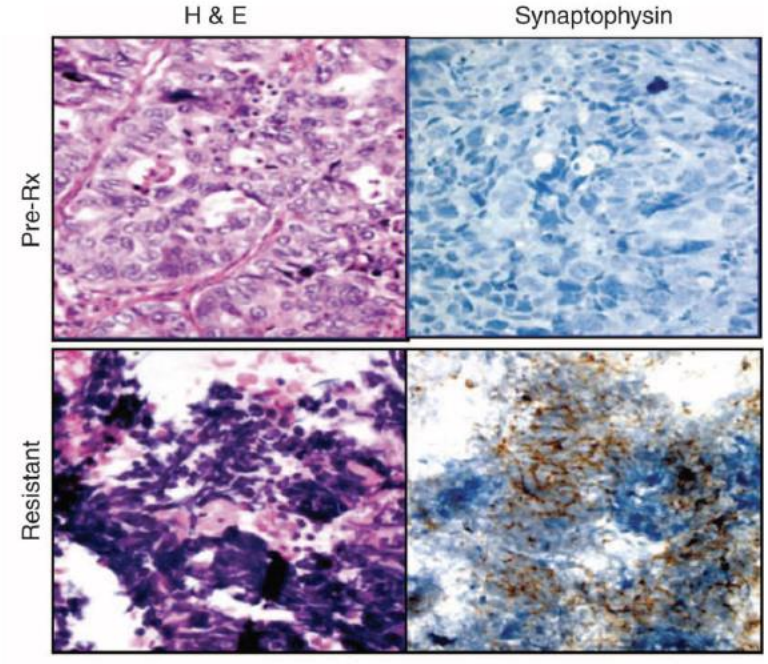
- A subset of SCLC mimickers (basaloid squamous cell carcinoma and LCNEC) express POU2F3
- p40 IHC and through morphologic assessment are important to differentiate NE-low SCLC from those

Baine et al. JTO 2022

Small Cell Transformation after EGFR TKI Therapy

Mechanisms of resistance to osimertinib





Choudhury NJ, et al. J Thorac Oncol 2023;18:463-75
Sequist LV, et al. Sci Transl Med 2011;3:75ra26

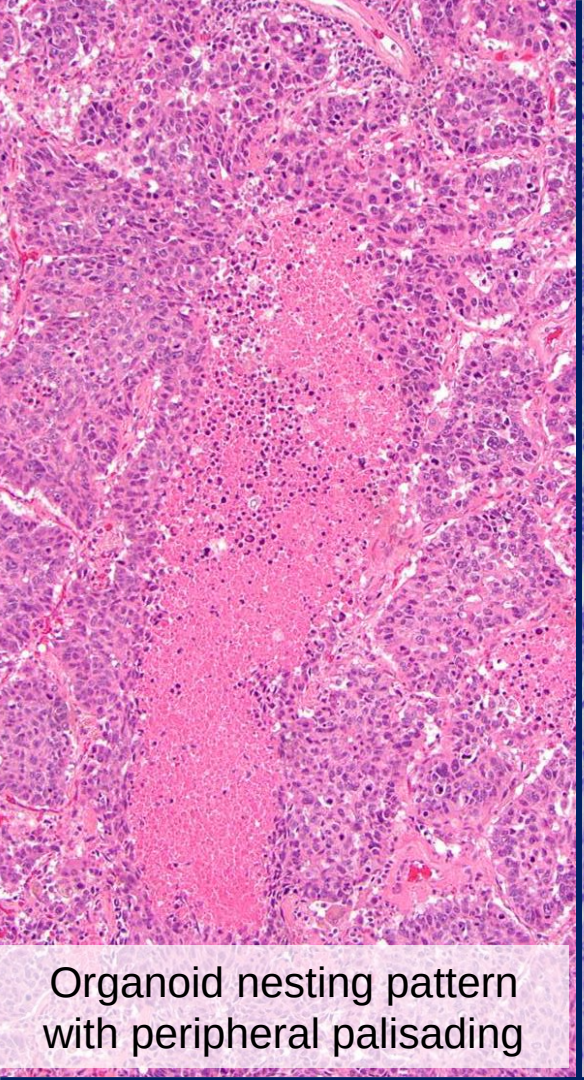
WHO 2021 Criteria for LCNEC of the Lung

Essential:

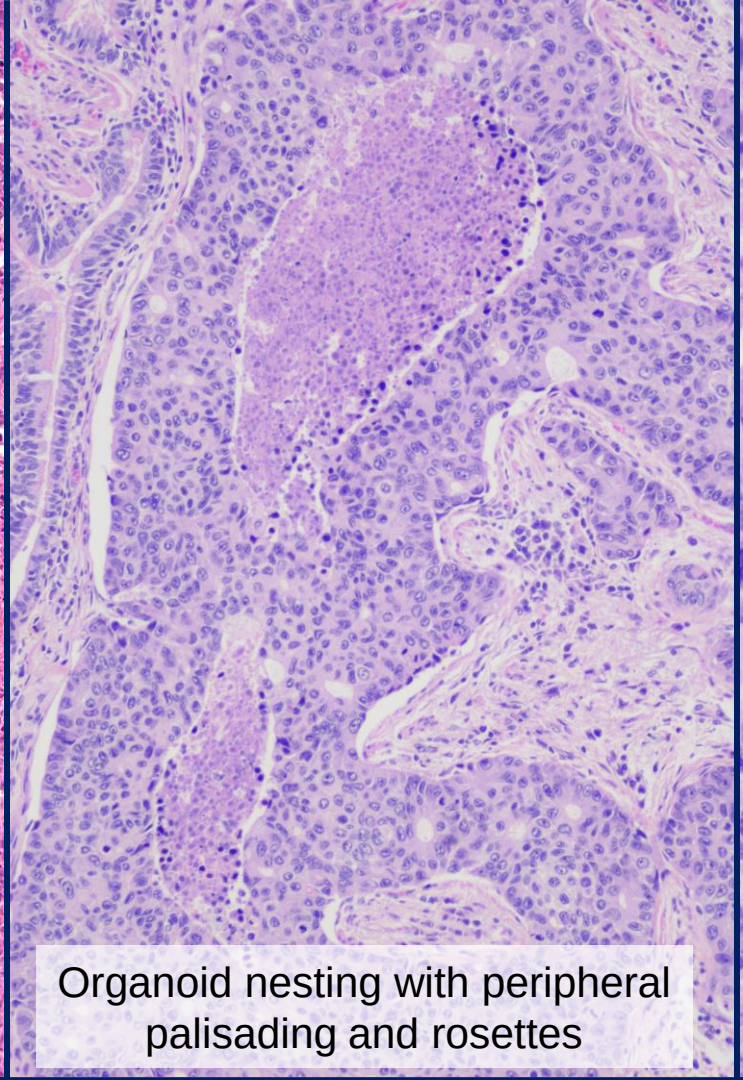
- NE morphology: organoid nesting, trabeculae, peripheral palisading, rosettes
- Non-small cell cytology: prominent nucleoli and/or moderate to abundant cytoplasm, larger cell size than SCLC (> 3 lymphocytes); chromatin may be either granular/stippled or vesicular
- High proliferation rate: > 10 mitoses/2 mm^2 (median: 70 mitoses/2 mm^2)
- One or more neuroendocrine marker (other than NSE) expressions

Desirable:

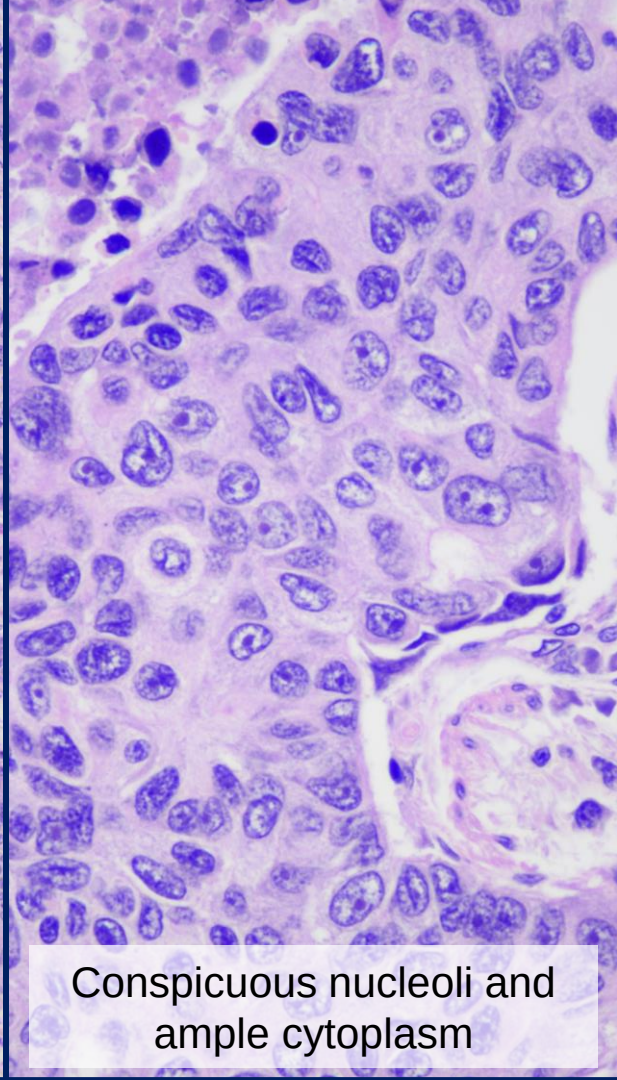
- Necrosis: generally extensive but may be limited to the center of tumor nests
- High Ki-67 index: > 30%, generally 40–80%
- Negative p40 immunohistochemistry



Organoid nesting pattern with peripheral palisading



Organoid nesting with peripheral palisading and rosettes



Conspicuous nucleoli and ample cytoplasm

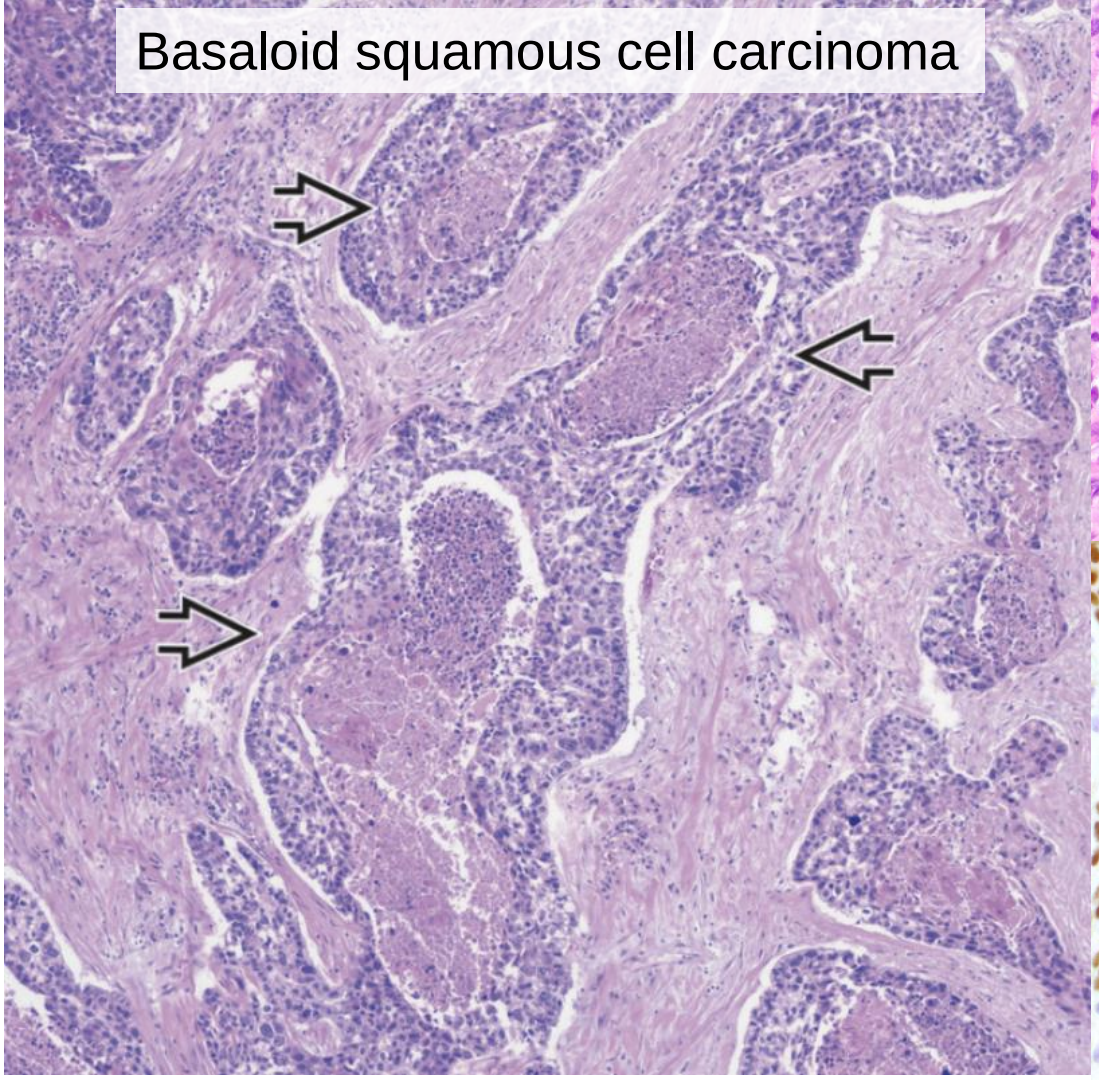
Diagnosis of LCNEC in Small Biopsy

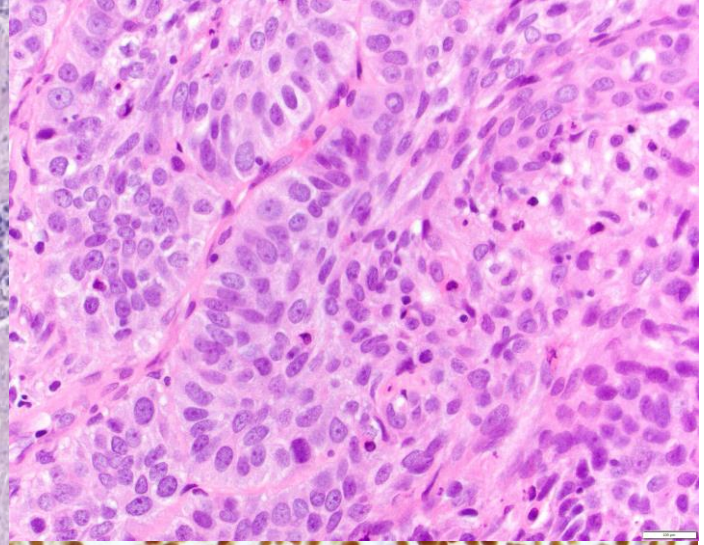
- Historically, the diagnosis of LCNEC was considered rarely feasible in small biopsies
- The definitive diagnosis of LCNEC is possible with biopsy size sufficient to assess NE morphology and IHC markers
- The diagnosis has become possible more frequently due to the recent trend of obtaining larger-size thoracic biopsies for molecular testing
- If NE morphology and/or marker expression is not definitive in scant or disrupted samples of NSCLC, the diagnosis of “NSCLC, possible LCNEC” is appropriate

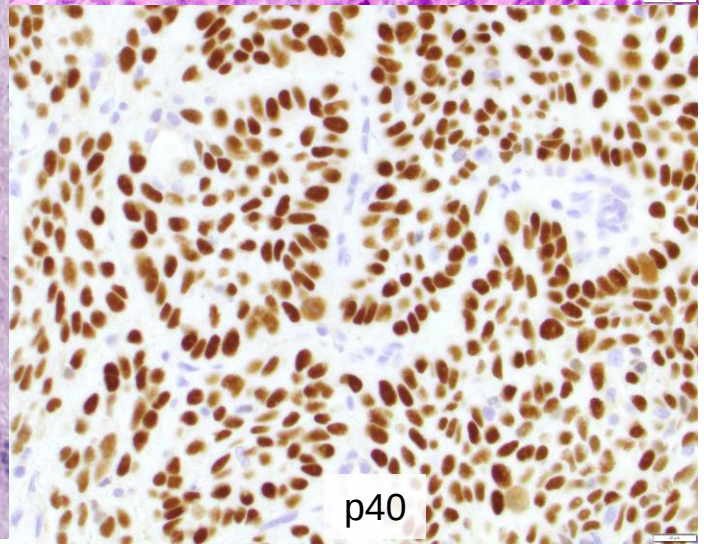
Differential Diagnosis of LCNEC

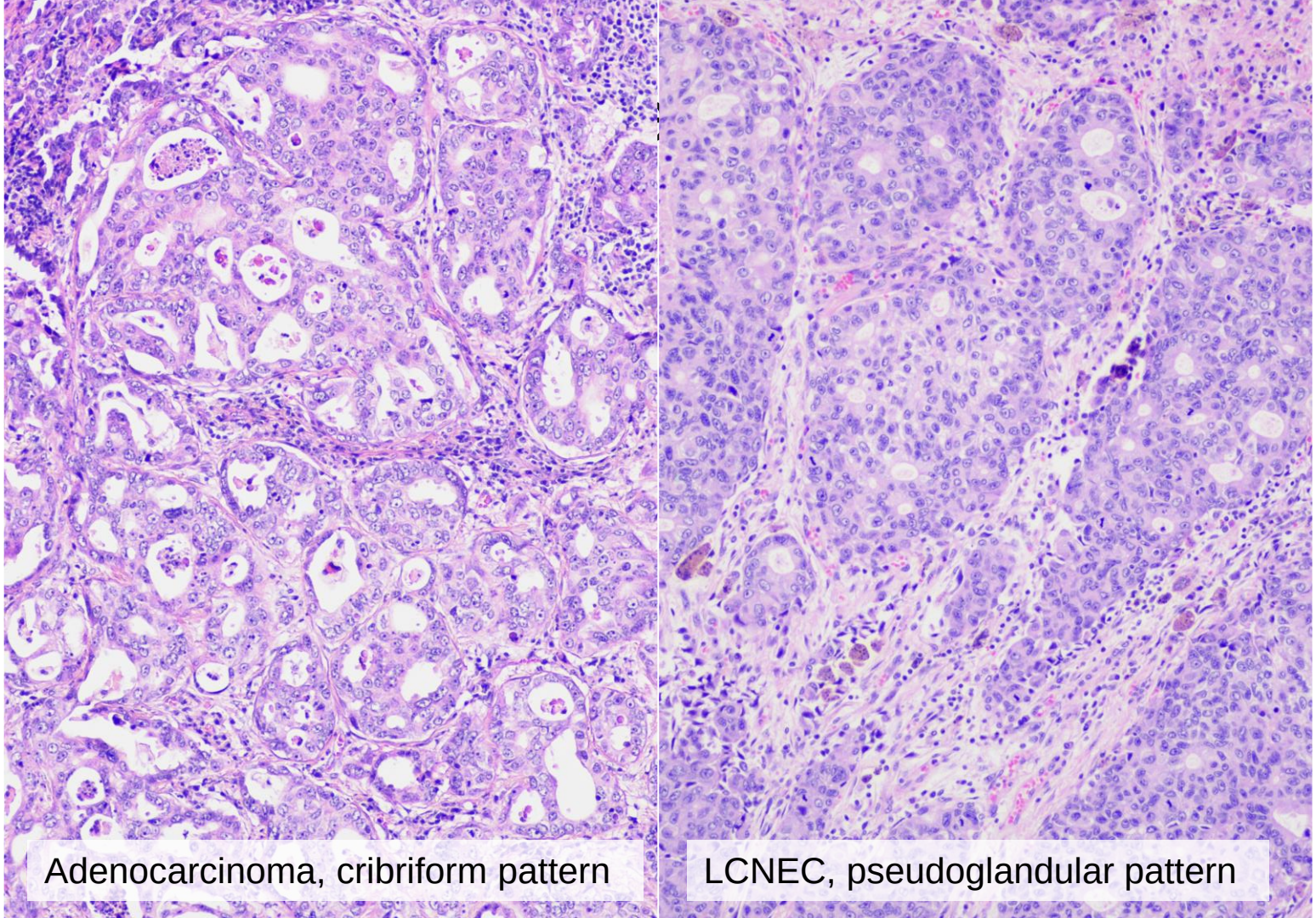
- Small cell carcinoma
- Basaloid squamous cell carcinoma
- Adenocarcinoma with a solid/nested or cribriform pattern
- Large cell carcinoma with a solid/nested pattern
- Atypical carcinoid
- Thoracic SMARCA4-deficient undifferentiated tumor

Basaloid squamous cell carcinoma





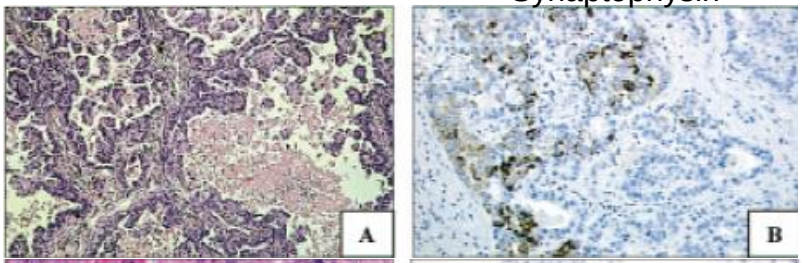



Adenocarcinoma, cribriform pattern
LCNEC, pseudoglandular pattern

LCNEC vs. NSCLC w/ NE morphology or differentiation

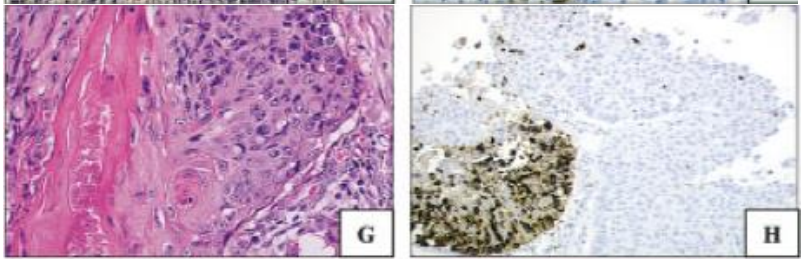
Synaptophysin

ADC (Stage I)



15/88 (17%)

SqCC (Stage I)



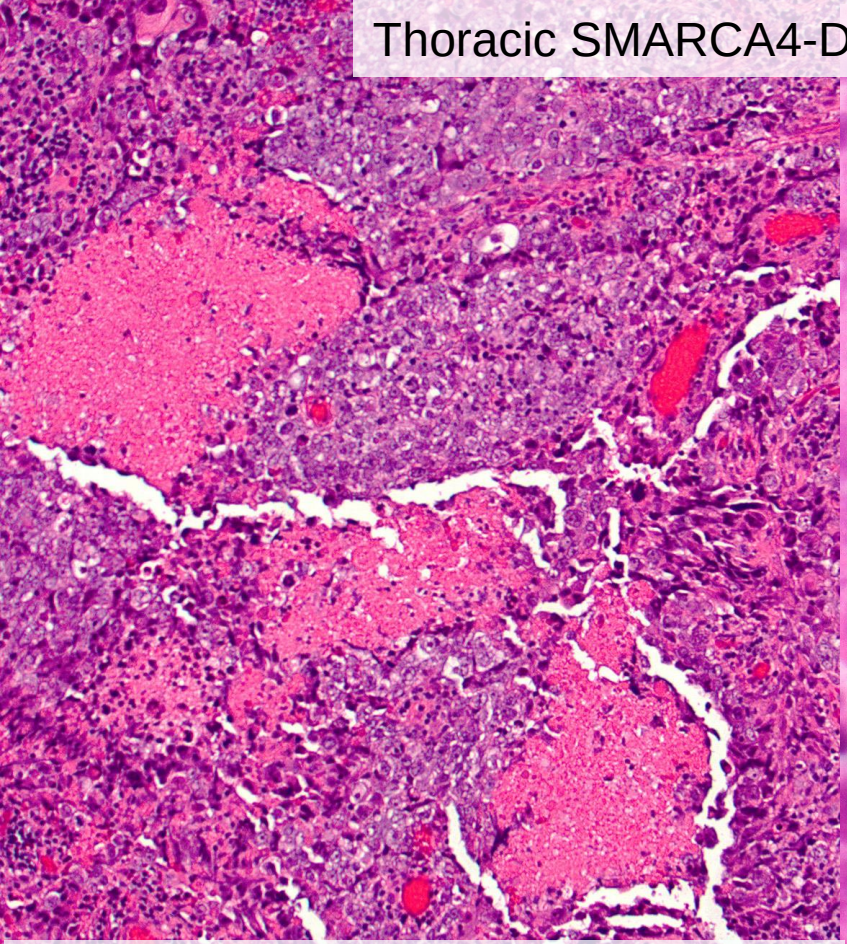
13/113 (11.5%)

*NE Morphology
- Organoid nesting trabecular pattern
- Palisading
- Rosettes

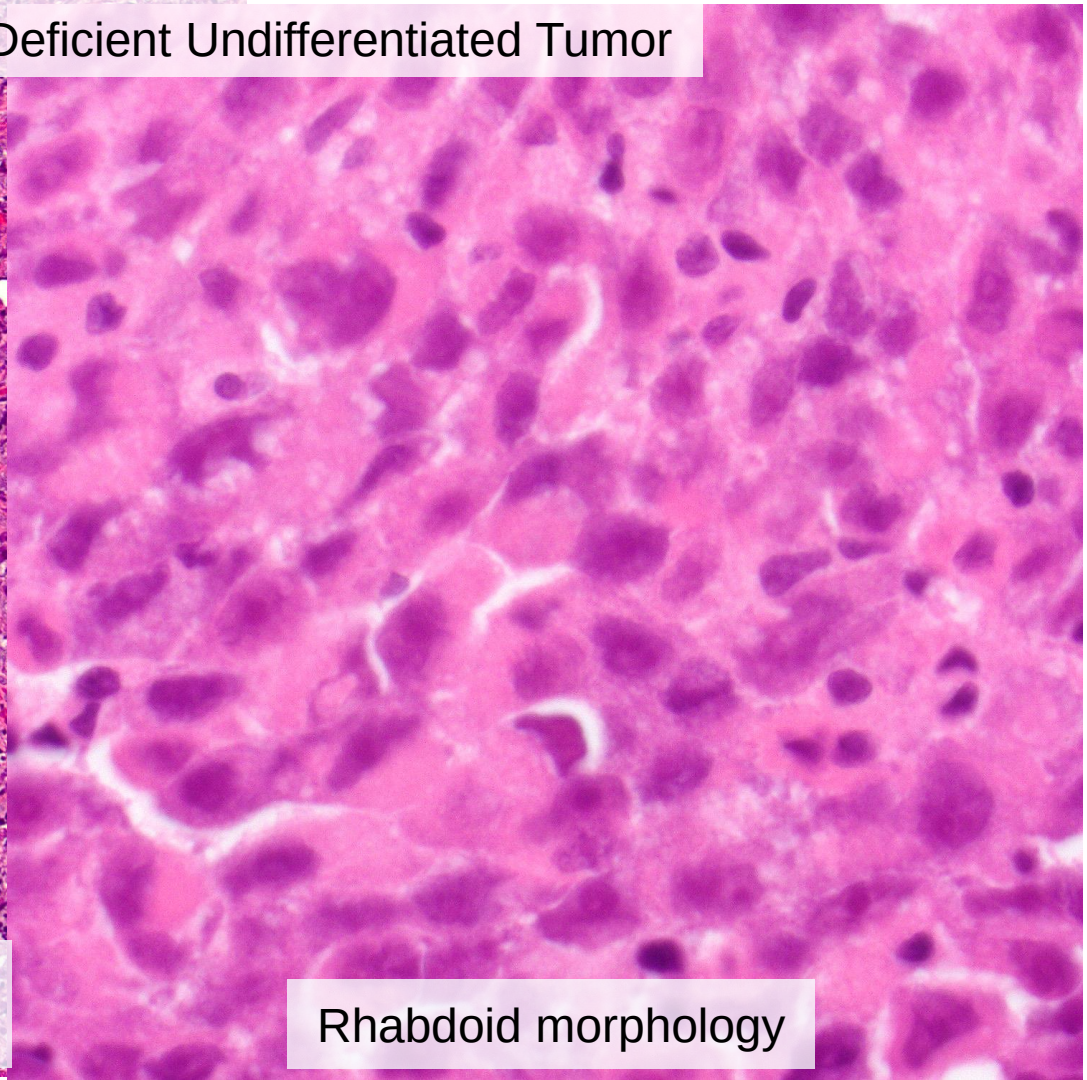
	LCNEC	NSCLC w/ NE morphology*	NSCLC w/ NE differentiation by IHC	NSCLC
NE morphology	+	+	-	-
NE marker expression	+	-	+	-
Clinical features	Aggressive	Aggressive (limited data)	Similar to conventional NSCLC	Better than LCNEC

Pelosi, Cancer 2003; Ionescu, Am J Surg Pathol 2007; Derks, Histopathology 2019; Baine, Am J Clin Pathol 2019

Thoracic SMARCA4-Deficient Undifferentiated Tumor

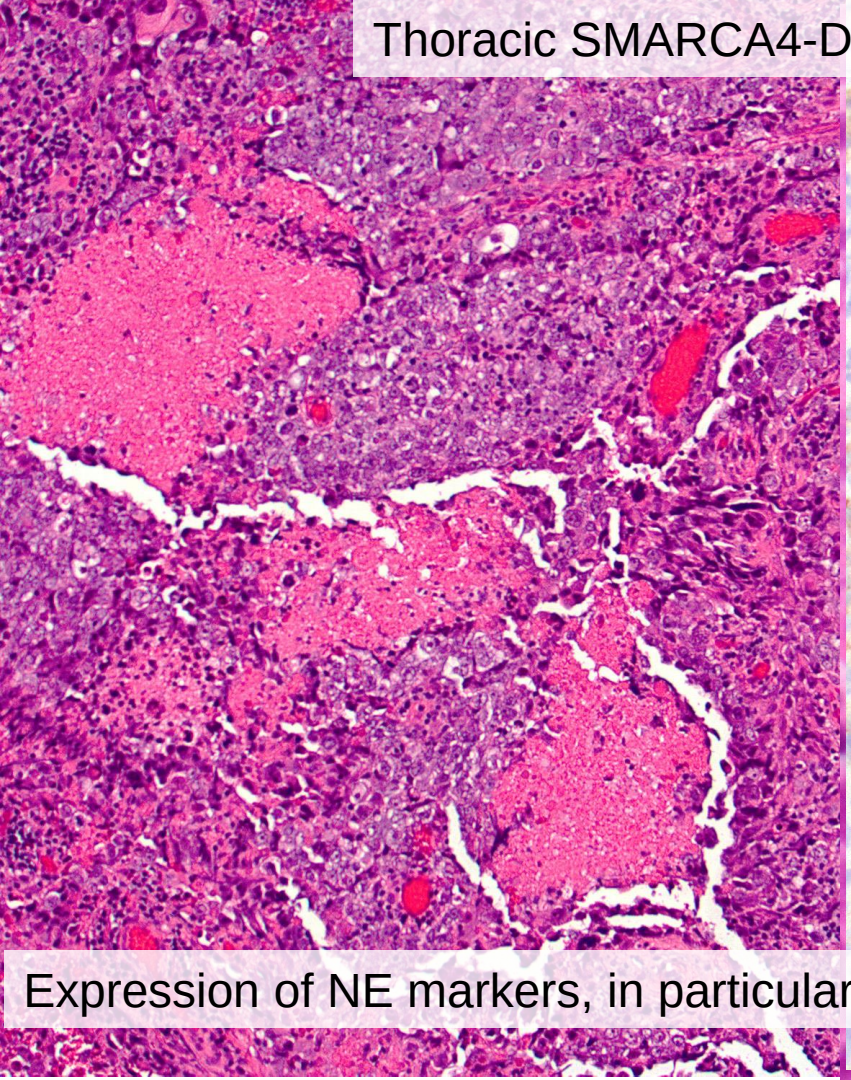


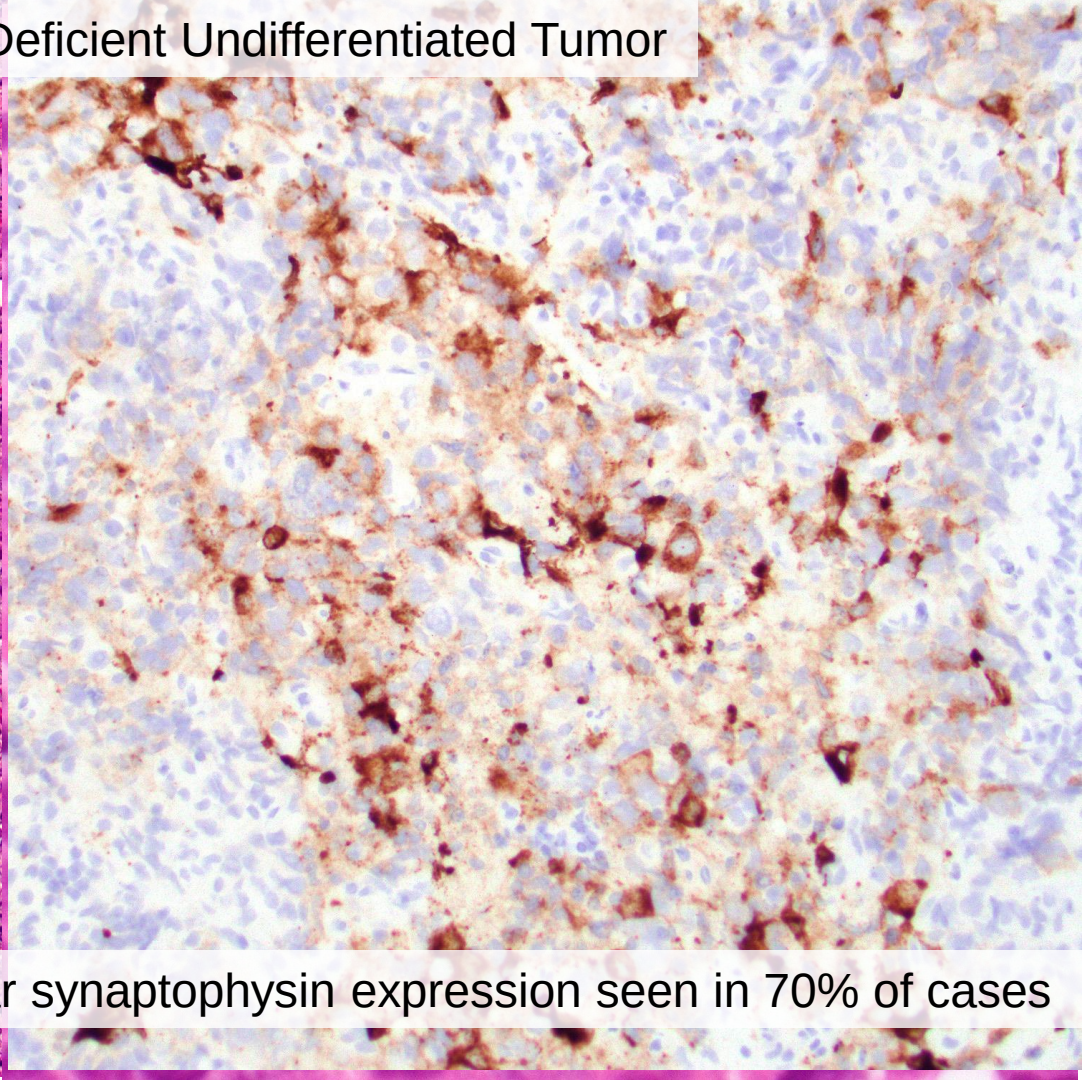
Sheet of undifferentiated round tumor cells with large areas of necrosis



Rhabdoid morphology

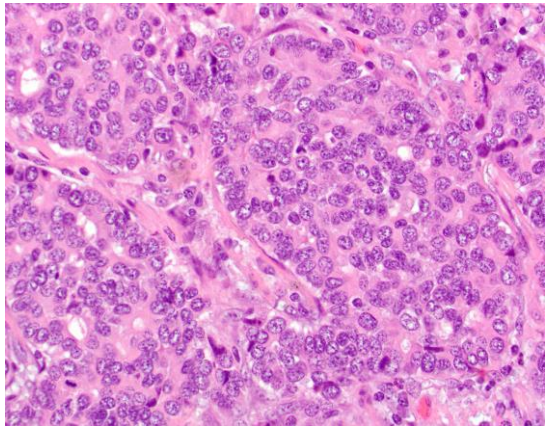
Thoracic SMARCA4-Deficient Undifferentiated Tumor



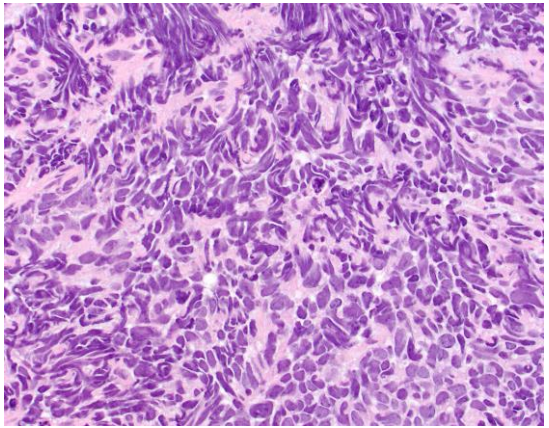


Expression of NE markers, in particular synaptophysin expression seen in 70% of cases

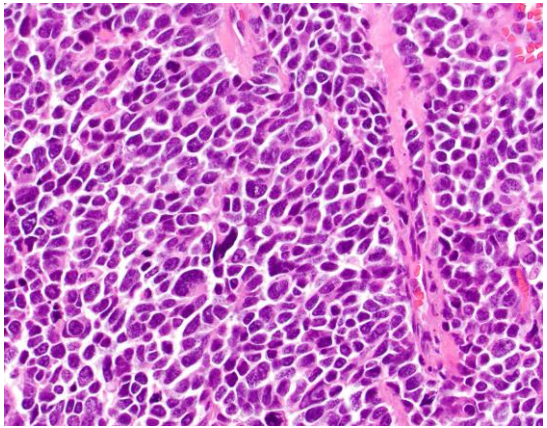
LCNEC vs. Small Cell Carcinoma (SCLC)



LCNEC



SCLC

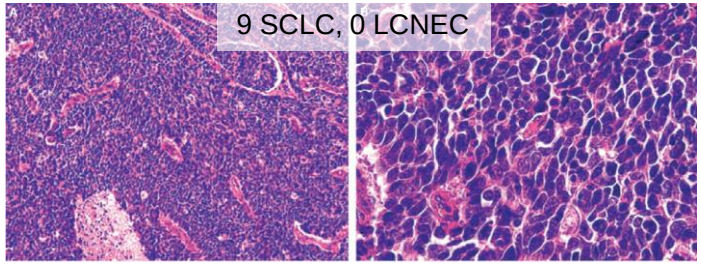


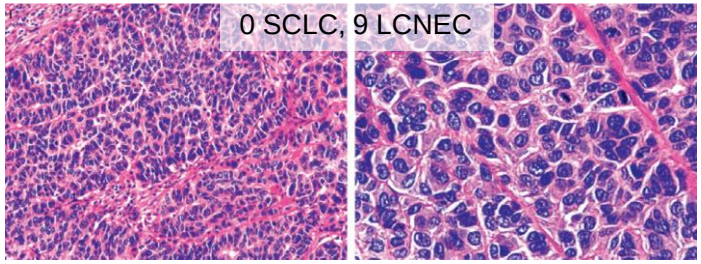
SCLC in resection

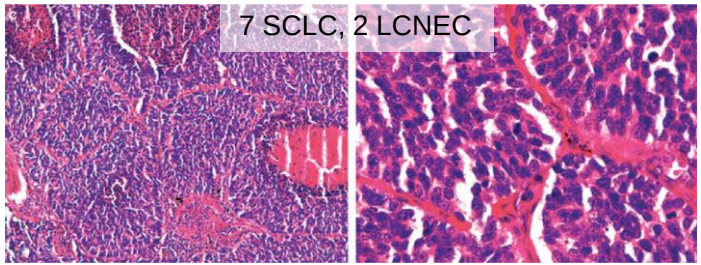
- LCNEC is distinguished from SCLC based on:
  - Presence of prominent nucleoli and/or abundant cytoplasm
  - Large cell size in most case
- Nested architecture or larger cell size alone should not be used as the sole criterion for the diagnosis of LCNEC over SCLC

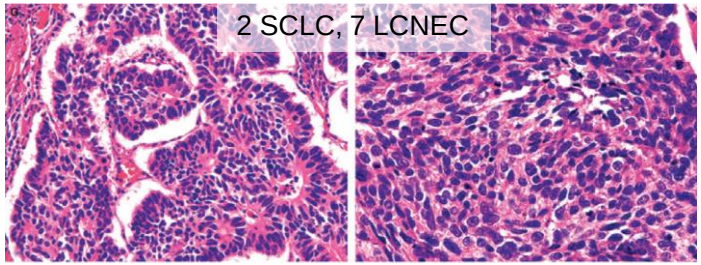
SCLC vs. LCNEC : Interobserver concordance

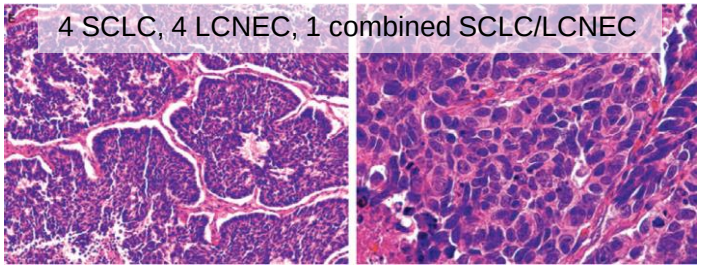
9 observers; 170 cases - overall κ value 0.40







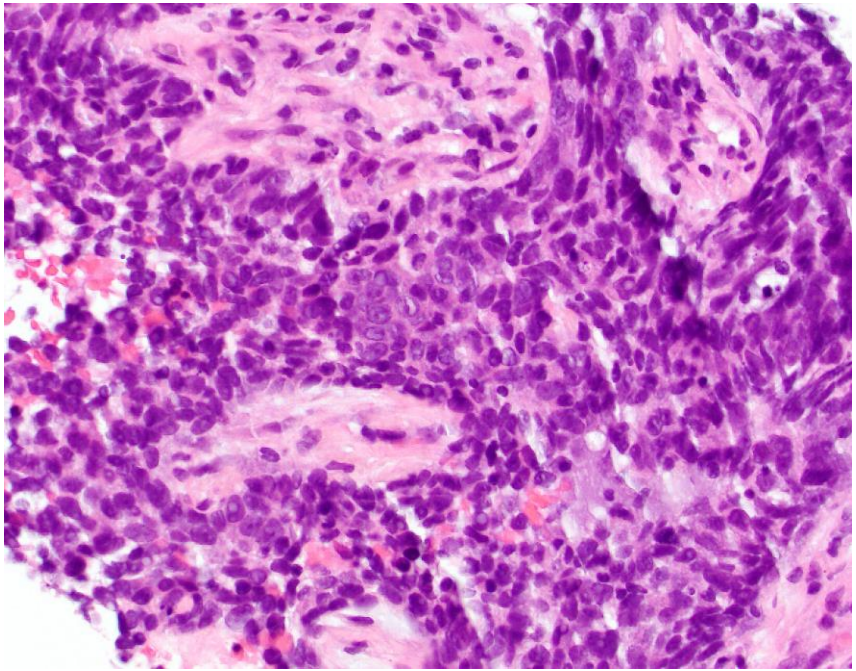




- Neuroendocrine growth patterns can be seen in SCLC
- Tumors with large cell size but otherwise classic SCLC cytology should be classified as SCLC

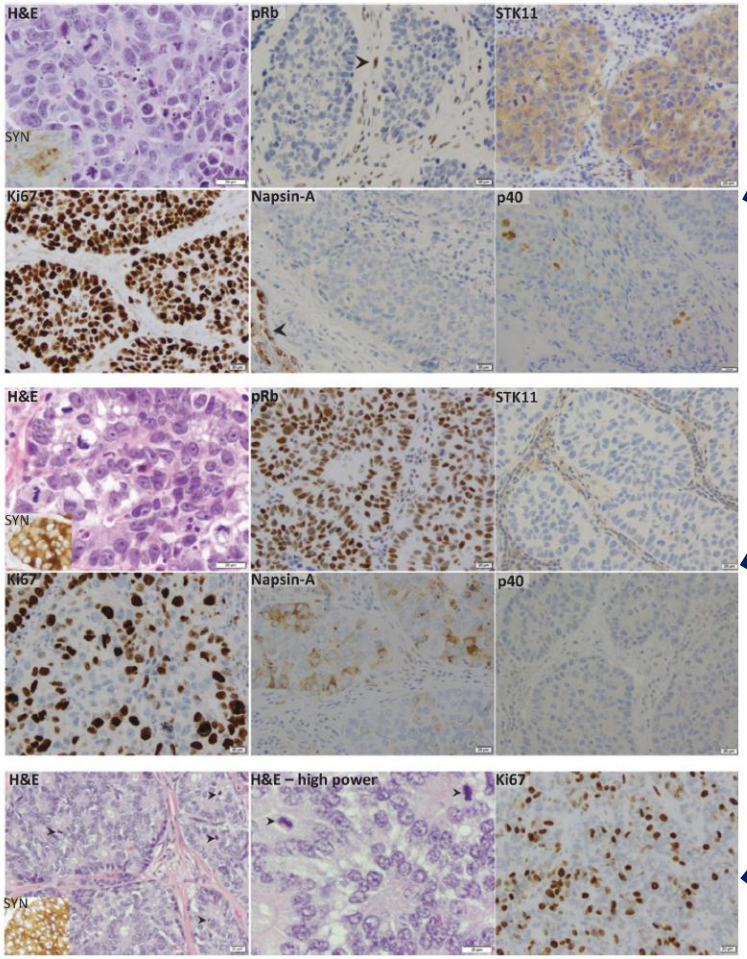
den Bakker MA, et al. Histopathology 2010;56:356-63

Some tumors are difficult to classify as LCNEC vs. SCLC, especially in biopsies



- High-grade NE carcinoma, NOS with a long note?
- Suggest the possibility of combined small cell and large cell NE carcinoma?
  - LCENC should make up ≥ 10% of tumor cells for the combined diagnosis
  - Any amount of SCLC in a predominant LCNEC qualifies the combined diagnosis

Molecular-based LCNEC subsets



SCLC-like molecular type LCNEC

- concurrent inactivating mutations in *TP53* and loss of *RB1* (typically seen in SCLC)

NSCLC-like molecular type LCNEC

- lack of concurrent*TP53* and *RB1* alterations
- nearly universal occurrence of NSCLC-type mutations (*SKT11, KRAS,* and/or *KEAP1*)

Carcinoid-like molecular type LCNEC

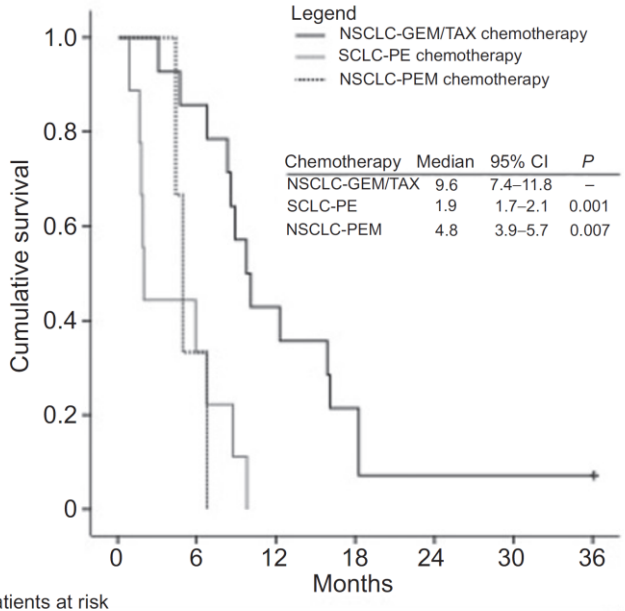
- well-differentiated morphology + increased mitotic activity
- *MEN1* mutations and low tumor mutation burden
- Formal diagnosis “to be determined”

Rekhtman N, et al. Clin Cancer Res 2016;56:3618-29

Rb Status as Predictive Marker in LCNEC

Netherlands Cancer Registry: 148 pt with stage IV LCNEC (79 with NGS, 109 with IHC)

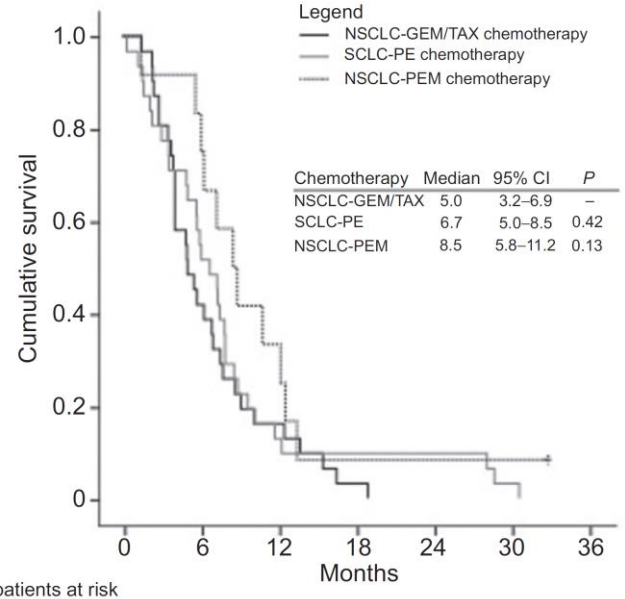
LCNEC with RB1 H-score ≥ 50



Chemotherapy	Median	95% CI	P
NSCLC-GEM/TAX	9.6	7.4–11.8	–
SCLC-PE	1.9	1.7–2.1	0.001
NSCLC-PEM	4.8	3.9–5.7	0.007

No. of patients at risk							
NSCLC-GEM/TAX	14	12	6	3	1	1	1
SCLC-PE	9	3	0	0	0	0	0
NSCLC-PEM	3	2	0	0	0	0	0

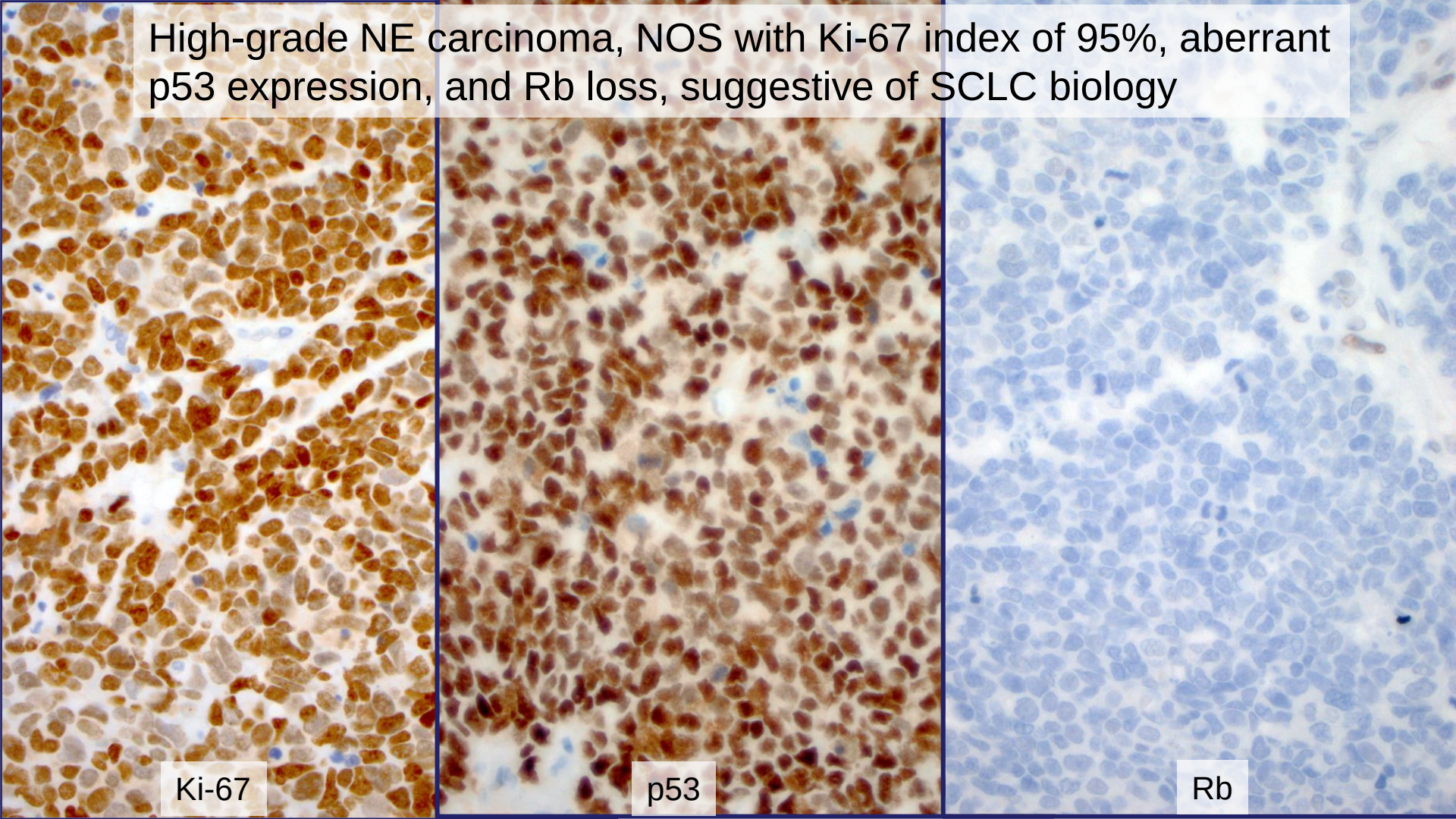
LCNEC with RBI H-score < 50



Chemotherapy	Median	95% CI	P
NSCLC-GEM/TAX	5.0	3.2–6.9	–
SCLC-PE	6.7	5.0–8.5	0.42
NSCLC-PEM	8.5	5.8–11.2	0.13

No. of patients at risk							
NSCLC-GEM/TAX	31	13	5	1	0	0	0
SCLC-PE	31	17	4	3	3	1	0
NSCLC-PEM	12	10	4	1	1	1	0

Derks et al. Clin Cancer Res 2017


High-grade NE carcinoma, NOS with Ki-67 index of 95%, aberrant p53 expression, and Rb loss, suggestive of SCLC biology
Ki-67
p53
Rb

Summary

- Carcinoids
  - Carcinoid terminology retained
  - Carcinoid tumor, NOS has been introduced
  - Established and emerging roles of Ki-67
  - Carcinoid tumors with increased proliferation vs. LCNEC –> stay tuned for updated terminology (equivalent to G3 in GI-pancreas NE tumor)

Summary

- SCLC
  - Role of IHC for diagnosis with POU2F3 as an emerging marker
  - Emerging molecular subtypes of SCLC with possible therapeutic implications
  - NE transformation post TKI therapy
- LCNEC
  - Differential diagnosis of LCNEC vs. NSCLC with morphologic or immunohistochemical NE feature
  - Diagnosis of LCNEC on biopsy
  - Molecular subtypes of LCENC with possible therapeutic implications


Thank you!

Rain forest in Costa Rica


Thank you!