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**Chaehwa Kim,**  
 Clinical Instructor and Thoracic Pathology Fellow  
 &  
**Jeffrey Myers,**  
 A. James French Professor of Diagnostic Pathology  
  
 Department of Pathology  
 Michigan Medicine, Ann Arbor, MI

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**Discussion Articles**

**Yu et al. An algorithmic approach utilizing CK7, TTF1, beta-catenin, CDX2, and SSTR2A can help differentiate between gastrointestinal and pulmonary neuroendocrine carcinomas. *Virchows Arch.* 2021;479:481-491.**

**Purpose:** To identify a panel of IHC stains useful in distinguishing pulmonary from GI neuroendocrine carcinomas (NECs) (*ie*, small cell carcinoma [SCC], large cell neuroendocrine carcinoma [LCNEC]).

**Methods:**

- test set – 2 TMAs
  - GI-NEC (13)
    - one 3-mm core (9); two 3-mm cores (4)
  - P-NEC (20)
    - one 3-mm core (19); two 3-mm cores (1)
- 21 IHC stains
  - 18 (AE1/3, CK7, CK20, SYN, CRG, CD56, INSM1, SSTR2A, CDX2, SATB2, TTF1, NAP, PR, GATA3, PAX8, ISL1,  $\beta$ -CAT, AFP) assessed using H-score: 3\*(% 3+) + 2\*(%2+) + 1\*(% 1+)
    - <50 = Negative;  $\geq$  50 = Positive
  - SMAD4 and Rb – complete loss of expression; P53 – mutant pattern (strong and diffuse/null)
- validation set – whole tissue sections of metastatic NEC (combined results with TMA for final statistical analysis)
  - GI-NEC (10); P-NEC (10)

**Results:**

- 7 stains showed statistically significant differences in mean H-scores; SCC  $\approx$  LCNEC

IHC stain	GI-NEC (13)	P-NEC (20)	<i>p</i> -value	Met GI-NEC (10)	Met-PI NEC (10)	<i>p</i> -value	All GI-NEC (23)	All P-NEC (30)	<i>p</i> -value
AE1/3	149	230	0.011						
CK7	30	209	<0.0001	108	200	0.12	64	206	<0.0001
SSTR2A	123	14	0.0021	72	16	0.079	101	14	0.0005
CDX2	43	0	0.33	5	0	0.33	27	0	0.022
SATB2	106	35	0.018						
TTF1	1	177	<0.0001	13	177	0.0002	6	177	<0.0001
$\beta$ -CAT	106	23	0.029	94	0	0.019	101	15	0.0018

- tested 5 markers in 3-step algorithm on validation set (correctly localized 17 of 20; 47 of 53 total cases).

								SENS	SPEC
CK7 + TTF1	→ POS/POS	→ P-NEC						67%	96%
	→ $\geq$ 1 NEG	→ $\beta$ -CAT + CDX2		→ $\geq$ 1 POS	→ GI-NEC			54%	90%
				→ NEG/NEG	→ SSTR2A	→ POS	GI-NEC	56%	100%
						→ NEG	P-NEC	NA	NA

**Take-home message:** Distinguishing P-NEC from G-NEC increasingly important and may be challenging in patients with advanced stage, multi-site disease. A simple algorithm with a small panel of antibodies will do the trick in most (but not all) circumstances.

**Wang et al. Expression of insulinoma-associated protein 1 in non-small cell lung cancers: a diagnostic pitfall for neuroendocrine tumors. *Hum Pathol.* 2021;115:104-111.**

**Purpose:** To assess the frequency of INSM1 staining in non-small cell non-neuroendocrine lung carcinomas (NSCNELC) by histologic subtype.

**Methods:**

- 324 case TMA (196 AdCs, 86 SqCCs, 42 “other” NSCNELCs)
- stained for INSM1, SYN, p40, CK5/6, and TTF1
- focal/positive INSM1 staining repeated on available surgical cases from 8 “focal” and 11 “positive” cases, and 8 randomly selected negative cases
- whole sections from 38 cases of SqCC with basaloid features
- INSM1 staining “focal” (< 10% tumor cells) or “positive” (10-50% “patchy”; >50% “diffuse”)

**Results:**

- Frequency of INSM1 expression (patchy, diffuse, focal) in 9-34% of NSCNELC

<b>TMA Cohort (324)</b>			
	<b>Positive</b>	<b>Focal</b>	<b>Negative</b>
<b>AdC (n = 196)</b>	10 (5.1%)	9 (4.7%)	177 (90.3%)
<b>SqCC (n = 86)</b>	8 (9.3%)	5 (5.8%)	73 (84.9%)
w basaloid (19)	3 (15.8%)	1 (5.3%)	15 (78.9%)
w/out basaloid (67)	5 (7.5%)	4 (6.0%)	58 (86.5%)
<b>Other (42)</b>	2 (4.8%)	2 (4.8%)	38 (90.4%)
<b>Whole Tissue Section SqCC w Basaloid Features Cohort (38)</b>			
<b>Biopsy (13)</b>	4 (30.8%)	2 (15.4%)	7 (53.8%)
<b>Resections (25)</b>	2 (8%)	5 (20%)	18 (72%)
<b>Total (38)</b>	6 (15.8%)	7 (18.4%)	25 (65.8%)

- Differences between AdC and SqCC, and SqCC with and without basaloid features, were not statistically significant
- Staining more frequent in poorly differentiated compared to moderately and well differentiated tumors.
- 1 “focal” positive (16) and 0 “positive” (20) AdCs, SqCCs, and Other was focally positive for SYN; 0 (of 38) INSM1 “positive” (6), “focal” positive (7), and “negative” (25) whole tissue sections of SqCC w/w’out basaloid features stained for SYN
- Surgical case “validation”: 8/8 negative cases were negative; 8/8 focal cases were focal; 6/11 positive cases remained positive and 5/11 were focal.

**Take-home message:** INSM1 is of limited (no?) value as a marker of neuroendocrine differentiation.

**Hiroshima et al. Membranous HEG1 expression is a useful marker in the differential diagnosis of epithelioid and biphasic malignant mesothelioma versus carcinomas. *Pathol Int.* 2021;71(9):604-613.**

**Purpose:** To evaluate the clinical applicability of HEG1 as a marker in the diagnosis of mesothelioma.

**Methods:**

- HEG1 immunoreactivity was evaluated in whole sections and tissue microarrays
- Whole Sections
  - 122 malignant mesotheliomas (57 epithelioid, 32 biphasic, 25 sarcomatoid, 8 other variants)
  - 1 well-differentiated papillary mesothelioma
  - 75 pulmonary carcinomas
  - 55 other carcinomas and 16 mesenchymal tumors
  - 24 reactive mesothelial proliferations
- Tissue microarrays (TMAs); 108 mesotheliomas (70 epithelioid, 36 biphasic, 2 sarcomatoid)
- IHC of monoclonal anti-sialylated HEG1 antibody with conventional mesothelial markers (calretinin, WT1, and podoplanin)
- Staining evaluation by scoring system (total score  $\geq 3$  of intensity 0-3 and extent 0-3) and staining pattern (membranous or cytoplasmic)

**Results:**

- HEG1 expression is higher than WT1 and podoplanin in epithelioid and biphasic mesothelioma in TMAs
- HEG1 showed membranous staining in 88.8% (79/89) of epithelioid components of epithelioid/biphasic mesothelioma. 80.0% (20/25) of sarcomatoid mesothelioma and 66.7% (2/3) of desmoplastic mesothelioma showed cytoplasmic staining of HEG1 without membranous staining. One well-differentiated papillary mesothelioma showed strong apical staining of HEG1.
- HEG1 was not expressed in any of pulmonary adenocarcinoma, mucoepidermoid carcinoma, or large cell neuroendocrine carcinomas. HEG1 was focally expressed in 21.7% (5/23) of pulmonary squamous cell carcinomas only with weak to moderate cytoplasmic staining. 66.7% (6/9) of ovarian serous carcinoma and all (3/3) thyroid carcinomas showed membranous HEG1 staining. Overall, membranous HEG1 staining had a sensitivity of 88.8% and a specificity of 92.3% in distinguishing epithelioid/biphasic mesotheliomas from carcinomas.
- 3 of 16 (18.8%) mesenchymal tumors studied showed membranous HEG1 staining. Membranous HEG1 had a low sensitivity in distinguishing sarcomatoid mesothelioma from mesenchymal tumors. Reactive mesothelial cells showed strong and diffuse apical HEG1 staining in all cases (3/3).

**Take-home message:** HEG1 staining in most epithelioid mesotheliomas and epithelioid component of biphasic mesotheliomas is membranous, strong, and diffuse. Knowing that staining pattern, membranous HEG1 staining has an excellent specificity (98.7% in the study) in distinguishing epithelioid/biphasic mesotheliomas from pulmonary carcinomas including squamous cell carcinomas which showed only focal cytoplasmic staining. Importantly, HEG1 was positive in 71.4% of the cases that stained positive with only one conventional mesothelial marker, so it can help challenging cases with weak or negative conventional mesothelial marker expression

**Naso et al. Utility of SOX6 and DAB2 for the Diagnosis of Malignant Mesothelioma. *Am J Surg Pathol.* 2021;45:1245-1251.**

**Purpose:** To assess the value of SOX6 and DAB2 immunostains compared to standard panels for distinguishing mesothelioma from carcinoma mimics.

**Methods:**

- TMA – 63 epithelioid (40) and sarcomatoid (23) mesotheliomas; 121 pulmonary adenocarcinomas (52), squamous cell (57) and large cell (12) carcinomas.
- SOX6 and DAB2 scored as positive with **nuclear (SOX6)** or **cytoplasmic (DAB2)** “staining of any intensity or diffuseness”, diffuseness scored <10%, 10-50%, and >50%.
- other meso stains calretinin, WT1, D2-40, CK5/6 and HEG1 (see previous, Hiroshima, Pathol Int 2021); carcinoma stains claudin-4, MOC31, BerEP4, TTF-1, and p40

**Results:**

- SOX6 and DAB2 highly sensitive for epithelioid (85-98%) but not sarcomatoid (NA-13%) mesos

		>50%	10-50%	<10%	None	% any pos
epith meso (40)	SOX6	25	6	3	6	85
	DAB2	34	4	1	1	98
sarc meso (23)	SOX6	1	2	0	20	13
	DAB2*	NA	NA	NA	NA	NA

\*difficulty in distinguishing staining in background stromal cells from tumor cells precluded reliable scoring; also stains macs

- SOX6 > DAB2 specificity for AdC (94% vs 77%) and LCC (92% vs 67%) but not SqCC (79% vs 86%)
- other meso markers had higher sensitivities for epithelioid and sarcomatoid mesos compared to SOX6 (except for WT1 for sarcomatoid)
  - highest sensitivities for SOX6 pairs
    - epith-meso: HEG1 (98%), WT1 (98%), D2-40 (100%)
    - sarc-meso: HEG1 (48%), D2-40 (65%); 1 case had only SOX6 staining
- SOX6 paired with broad spectrum carcinoma markers solved most (but not all) problems.

SOX6	claudin-4	meso (40)	AdC (52)	SqCC (57)	LCC (12)	Specificity	Sensitivity	Interp
POS	NEG	34 (85%)	1 (2%)	3 (5%)	0	95-100%	85%	meso likely
POS	POS	0	2 (4%)	9 (16%)	1 (8%)	100%	4-16%	carcinoma
NEG	POS	0	44 (85%)	36 (63%)	8 (67%)	100%	63-85%	carcinoma
NEG	NEG	6 (15%)	5 (10%)	9 (16%)	3 (25%)	NA	NA	uninformative
SOX6	BerEP4							
POS	NEG	25 (63%)	0	2 (4%)	0	96-100%	63%	meso likely
POS	POS	9 (23%)	3 (6%)	10 (18%)	1 (8%)	NA	NA	uninformative
NEG	POS	3 (8%)	46 (88%)	39 (68%)	9 (75%)	93%	68-88%	carcinoma likely
NEG	NEG	3 (8%)	3 (6%)	6 (11%)	2 (17%)	NA	NA	uninformative

**Take-home message:** The combination of SOX6 (nuclear) and claudin-4 (membranous) provided the highest number of interpretable results and could be designed as dual staining. HEG1 may have better sensitivity and specificity than SOX6 but is not yet available outside of Japan. Rare AdC and SqCC SOX6-pos/claudin-neg for which p40 and TTF-1 may be helpful. Sarcomatoid mesotheliomas still a problem for which combining SOX6 with CK5/6, D2-40, calretinin, WT-1 and HEG1 may help (GATA-3 oddly missing from this discussion?).

## Articles for notation

### NSCLC

**Amemiya K et al. Actionable Driver DNA Variants and Fusion Genes Can Be Detected in Archived Cytological Specimens With the Oncomine Dx Target Test Multi-CDx System in Lung Cancer. *Cancer Cytopathol* 2021;129:729-738.**

**Purpose:** Authors collected 18 archived cytological specimens (ACS) and 15 formalin-fixed, paraffin-embedded (FFPE) tumor tissues from 15 patient with lung cancer and investigated genomic profiles with the Oncomine Dx Target Test Multi-CDx system, which is an integrated NGS platform for four target genes including EGFR, BRAF, ALK, and ROS.

**Take-home message:** There were no differences in sequencing quality control between ACSs and FFPE tissues. A total of 21 variants were detected in both ACSs and FFPE tissues with 100% concordance. Authors concluded ACSs could be a feasible alternative to identify mutations and fusion genes via the Oncomine DX Target Test Multi-CDx system.

**Chen CC et al. Evaluation of the Diagnostic Accuracy of Bronchial Brushing Cytology in Lung Cancer: A Meta-Analysis. *Seminars in Diagnostic Pathology* 2021;38:90–97.**

**Purpose:** To evaluate the diagnostic accuracy of bronchial brushing cytology (BBC) for lung cancer diagnosis by a meta-analysis of 17 studies with 2538 patients.

**Take-home message:** The meta-analysis of BBC showed moderate sensitivity (0.67) and high specificity (0.91). Authors suggest that bronchoscopy in combination with BBC which has high specificity may complement low dose CT (LDCT) which has high sensitivity but low specificity, and improve the overall capacity of LDCT-based lung cancer screening.

**Spira AI et al. A retrospective observational study of the natural history of advanced non–small-cell lung cancer in patients with KRAS p.G12C mutated or wild-type disease. *Lung Cancer* 2021;159:1-9.**

**Purpose:** To understand clinical and pathologic characteristics and outcomes in patients with KRAS p.G12C mutation-positive advanced NSCLC. This study included 7,069 advanced NSCLC patient including 743 KRAS p.G12C mutation positive patients and 3,957 KRAS/EGFR/ALK wild type cases from de-identified nationwide (US-based) NSCLC clinic-genomic database from 2011 to 2019.

**Take-home message:** KRAS p.G12C mutation positive patients have more history of current or former smoking and more non-squamous histology. The KRAS mutation was mutually exclusive with other actionable driver mutations, and co-mutation with STK11 and KEAP1 occurred in 20% and 7% with KRAS p/G12C mutation respectively, both of which were associated with lower overall survival and treatment resistance. In advanced NSCLC, KRAS p.G12C mutation is not predictive of outcome, however in earlier stage NSCLC, KRAS p.G12C mutation is associated with shorter overall survival than those without KRAS mutations. Still there is an unmet need for KRAS mutated NSCLC.

**Zhao M et al. Proposal for Rib invasion as an independent T descriptor for non-small cell lung cancer: A propensity-score matching analysis. *Lung Cancer* 2021;159:27–33.**

**Purpose:** To evaluate the difference of prognosis between patients of NSCLC with different depth of chest wall invasion, and estimate the impact of rib invasion on the pathological T classification.

**Take-home message:** There were multiple studies on the prognostic impact of chest wall invasion depth of NSCLC, however they were not consistent and none of them has investigated the impact of rib invasion on the T classification. Authors selected 521 patients with surgical resection for pT3-4 NSCLC and retrospectively reviewed their prognosis with propensity-score matching analysis balancing the known confounders of the prognosis. Authors reported that survival outcomes of rib invasion were worse than those of matched pT3 cases and were similar to those of pT4 NSCLC. Reclassification of rib invasion as pT4 disease should be further validated and discussed in the forthcoming edition of WHO tumor classification.

**Reviews and Guidelines**

**Caramaschi et al. Histopathological findings and clinicopathologic correlation in COVID-19: a systematic review. *Mod Pathol.* 2021;34:1614-1633.**

**Purpose:** Large meta analysis in autopsy and tissue specimens from living patients that “at the time of this manuscript preparation” included analysis of “508 decedents from 43 studies” (ie, not the 662 patients from 58 studies advertised in the abstract).

**Take-home message:** SARS-CoV-2 causes ARDS/DAD in the majority of patients with COVID-19 pneumonia with a higher frequency of concomitant large vessel thrombi/thromboembolism and variable viral detection rates in pneumocytes, alveolar macrophages, tracheobronchial epithelium, and minimally in endothelial cells. Viral detection depends on method used (RT-PCR, IHC, ISH, NGS, TEM, culture) and timing from onset of symptoms.

**Metovic et al. Recent advances and current controversies in lung neuroendocrine neoplasms. *Semin Diagn Pathol.* 2021;38(5):90-97.**

**Purpose:** An update on ongoing controversies and challenges with NENs including 1) criteria and terminology options for biopsies/cytologies, 2) carcinoid tumors with elevated Ki67 indices, 3) relevance of the molecular landscape, and an alternative view/proposal regarding development of lung NENs.

**Take-home message:** Confusion persists regarding the relative hierarchy and cutoff criteria for carcinoid tumors (NET G1), carcinoid tumors with increased mitotic rates and/or Ki67 indices (NET G2/G3), and atypical carcinoid tumors (NET G2/G3). A subset of gray zone tumors (“carcinoid-like LCNEC”) contribute to the confusion and have carcinoid/atypical carcinoid histology but mitotic rates  $> 10/2 \text{ mm}^2$  and/or extensive necrosis with Ki67 indices of 20-30% and a molecular profile resembling carcinoid tumors including *MEN1*, *ARID1A/B*, and *KDM5C* mutations common to carcinoids without the *RBI* or *TP53* inactivation and high tumor mutational burden (TMB) characteristic of NECs. Molecular profiling segregates LCNEC into not only *carcinoid-like* but also *NSCLC-like* and *SCLC-like* subsets (thus self-immolating which may be its destiny). Molecular subsets characterized by distinct gene expression profiles with potential implications for survival/treatment have also been proposed for SCLC. This heterogeneity and overlapping gray zones in the genetic landscape of lung NENs suggests multiple evolutionary pathways with imperfect links to morphology/histology, which remains the cornerstone for clinical decision making.

**Moran CA. Thymoma Staging: An Analysis of the Different Schemas. *Adv Anat Pathol.* 2021;28:298-306.**

**Purpose:** To compare and contrast previously proposed staging schemas with the currently employed 8<sup>th</sup> edition AJCC TNM staging system, and challenge the assumption that the same schema should be applied to thymomas as well as thymic carcinomas.

**Take-home message:** The current AJCC staging system is a reorganization of the Masaoka and Moran systems without benefit of new robust data to support it, and includes an N category which is largely irrelevant to thymomas. However you stage it, capacity to invade and resectability remain the key predictors of outcome.