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High-Grade Neoplasms of Uncertain Lineage:
Do's and Don'ts of "Ancillary" Testing

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Disclosures and Shameless Plugs

- Nothing to disclose

- @IHC_guy
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- @UIPathology

REVIEW ARTICLE

An Algorithmic Immunohistochemical Approach to Define Tumor Type and Assign Site of Origin

Andrew M. Bellizzi, MD

Abstract: Immunohistochemistry represents an indispensable complement to an epidemiology and morphology-driven approach to tumor diagnosis and site of origin assignment. This review reflects the state of my current practice, based on 15-years' experience in Pathology and a deep-dive into the literature, always striving to be better equipped to answer the age old questions, "What is it, and where is it from?" The tables and figures in this manuscript are the ones I "pull up on the computer" when I am teaching at the microscope and turn to myself when I am (frequently) stuck. This field is so exciting because I firmly believe that, through the application of next-generation immunohistochemistry, we can provide better answers than ever before. Specific topics covered in this review include (1) broad tumor classification and associated screening markers; (2) the role of cancer epidemiology in determining pretest probability; (3) broad-spectrum epithelial markers; (4) noncanonical expression of broad tumor class screening markers; (5) a morphologic pattern-based approach to poorly to undifferentiated malignant neoplasms; (6) a morphologic and immunohistochemical approach to define 4 main carcinoma types; (7) CK7/CK20 coordinate expression; (8) added value of semiquantitative immunohistochemical stain assessment; algorithmic immunohistochemical approaches to (9) "garden variety" adenocarcinomas presenting in the liver, (10) large polygonal cell adenocarcinomas, (11) the distinction of primary surface ovarian epithelial tumors with mucinous features from metastasis, (12) tumors presenting at alternative anatomic sites, (13) squamous cell carcinoma versus urothelial carcinoma, and neuroendocrine neoplasms, including (14) the distinction of pheochromocytoma/paraganglioma from well-differentiated neuroendocrine tumor, site of origin assignment in (15) well-differentiated neuroendocrine tumor and (16) poorly differentiated neuroendocrine carcinoma, and (17) the distinction of well-differentiated neuroendocrine tumor G3 from poorly differentiated neuroendocrine carcinoma; it concludes with (18) a discussion of diagnostic considerations in the broad-spectrum keratin/CD45/S-100-"triple-negative" neoplasm.

Key Words: immunohistochemistry, tumor classification, carcinoma of unknown primary, site of origin, differential diagnosis

(*Adv Anat Pathol* 2020;00:000–000)

NEXT-GENERATION IMMUNOHISTOCHEMISTRY AND THE PRIMACY OF LINEAGE-RESTRICTED TRANSCRIPTION FACTORS

"Next-generation immunohistochemistry" refers to the mining of the molecular genetic and developmental biology literature to "discover" new immunohistochemical markers, including those identified through gene expression profiling, protein correlates of molecular genetic events, and lineage-restricted transcription factors. While historically our diagnostic armamentarium was geared toward cytoplasmic or membranous differentiation markers, which often demonstrate reduced expression and, thus, reduced sensitivity in poorly differentiated tumors, transcription factors tend to be strongly expressed regardless of differentiation. Table 1 lists the next-generation immunohistochemical markers discussed in this review, associated diagnostic applications, and their "qualifications" as next-generation markers.

There are "immuno-optimists" and "immuno-pessimists." I like to think I am an "immuno-realist." There is no "perfect" immunohistochemical marker, and in most instances a panel of immunohistochemical stains should be applied to adjudicate an epidemiology and morphology-driven differential diagnosis. The "immuno-pessimists" are perfectly fine with an *EWSR1* rearrangement driving Ewing sarcoma, clear cell sarcoma, desmoplastic small round cell tumor, angiomatoid fibrous histiocytoma, extraskeletal myxoid chondrosarcoma, and sclerosing epithelioid fibrosarcoma but have the unrealistic expectation that a single marker, especially a lineage-restricted transcription factor, will have a single diagnostic application. Even an "old school" next-generation marker like TTF-1 is expressed by lung and thyroid (and mesonephric-like adenocarcinoma, by the way).[1,2] Just like that *EWSR1* rearrangement, transcription factors are "allowed" to exert differential effects in a cell-type-specific manner.

A colleague recently remarked "GATA-3 is ruined" when I let her know that it was the best widely available marker to distinguish pheochromocytoma/paraganglioma from well-differentiated neuroendocrine tumor. Expression in this tumor type is not "random;" it is predicted by developmental biology, in which GATA-3 participates in a complex transcriptional network to regulate development of the autonomic nervous system.[3,4] Large-scale immunohistochemical surveys of emerging markers not only confirm what we already know, but provide the opportunity to discover additional "tools." For example, when Miettinen and colleagues described SOX10 expression in 12% of 486 invasive ductal carcinomas of breast origin, it was not "aberrant" staining, but rather, a signal demanding an explanation. It turns out that SOX10 expression is restricted to estrogen receptor (ER)-negative breast cancers and that SOX10-positivity is, thus, incredibly useful in the diagnosis of triple-negative breast cancer.

My favorite immunohistochemical markers are oligospecific transcription factors. I refer to them as the "Swiss Army Knives" of immunopathology, capable of "solving" multiple differential diagnoses. GATA-3 is a classic example, and Miettinen et al[5] highlighted 9 unique diagnostic contexts in which GATA-3 could be useful! In addition to the familiar ones in which GATA-3 functions as a positive marker of breast and urothelial carcinoma,

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What I want you to Remember From this Lecture

- Age, Gender, Anatomic Location
- Primary vs Metastasis
- Screening Markers (Keratin, CD45, SOX10, SALL4)
- Differentiation Markers (everything else)

- Don’t start ordering differentiation markers if you’re unsure about the broad tumor class

- Don’t be a hero: use work aids; show a colleague

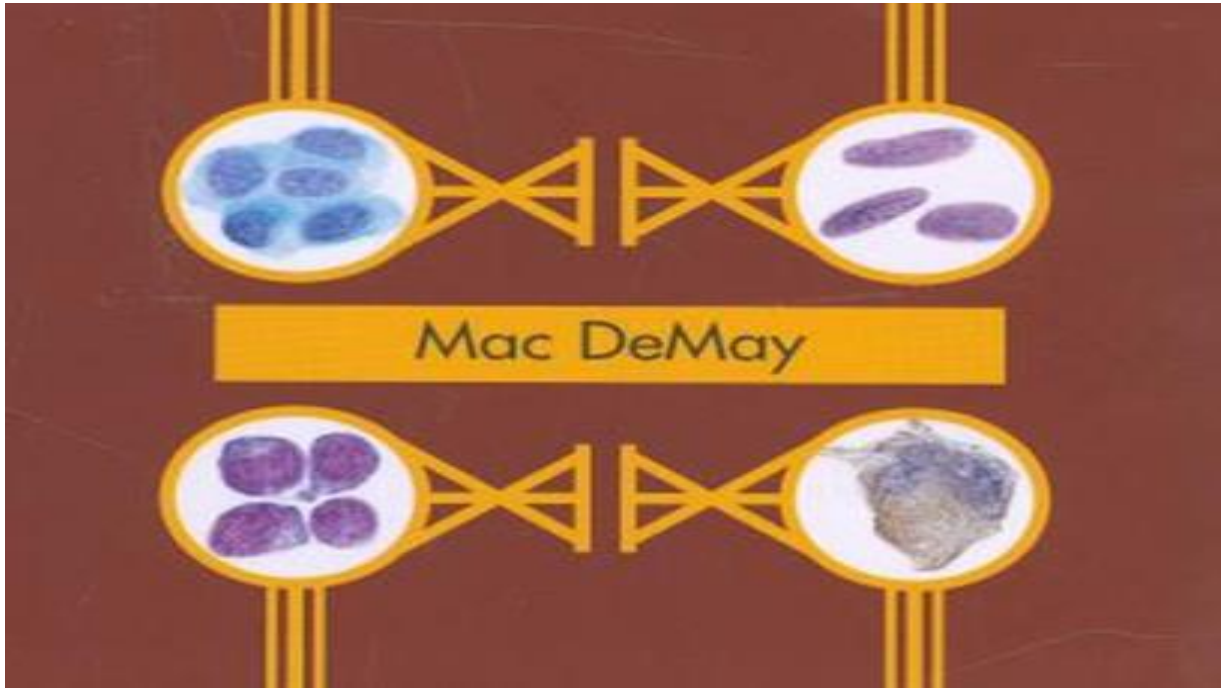
Don’t Be Penny Wise and Pound Foolish

- Divide tumor biopsies into (at least) 2 blocks
- Every biopsy is a potential molecular specimen
- Reserve 1 best block for molecular testing
- When ordering IHC cut extra unstained up front

Outline

- Screening Markers for Broad Tumor Classes
- Non-Canonical Expression of Broad Tumor Class Markers
- Everything Dedifferentiates
- IHC Workup of Small Round Blue Cell Sarcoma
- RNA Fusion Profiling
- Gene Expression Profiling for Tumor of Uncertain Lineage/Carcinoma of Unknown Primary

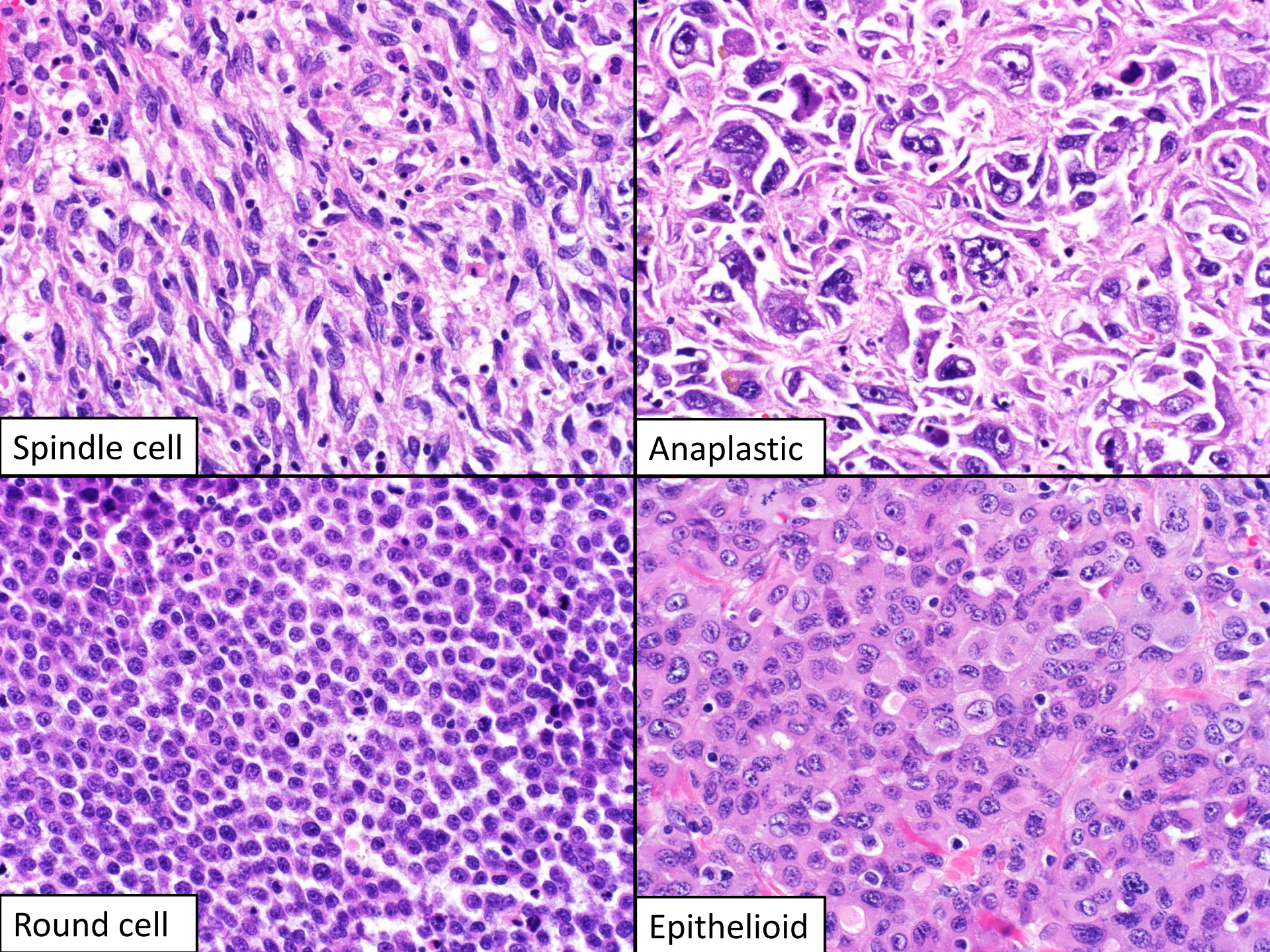
Diagnosis of Broad Tumor Class



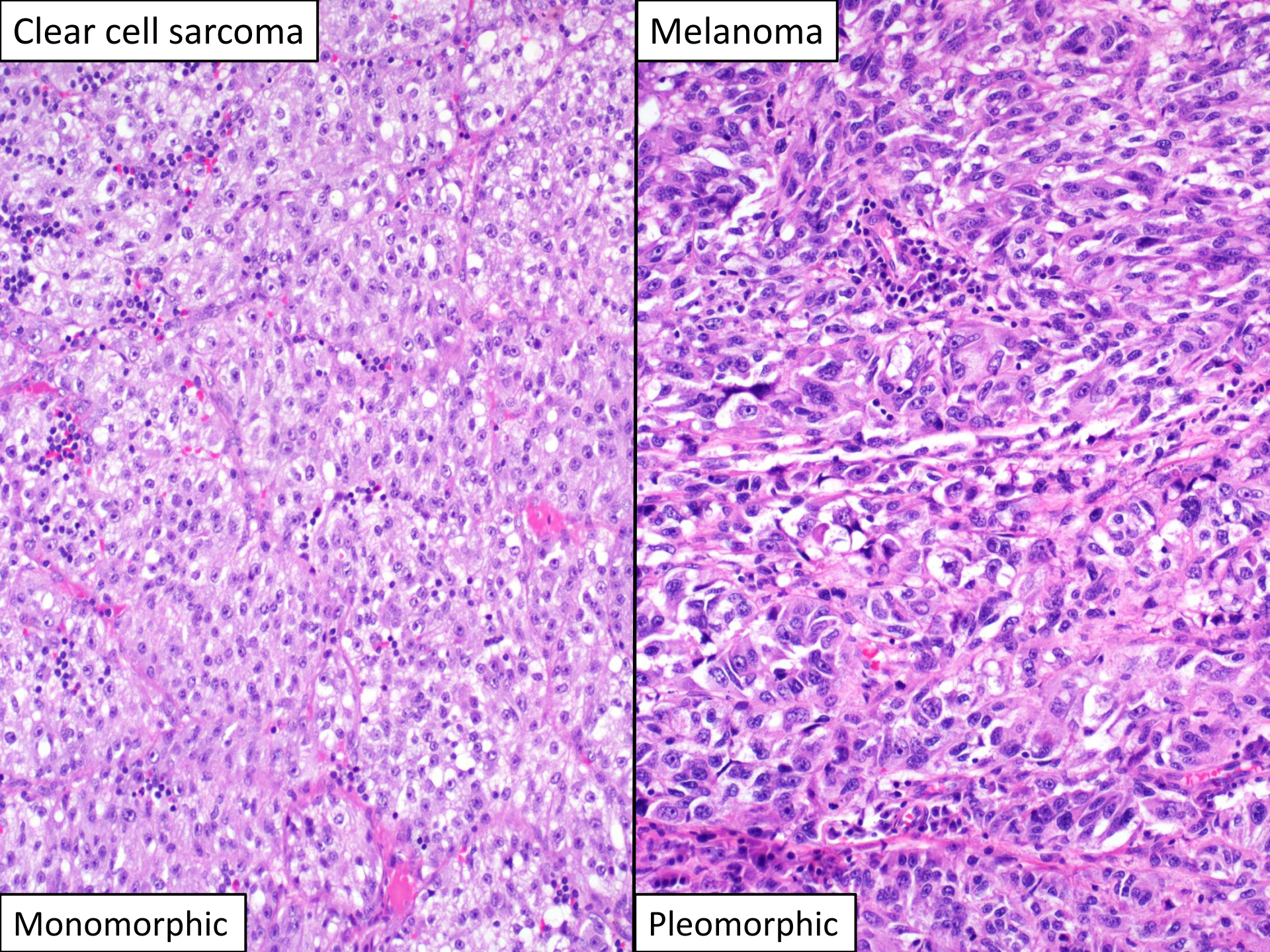
Morphologic “Boxes”

- Spindle cell (sarcoma, sarcomatoid carcinoma)
- Anaplastic (anything)
- Round cell (lymphoma, sarcoma)
- Epithelioid (carcinoma, melanoma)

- Monomorphic
- Pleomorphic


Spindle cell
Anaplastic
Round cell
Epithelioid

Pattern	Principal Diagnostic Considerations	Initial Screening Panel
Epithelioid	Carcinoma, melanoma, large cell lymphoma	Broad-spectrum keratin, CD45, SOX10
Round cell	Round cell sarcoma, lymphoma, small cell carcinoma	CD99, NKX2.2, desmin, myogenin, CD45, TdT, INSM1, broad-spectrum keratin, SOX10
Spindle cell	Sarcomatoid carcinoma, sarcoma, spindle cell/desmoplastic melanoma	Broad-spectrum keratin, p40, SMSA, desmin, SOX10
Anaplastic	Anything (usually not lymphoma)	Broad-spectrum keratin, CD45, SOX10


Clear cell sarcoma
Monomorphic
Melanoma
Pleomorphic

Monomorphic vs Pleomorphic: Application to Differential Diagnosis

Monomorphic

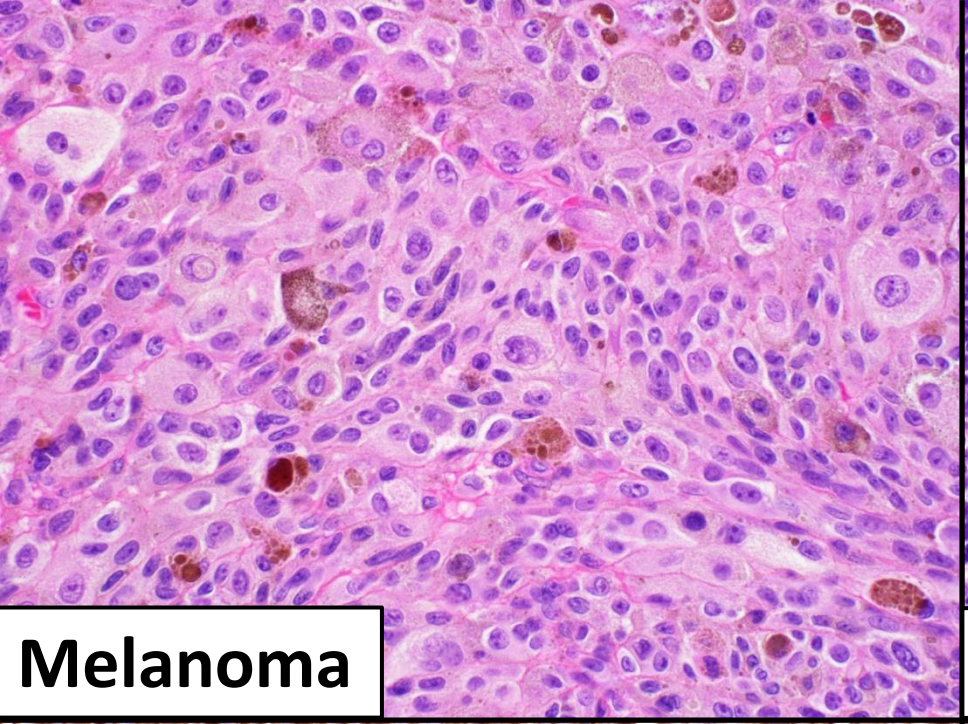
- Clear cell sarcoma
- Burkitt lymphoma
- Mesothelioma (epithelioid or sarcomatoid)
- Prostate cancer
- GIST
- Ewing sarcoma
- Synovial sarcoma
- INI1-deficient tumors
- Follicular dendritic cell tumors

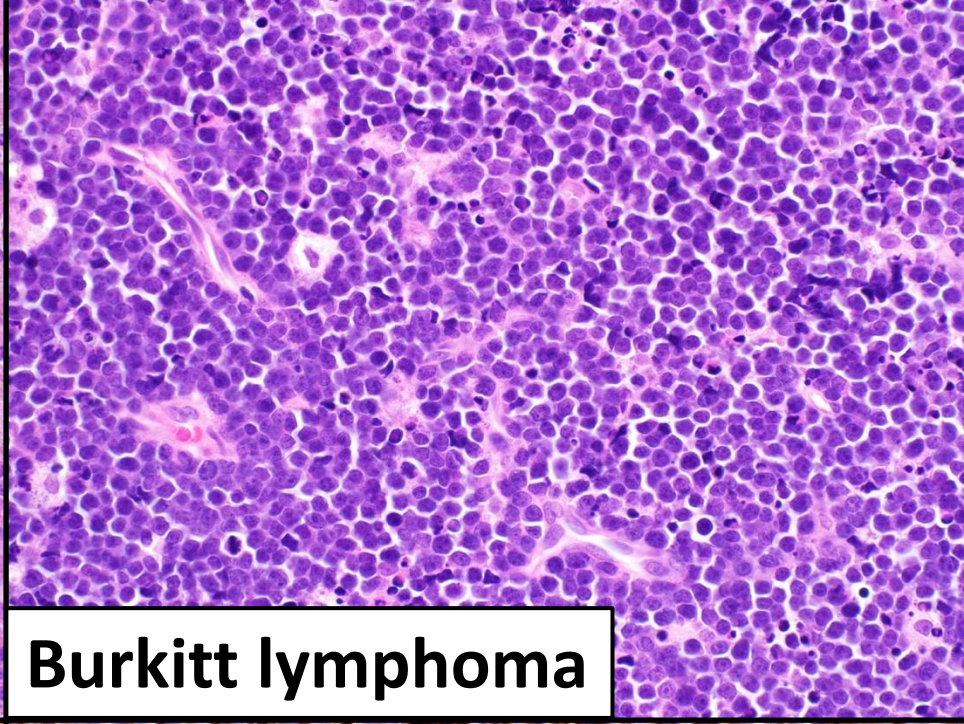
Pleomorphic

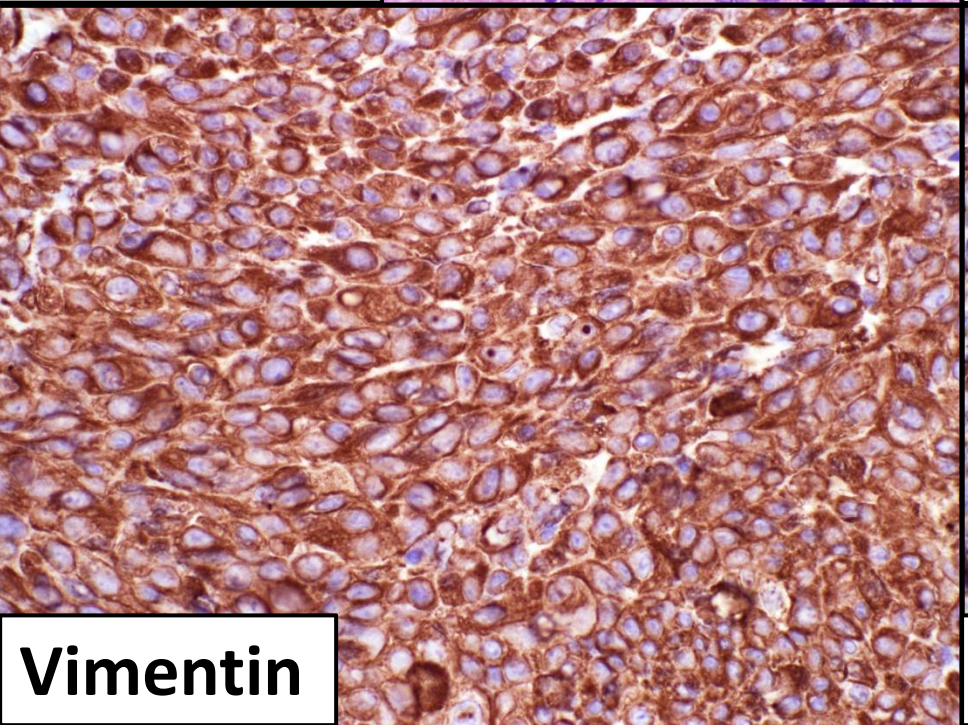
- Melanoma
- DLBCL
- Adenocarcinoma (esp. serous and pancreatic)
- Urothelial carcinoma
- Sarcomatoid carcinoma
- *CIC* and *BCOR* sarcoma

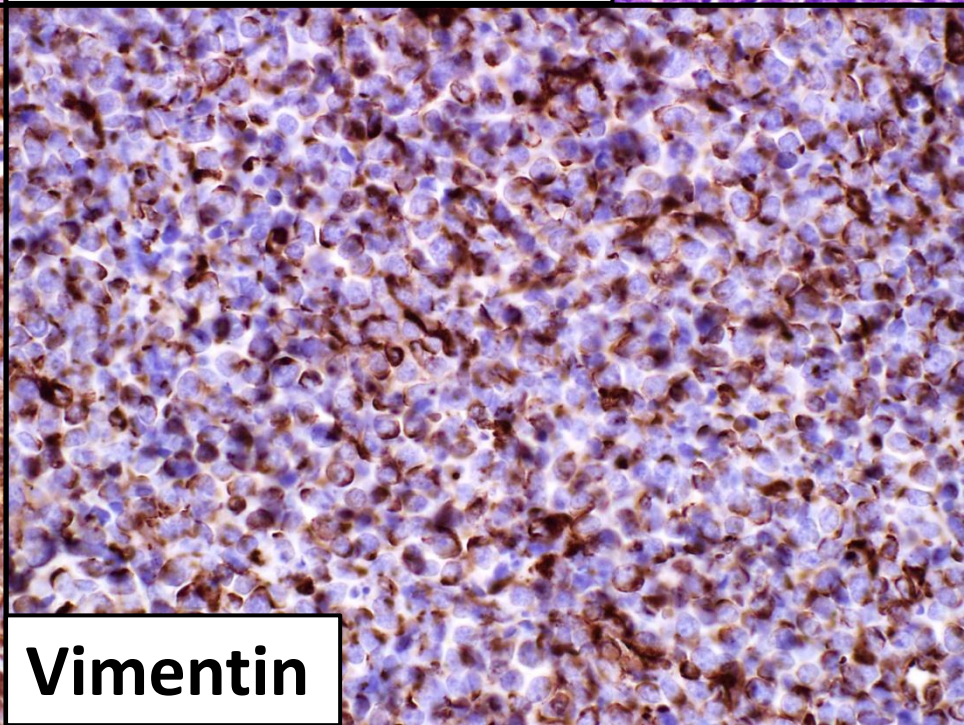
Broad Tumor Classes with Associated Screening Markers

Broad Tumor Class	Screening Markers	When to Consider	Confirmatory Markers
Carcinoma	**Broad-spectrum keratin**; EpCAM (i.e., MOC-31, Ber-EP4), EMA, claudin-4	Always	See additional algorithms
Hematolymphoid	**CD45**	Always; re-consider in a "triple-negative" neoplasm	CD45-negative lymphoma panel: CD43, CD79a, MUM1, ALK, CD30
Melanoma	**SOX10** or S-100	Always	Melan A, HMB-45, tyrosinase, BRAF V600E
Sarcoma	None	Spindle cell morphology; tumor in mediastinum, retroperitoneum, or somatic soft tissue	Unclassified malignant neoplasm in mediastinum, retroperitoneum, paratestis: MDM2/CDK4 (DDLPS) Epithelioid neoplasm defying typing: ERG (angiosarcoma), INI1 (epithelioid sarcoma) Last ditch effort: CD34 (rarely + in carcinoma)


Melanoma


Burkitt lymphoma


Vimentin


Vimentin

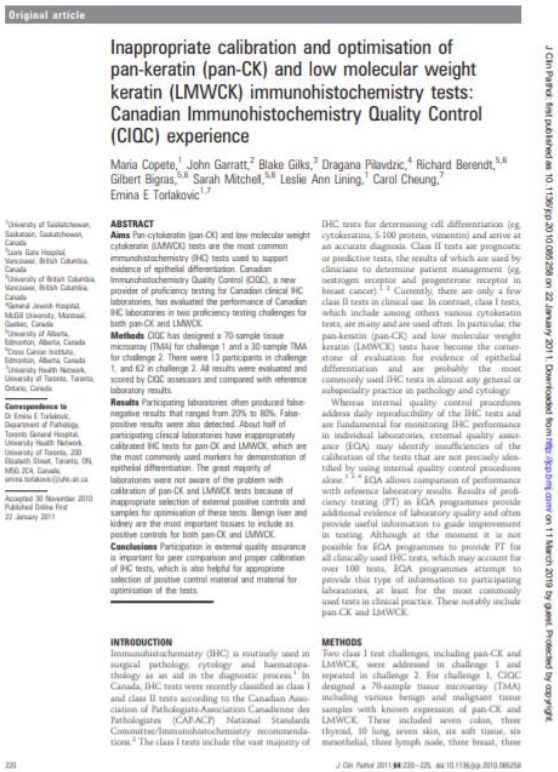
Which Screening Keratin Should I Use?

Clone	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AE1/AE3	X	X	X	X	X	X		X		X				X	X	X			X
OSCAR							X	X										X	X
MAK-6								X						X	X	X		X	X
MNF116					X	X		X									X		
CAM5.2							X	X											
KL1	X	X			X	X	X	X			X			X		X	X	X	
34βE12	X				X					X				X					

- In general, any of these are acceptable
- It's not the number of keratins, per se, but rather the affinity (e.g., CAM5.2 vs AE1/AE3 in HCC/RCC)
- Stratified epithelia: K1-6, 9-17
- Simple epithelia: 7, 8, 18, 19, 20

Ordóñez NG. *Hum Pathol*. 2013 Jul;44(7):1195-215.

It Doesn’t Matter How Many Keratins Your Pan-Keratin Reacts with if it isn’t Well-Optimized



Original article

Inappropriate calibration and optimisation of pan-keratin (pan-CK) and low molecular weight keratin (LMWCK) immunohistochemistry tests: Canadian Immunohistochemistry Quality Control (CIQC) experience

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ABSTRACT

Aims Pan-cytokeratin (pan-CK) and low molecular weight cytokeratin (LMWCK) tests are the most common immunohistochemistry (IHC) tests used to support evidence of epithelial differentiation. Canadian Immunohistochemistry Quality Control (CIQC), a new provider of proficiency testing for Canadian clinical IHC laboratories, has evaluated the performance of Canadian IHC laboratories in two proficiency testing challenges for both pan-CK and LMWCK.

Methods CIQC has designed a 70-sample tissue microarray (TMA) for challenge 1 and a 30-sample TMA for challenge 2. There were 13 participants in challenge 1, and 62 in challenge 2. All results were evaluated and scored by CIQC assessors and compared with reference laboratory results.

Results Participating laboratories often produced false-negative results that ranged from 20% to 80%. False-positive results were also detected. About half of participating clinical laboratories have inappropriately calibrated IHC tests for pan-CK and LMWCK, which are the most commonly used markers for demonstration of epithelial differentiation. The great majority of laboratories were not aware of the problem with calibration of pan-CK and LMWCK tests because of inappropriate selection of external positive controls and samples for optimisation of these tests. Benign liver and kidney are the most important tissues to include as positive controls for both pan-CK and LMWCK.

Conclusions Participation in external quality assurance is important for peer comparison and proper calibration of IHC tests, which is also helpful for appropriate selection of positive control material and material for optimisation of the tests.

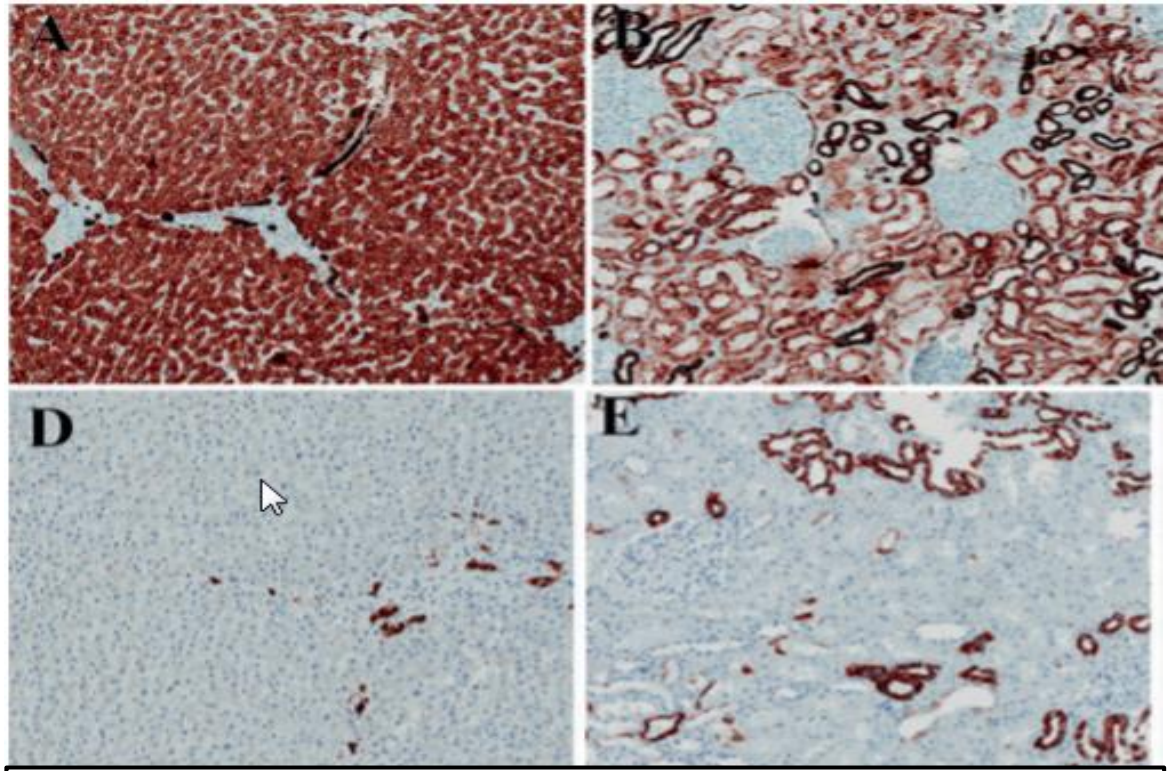
INTRODUCTION

Immunohistochemistry (IHC) is routinely used in surgical pathology, cytology and haematopathology as an aid in the diagnostic process.[1] In Canada, IHC tests were recently classified as class I and class II tests according to the Canadian Association of Pathologists-Association Canadienne des Pathologistes (CAP-ACP) National Standards Committee/Immunohistochemistry recommendations.[2] The class I tests include the vast majority of IHC tests for determining cell differentiation (eg, cytokeratins, S-100 protein, vimentin) and arrive at an accurate diagnosis. Class II tests are prognostic or predictive tests, the results of which are used by clinicians to determine patient management (eg, oestrogen receptor and progesterone receptor in breast cancer).[1 2] Currently, there are only a few class II tests in clinical use. In contrast, class I tests, which include among others various cytokeratin tests, are many and are used often. In particular, the pan-keratin (pan-CK) and low molecular weight keratin (LMWCK) tests have become the cornerstone of evaluation for evidence of epithelial differentiation and are probably the most commonly used IHC tests in almost any general or subspecialty practice in pathology and cytology.

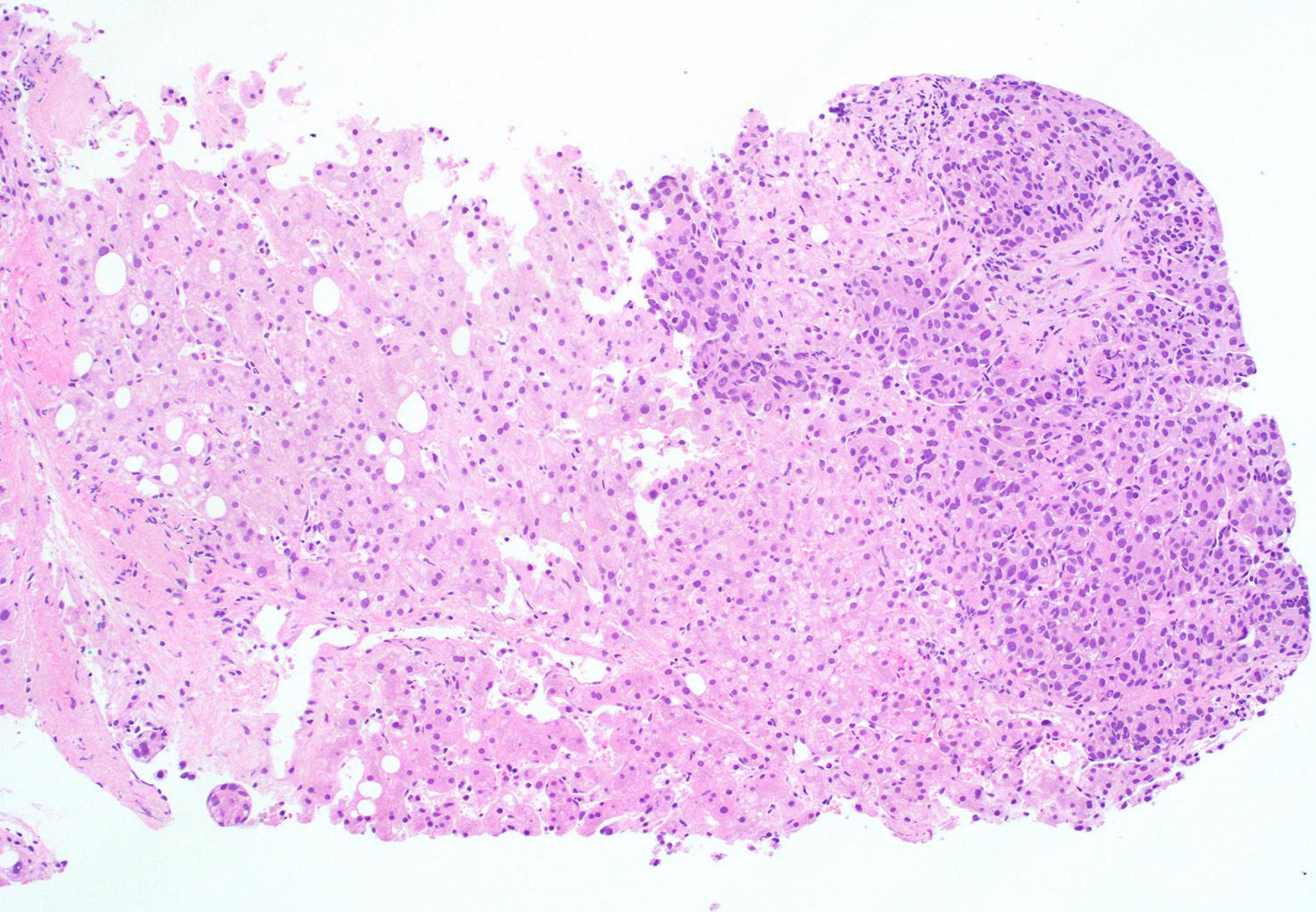
Whereas internal quality control procedures address daily reproducibility of the IHC tests and are fundamental for monitoring IHC performance in individual laboratories, external quality assurance (EQA) may identify insufficiencies of the calibration of the tests that are not precisely identified by using internal quality control procedures alone.[1 2 3] EQA allows comparison of performance with reference laboratory results. Results of proficiency testing (PT) in EQA programmes provide additional evidence of laboratory quality and often provide useful information to guide improvement in testing. Although at the moment it is not possible for EQA programmes to provide PT for all clinically used IHC tests, which may account for over 100 tests, EQA programmes attempt to provide this type of information to participating laboratories, at least for the most commonly used tests in clinical practice. These notably include pan-CK and LMWCK.

METHODS

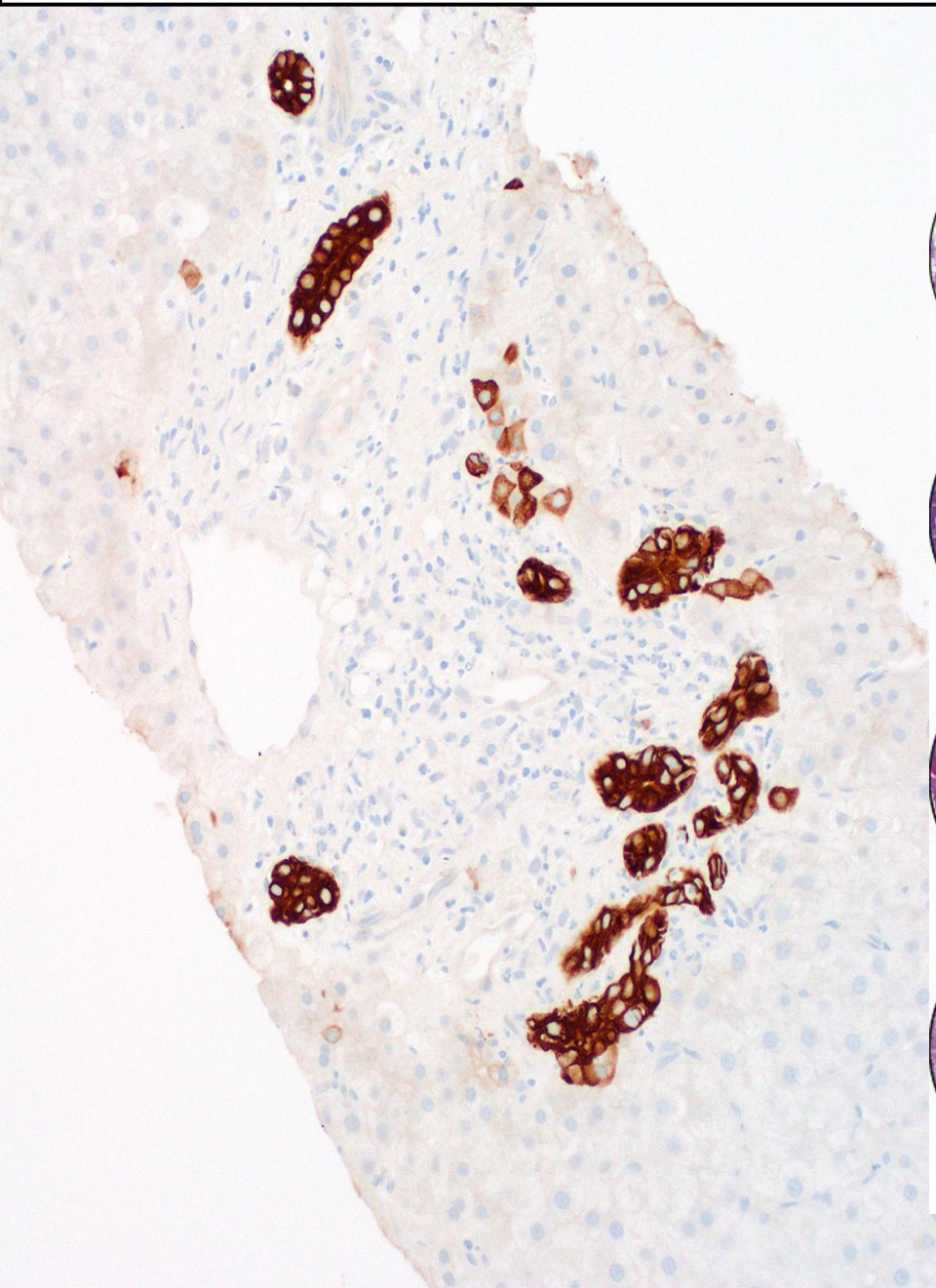
Two class I test challenges, including pan-CK and LMWCK, were addressed in challenge 1 and repeated in challenge 2. For challenge 1, CIQC designed a 70-sample tissue microarray (TMA) including various benign and malignant tissue samples with known expression of pan-CK and LMWCK. These included seven colon, three thyroid, 10 lung, seven skin, six soft tissue, six mesothelial, three lymph node, three breast, three

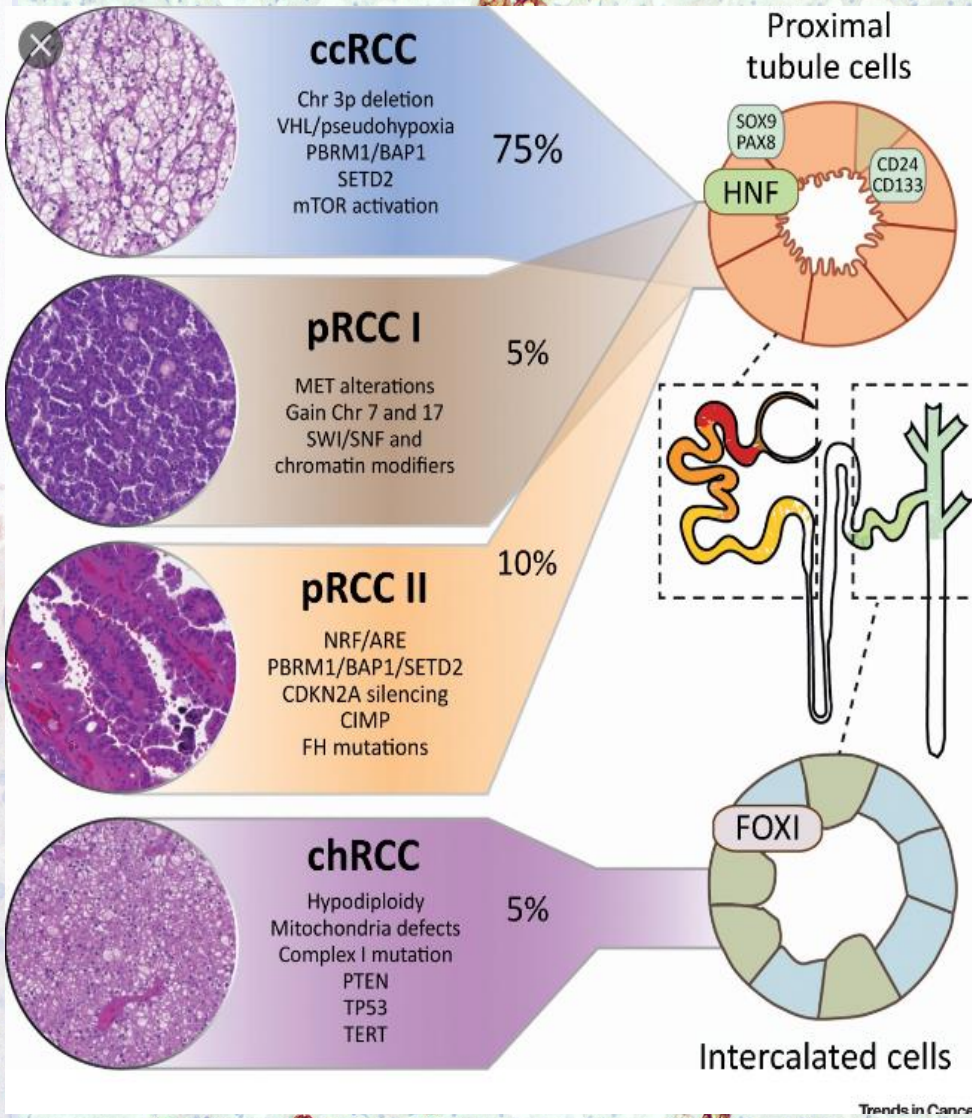


Keratin AE1/AE3 in two different labs; liver A, D and kidney B, E

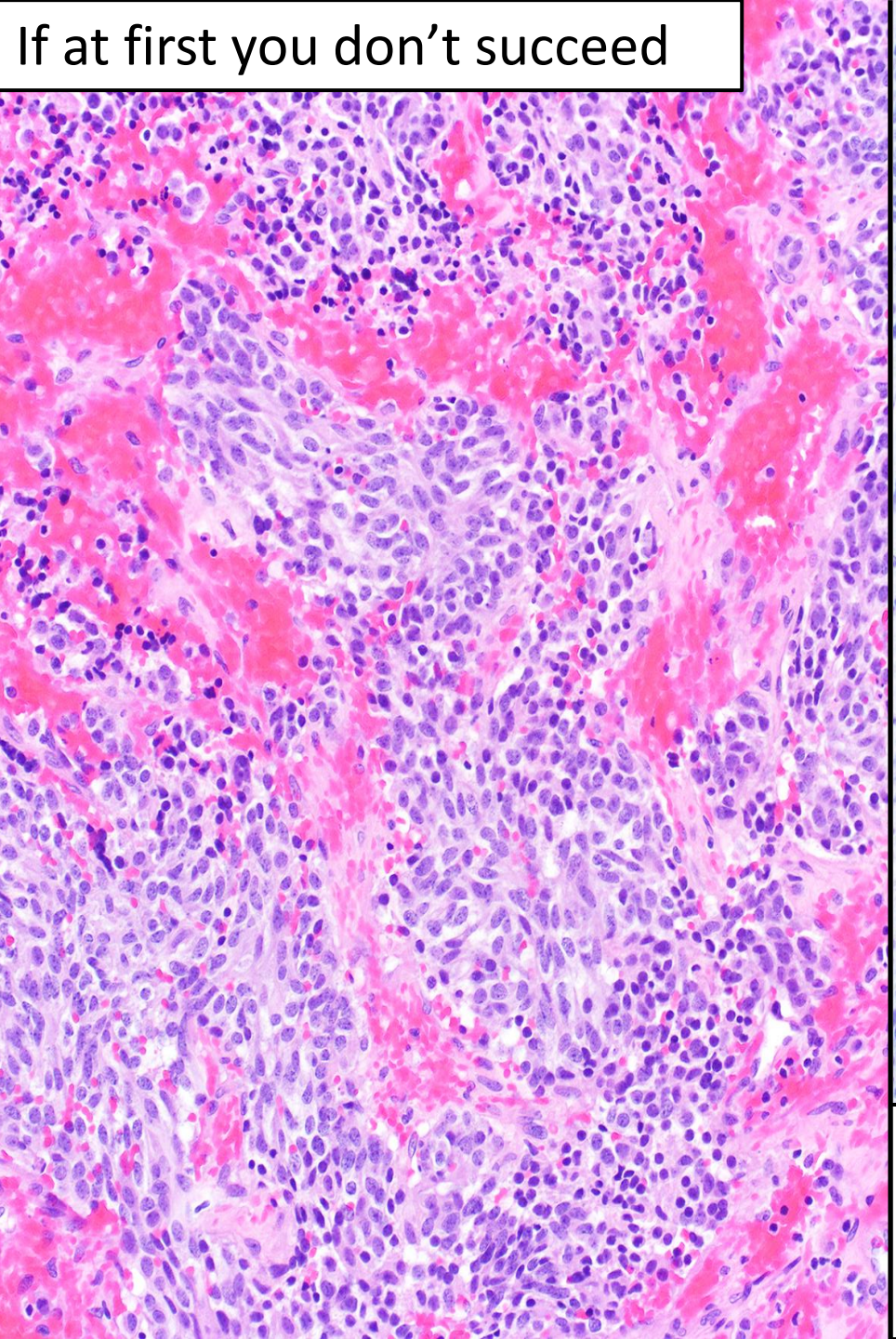


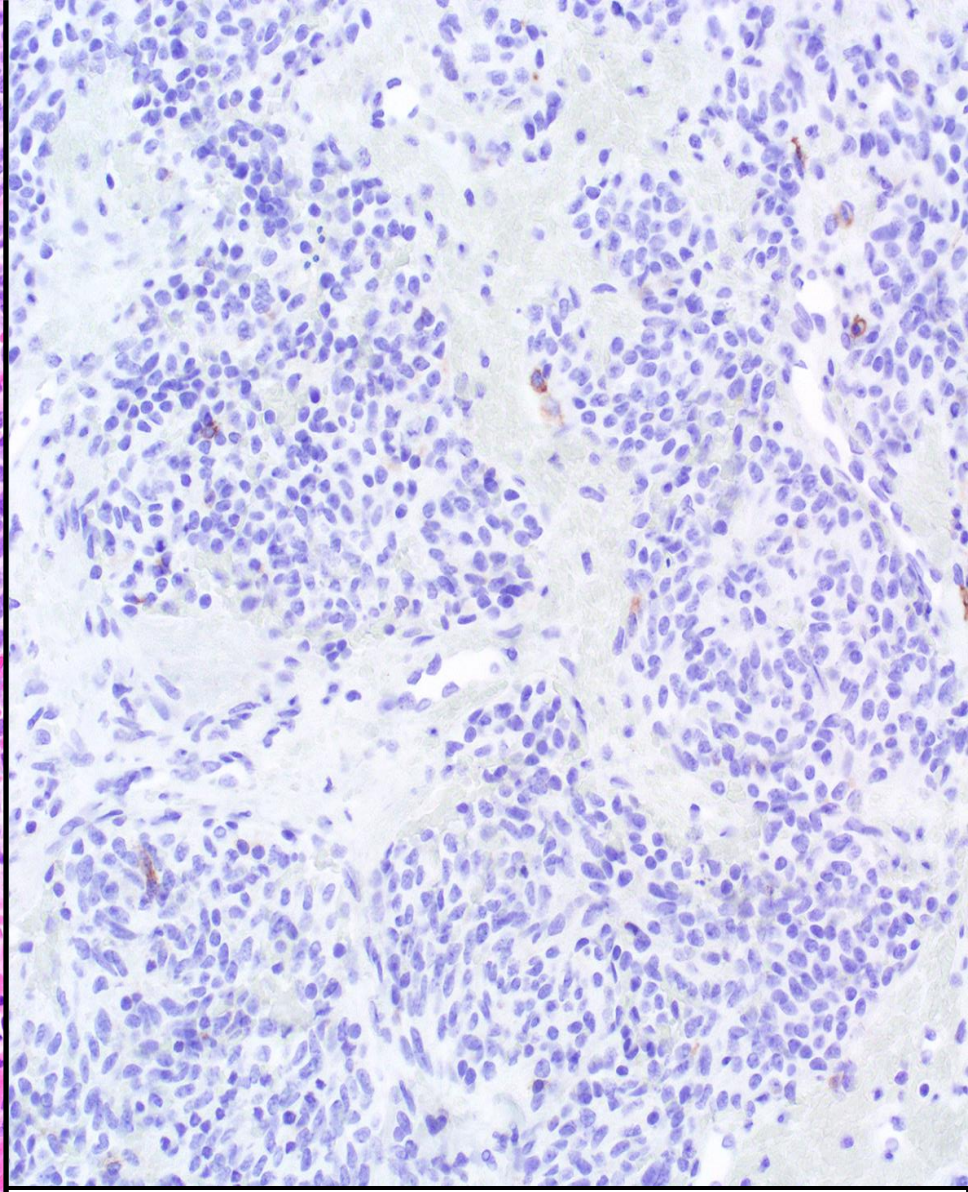
Don’t Diagnose with One Hand Tied Behind Your Back



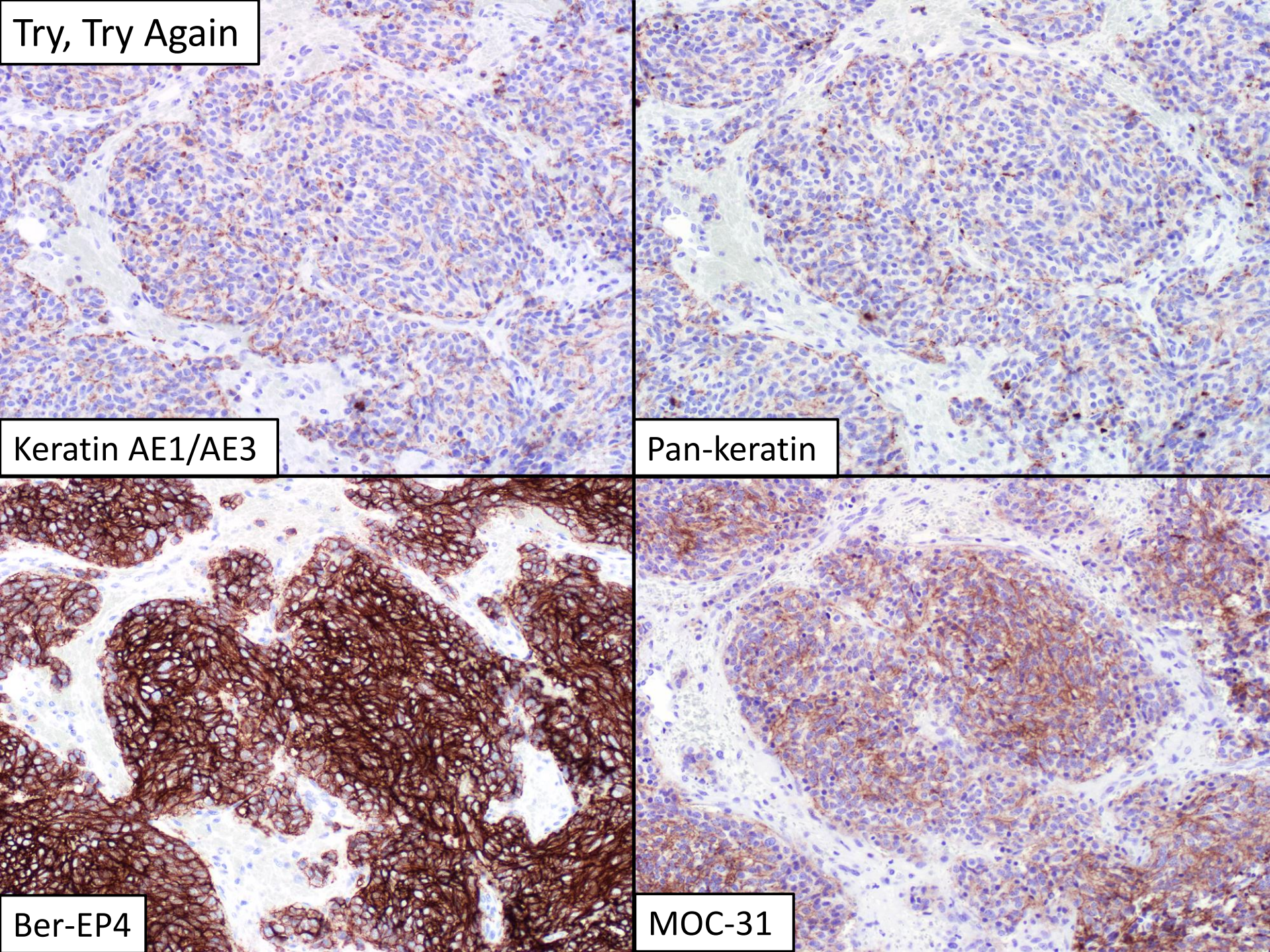


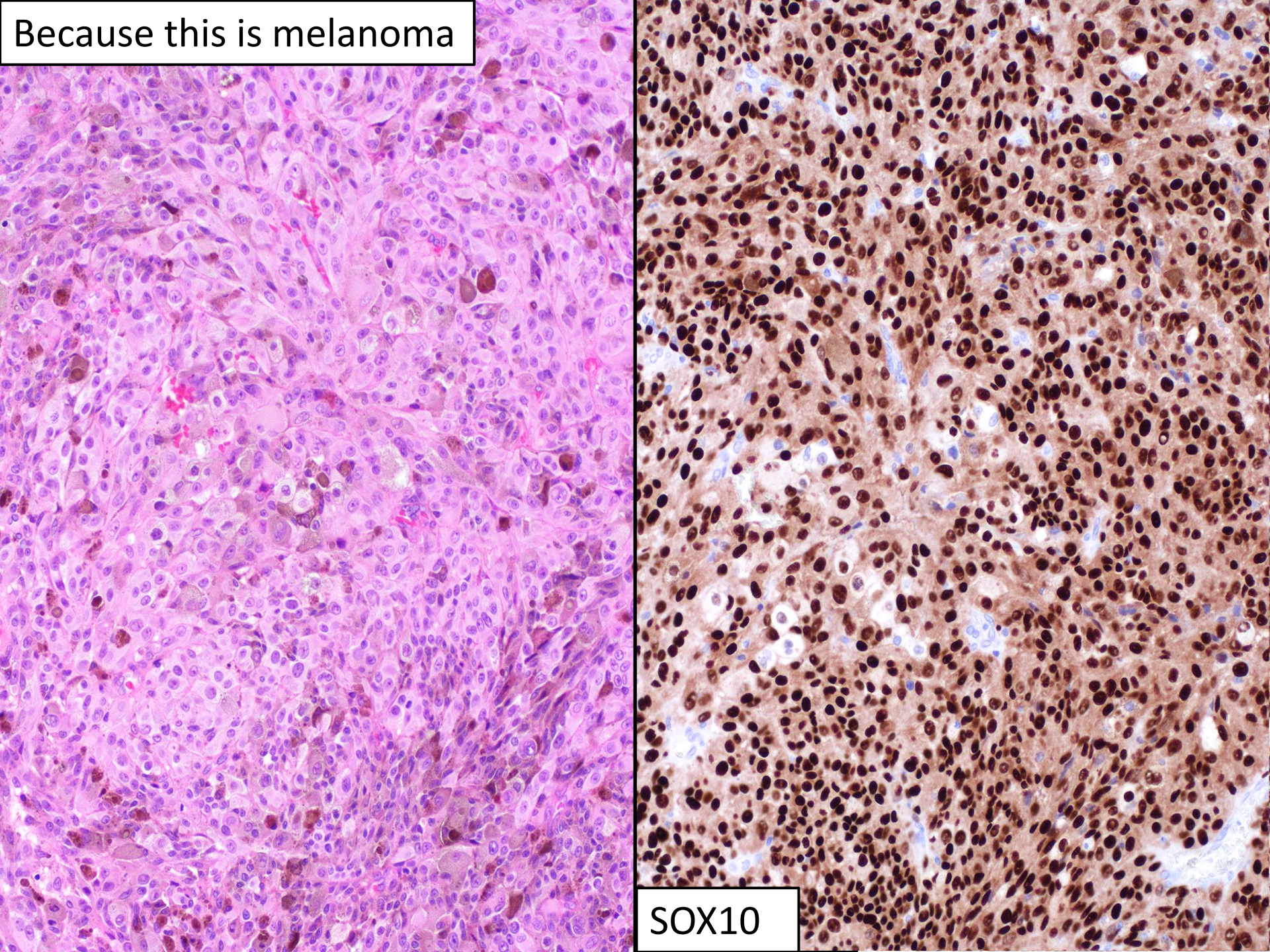
If at first you don’t succeed





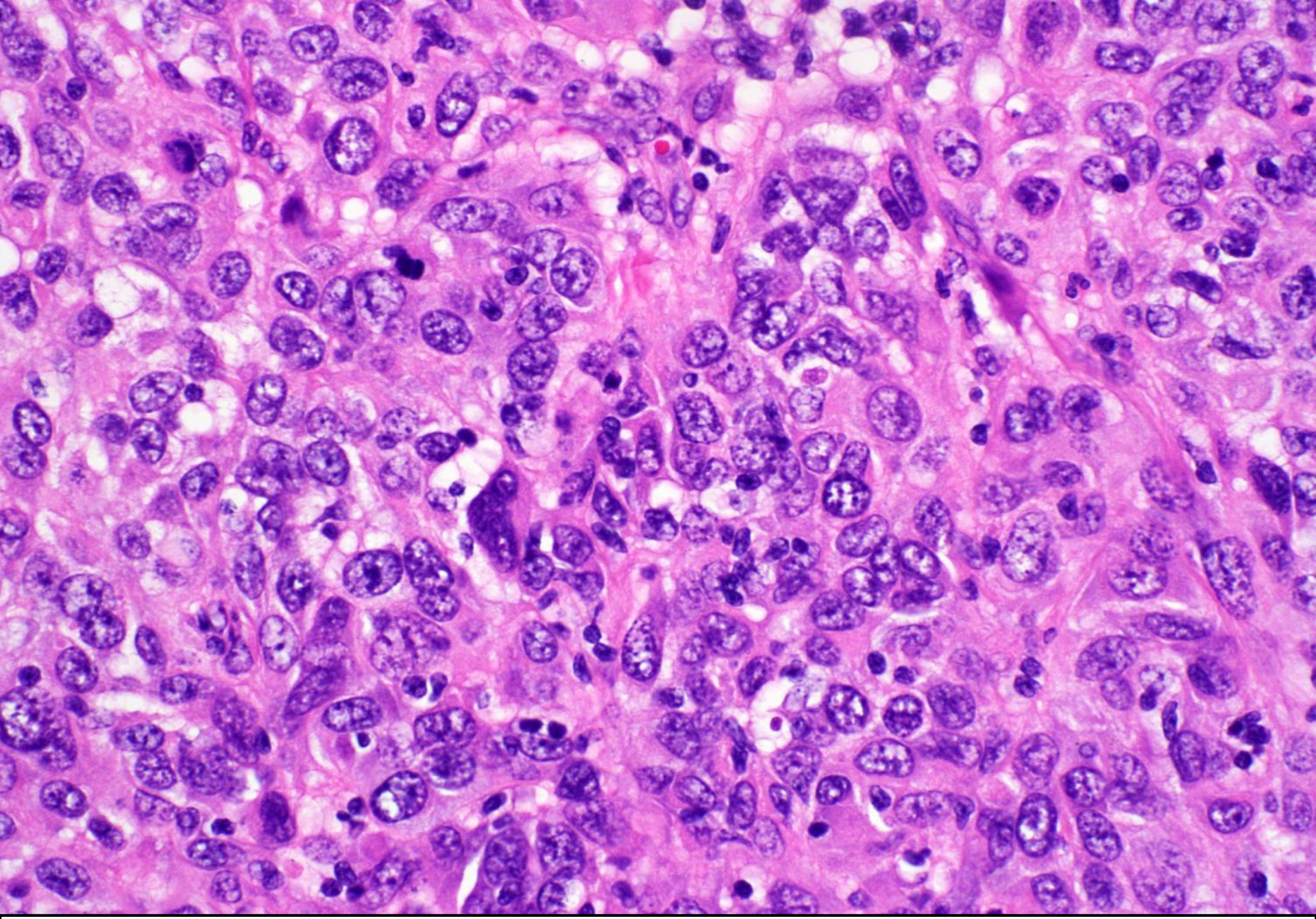
EMA: my least favorite broad-spectrum epithelial marker; expressed by plasma cells here; often positive in LCA-negative lymphomas (plasmacytomas; ALCL, plasmablastic lymphoma)


Try, Try Again
Keratin AE1/AE3
Pan-keratin
Ber-EP4
MOC-31


Because this is melanoma
SOX10

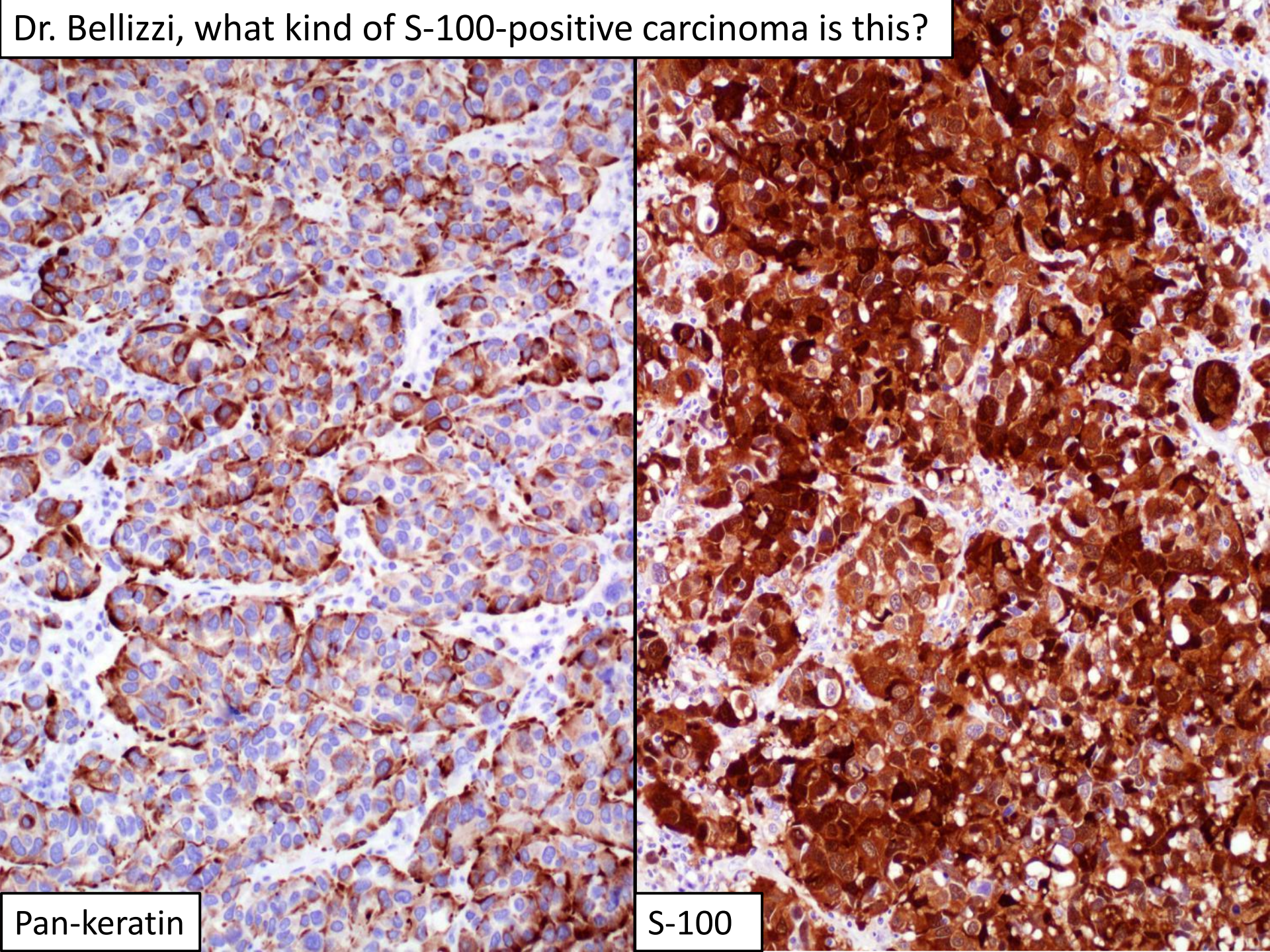
Magnesium alloy top and rear covers

Aluminum and polycarbonate resin body



78-year-old man p/w word-finding difficulty: L temporal lobe mass

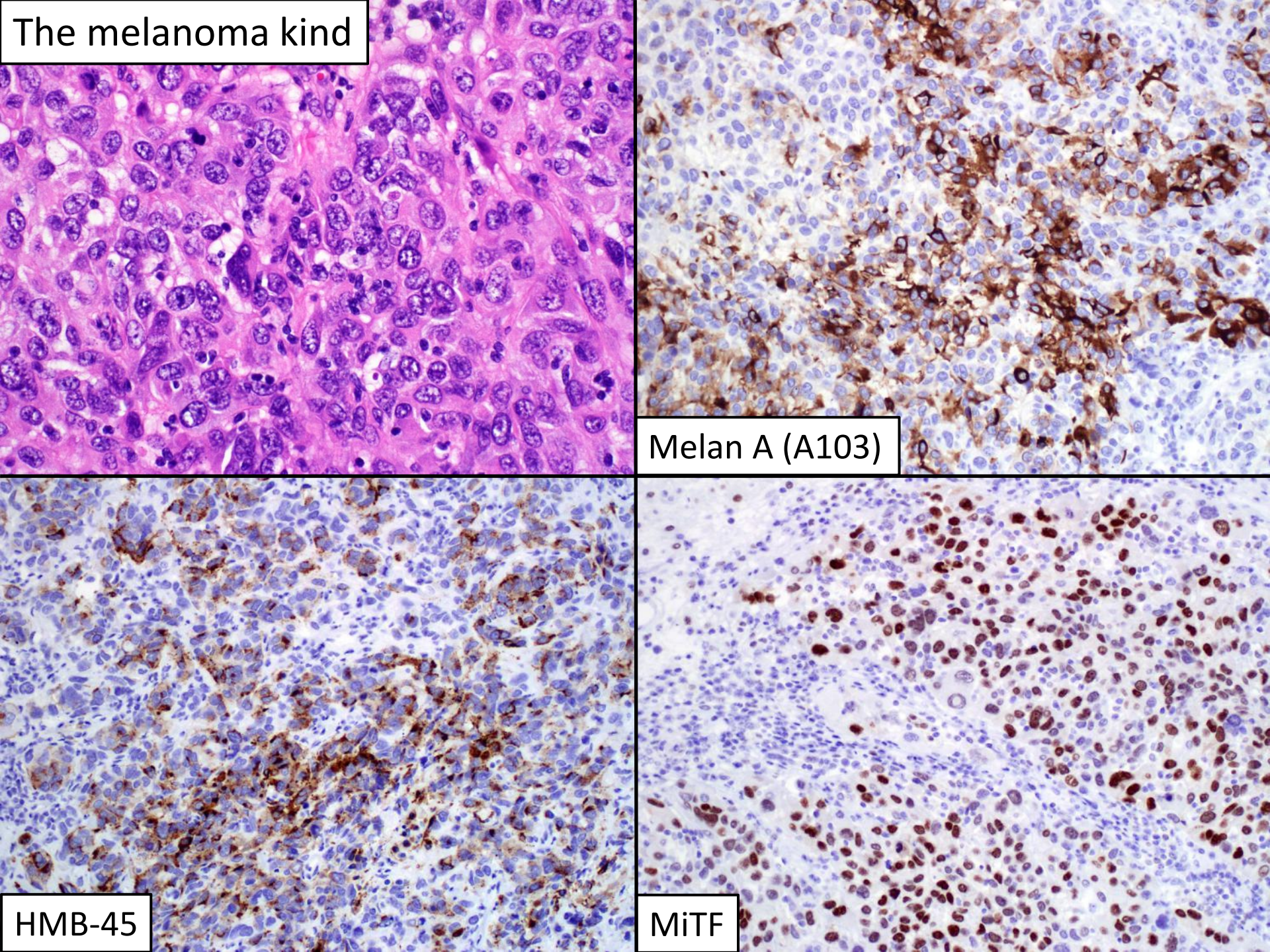
Dr. Bellizzi, what kind of S-100-positive carcinoma is this?



S-100 Expression in Adenocarcinoma

	Primary Tumors	Metastatic Tumors
Salivary gland	80% (n=15)	75% (n=4)
Lung	7% (n=27)	12% (n=25)
Breast	60% (n=20)	62% (n=8)
Esophagus	0% (n=8)	0% (n=2)
Stomach	20% (n=10)	25% (n=8)
Gallbladder	0% (n=1)	0% (n=1)
Colorectum	25% (n=28)	23% (n=13)
Pancreas	0% (n=8)	0% (n=5)
Kidney	65% (n=23)	66% (n=3)
Endometrium	78% (n=36)	64% (n=14)
Ovary	84% (n=24)	87% (n=22)
Prostate	0% (n=27)	0% (n=8)
Unknown origin		22% (n=9)
Total	43% (n=228)	39% (n=122)

Herrera GA, et al. *Am J Clin Pathol*. 1988 Feb;89(2):168-76.


The melanoma kind
Melan A (A103)
HMB-45
MiTF

Broad-Spectrum Epithelial Marker Expression in Melanoma

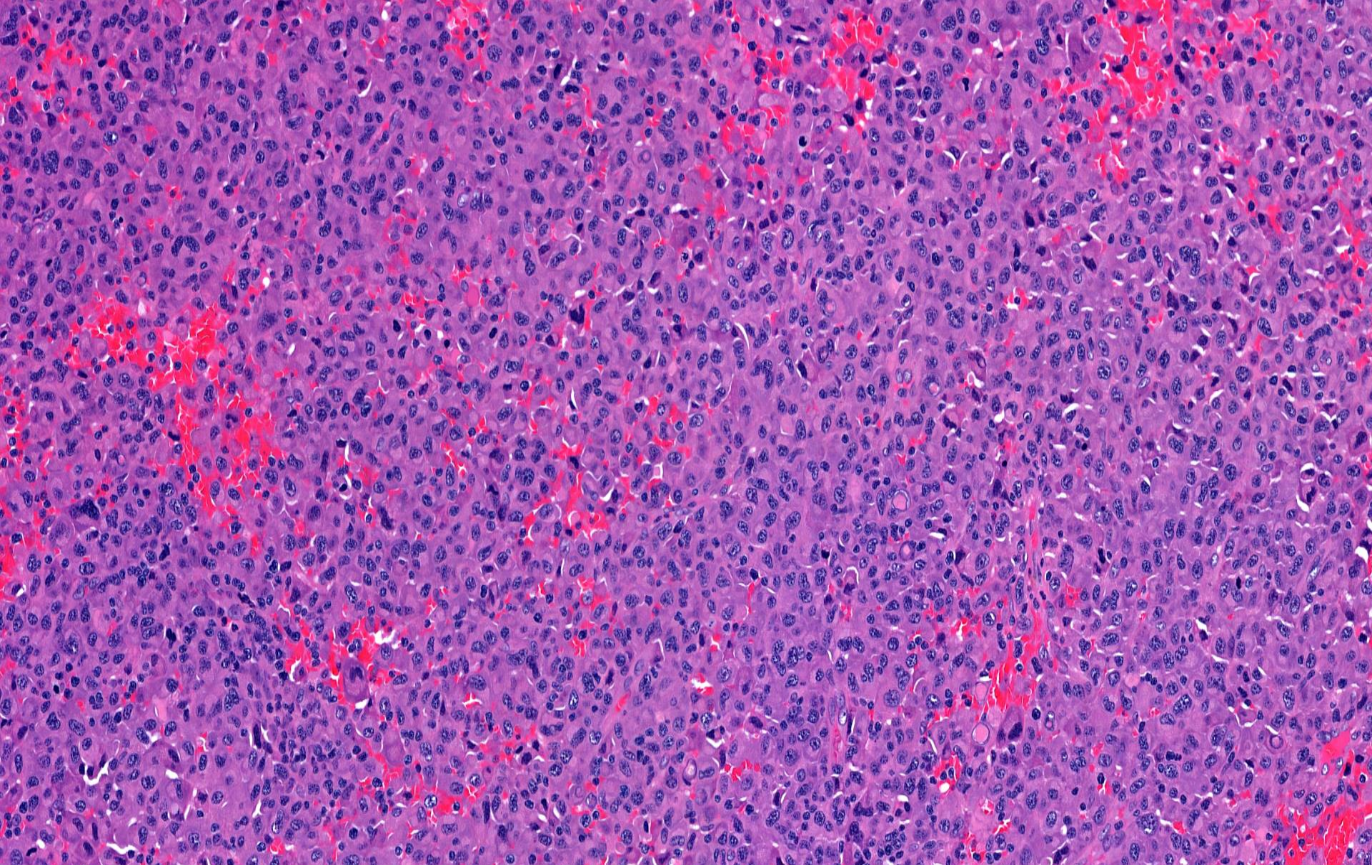
	E-cadherin % positive (median H-score)	EMA % positive (median H-score)	AE1/AE3 % positive (median H-score)
Primary (n=137)	77% (80)	18% (17)	7% (23)
Metastasis (n=139)	74% (112)	14% (7)	10% (20)

MOC-31 and Ber-EP4 were uniformly negative

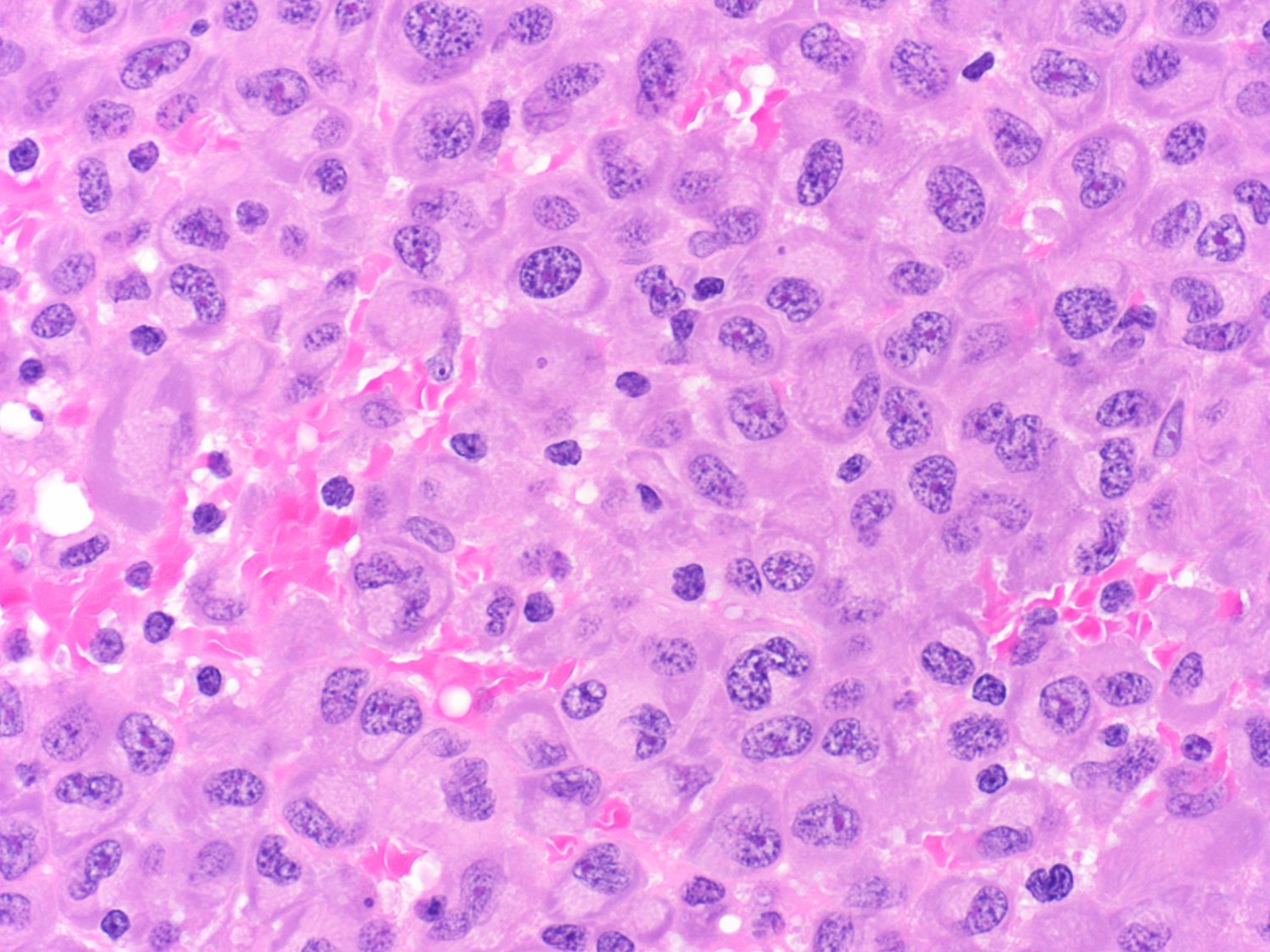
When Two Broad Tumor Class Screening Markers are at Odds

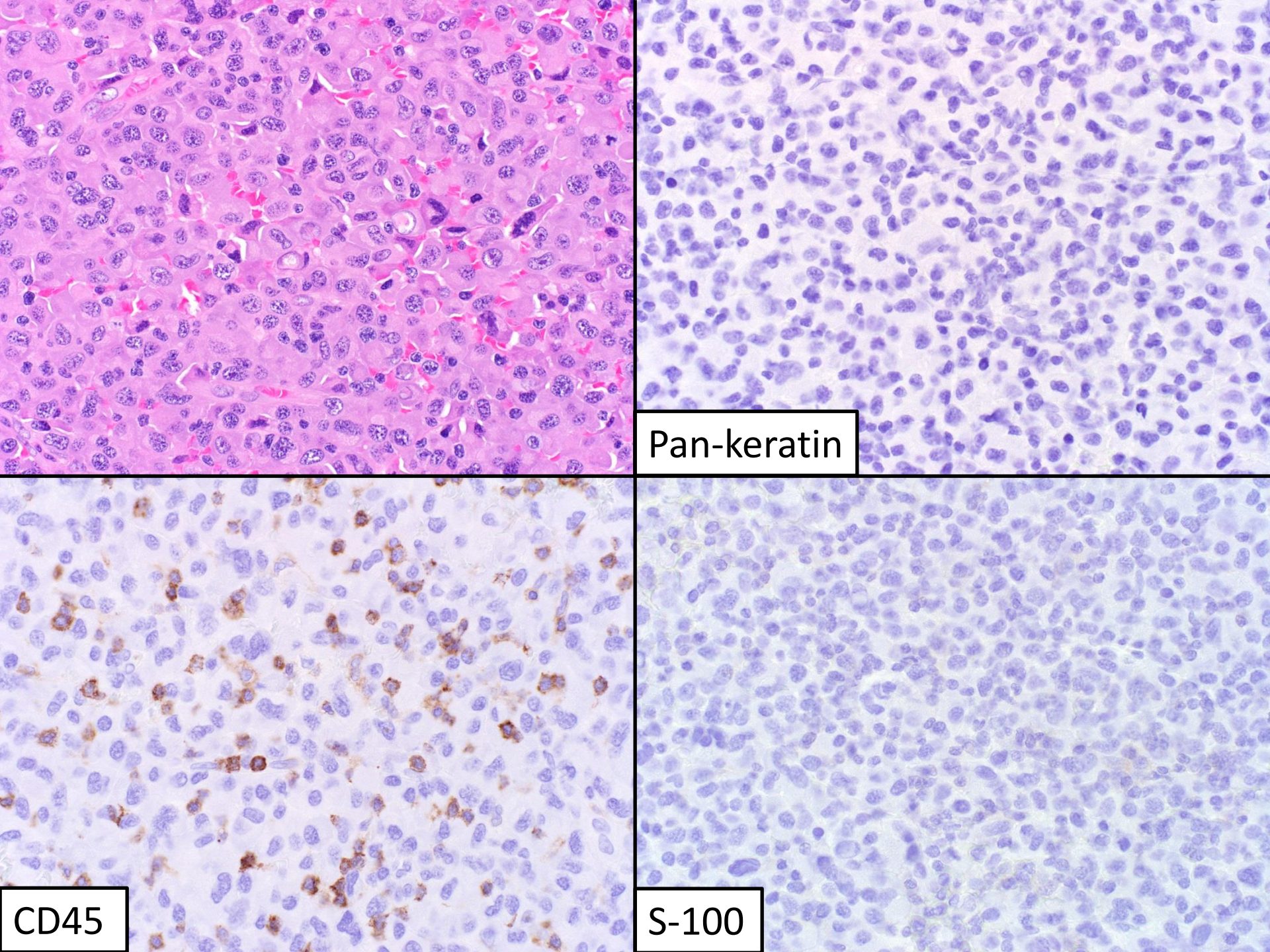
(more generally, when markers for both of the entities in your differential are expressed):

- You have to solve the differential diagnosis
- The stronger marker often wins

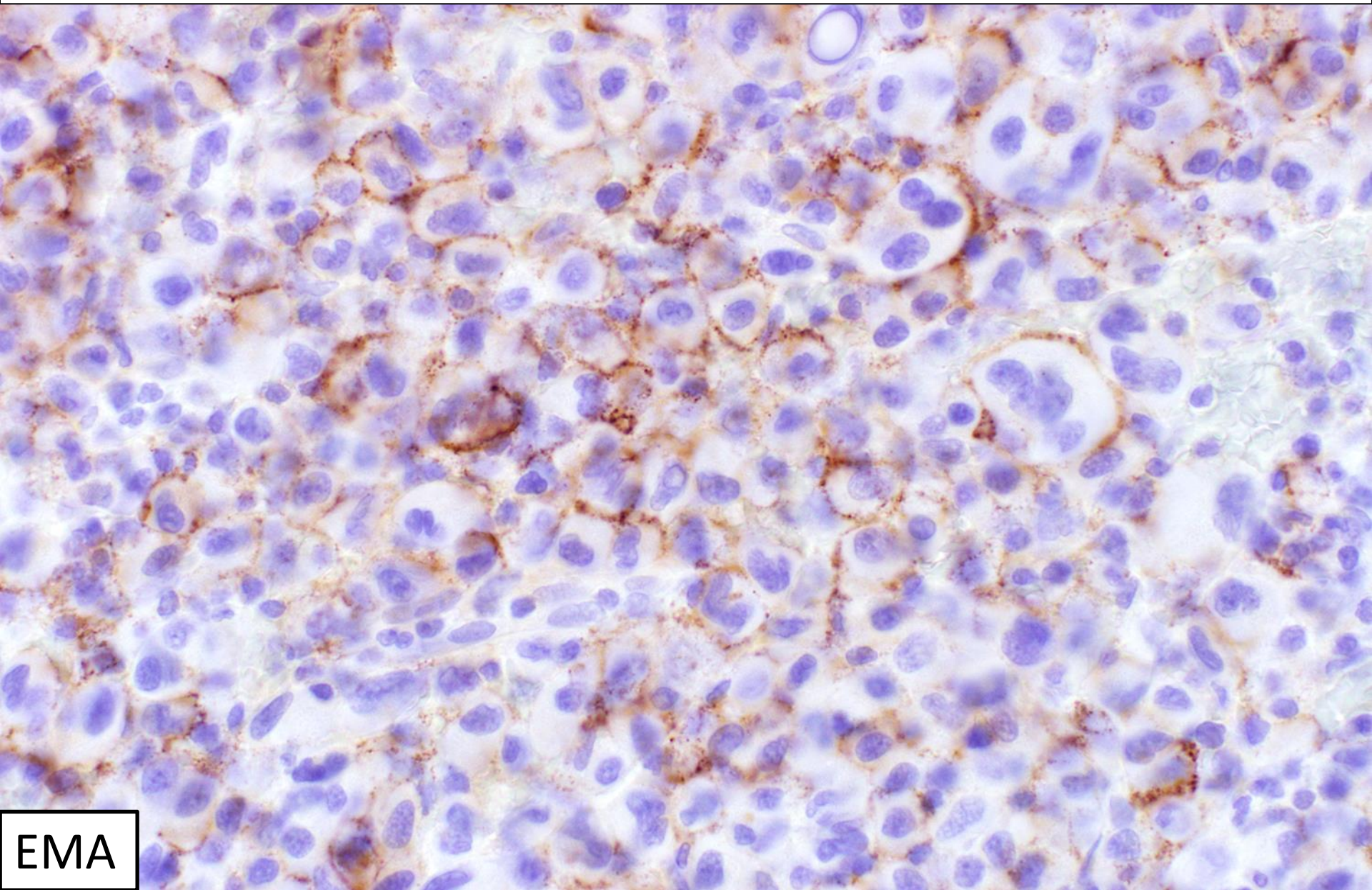


73-year-old man with large naval cavity mass. This case was referred by a former trainee. Her morphologic impression was melanoma and her immunopanel included a broad-spectrum keratin, S-100, and melan A

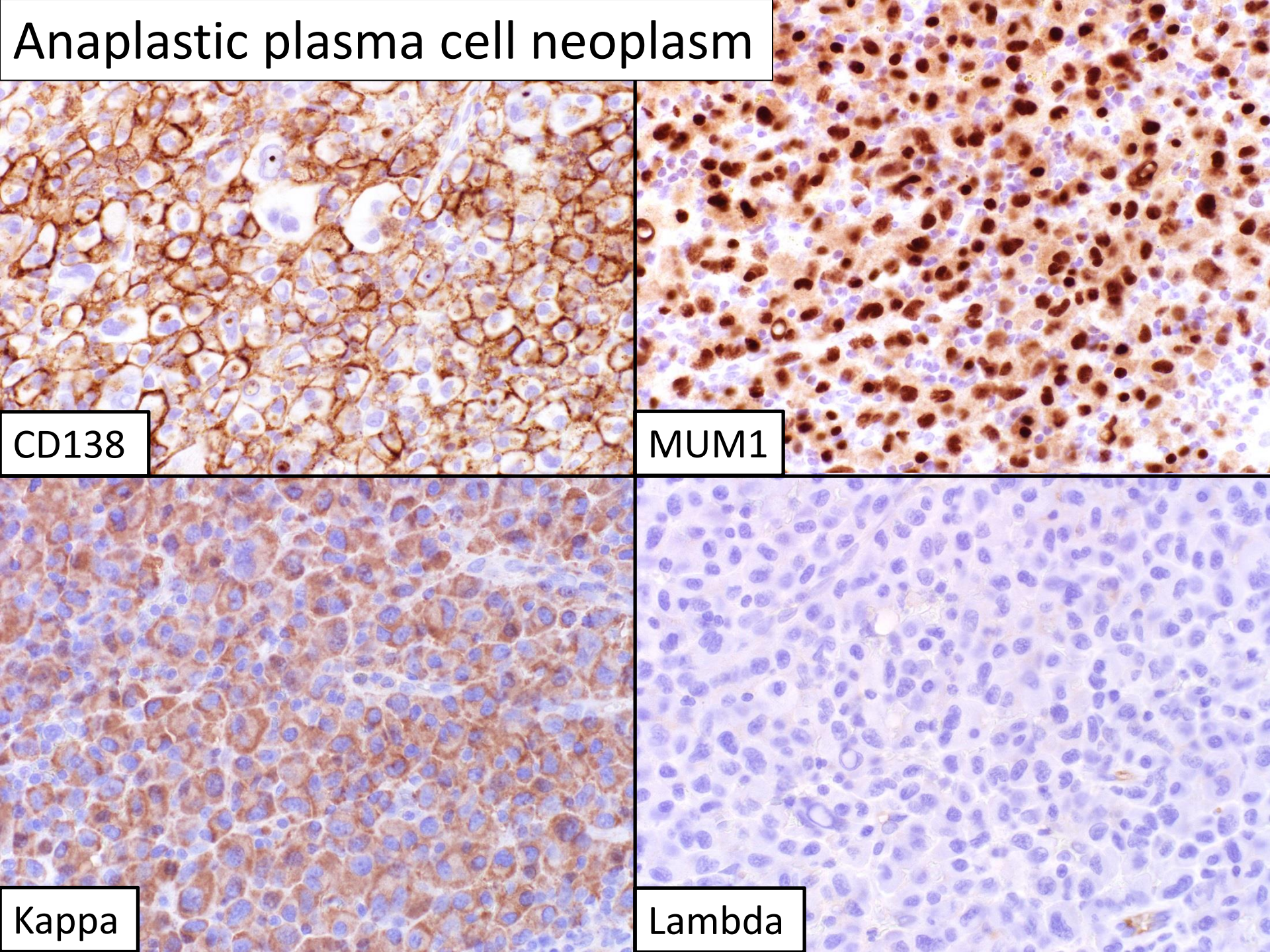



Pan-keratin
CD45
S-100

My smarty pants fellow thought this might be a SMARCB1-deficient sinonasal carcinoma, but INI1 was intact

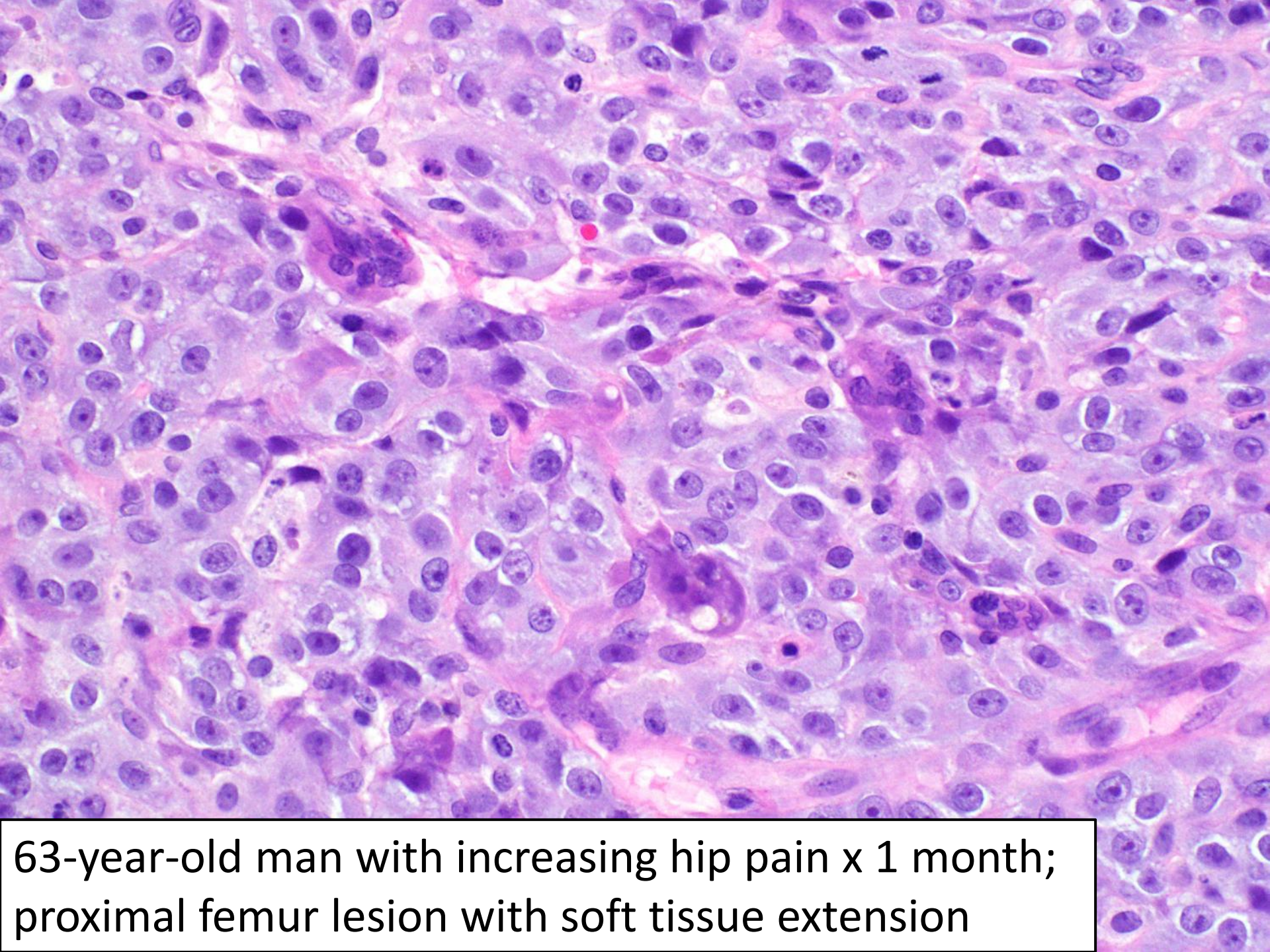


Anaplastic plasma cell neoplasm



EMA+ Only: Beware

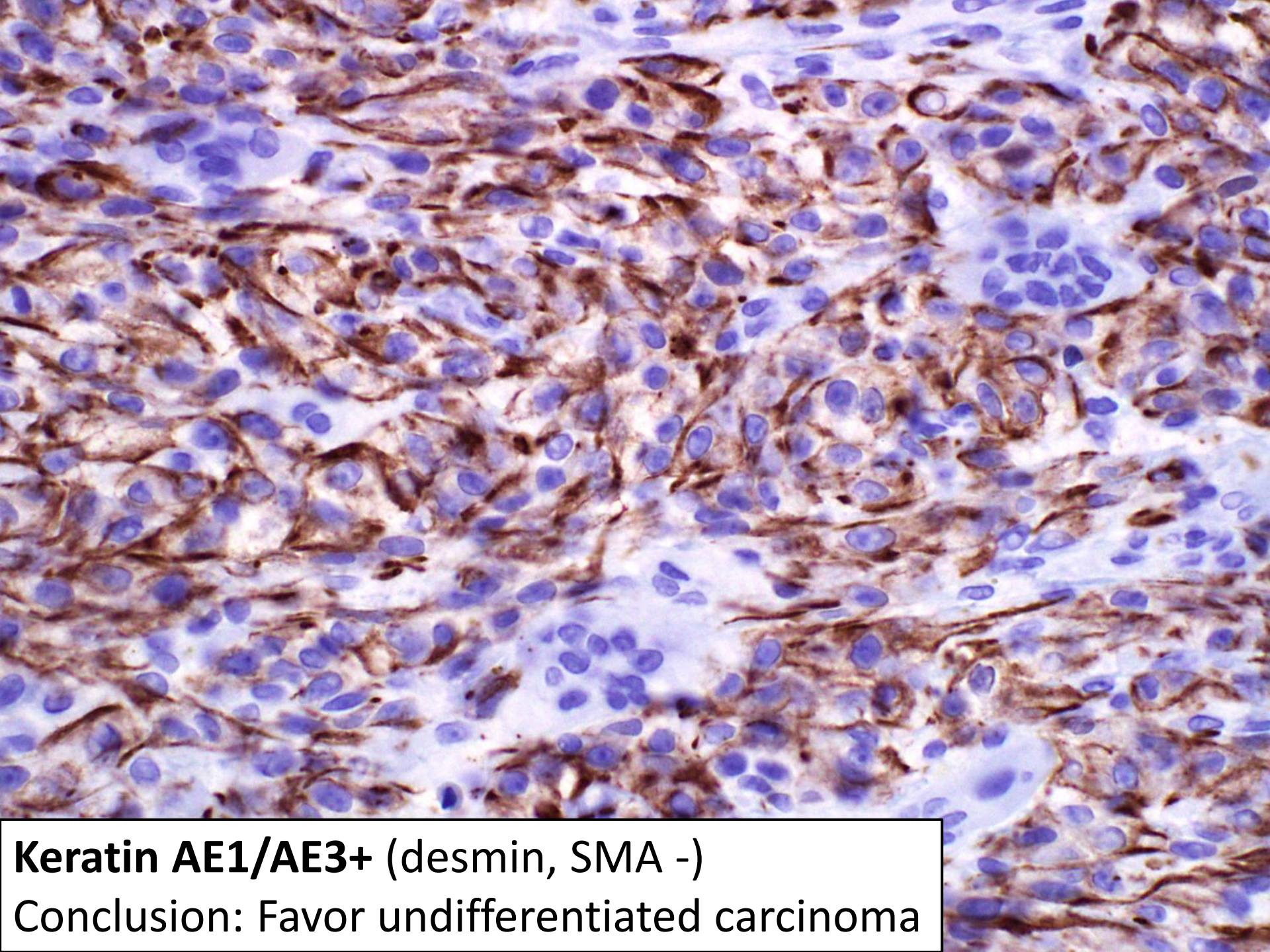
EMA-Positive Hematolymphoid Neoplasms	LCA-Weak to Negative Hematolymphoid Neoplasms
	Lymphoblastic leukemia/lymphoma
	Classical Hodgkin lymphoma
Plasma cell neoplasm	**Plasma cell neoplasm**
Plasmablastic lymphoma	**Plasmablastic lymphoma**
Anaplastic large cell lymphoma	**Anaplastic large cell lymphoma**
ALK+ DLBCL	**ALK+ DLBCL**
Follicular dendritic cell sarcoma	**Follicular dendritic cell sarcoma**
T-cell/histiocyte rich DLBCL	
Primary effusion lymphoma	



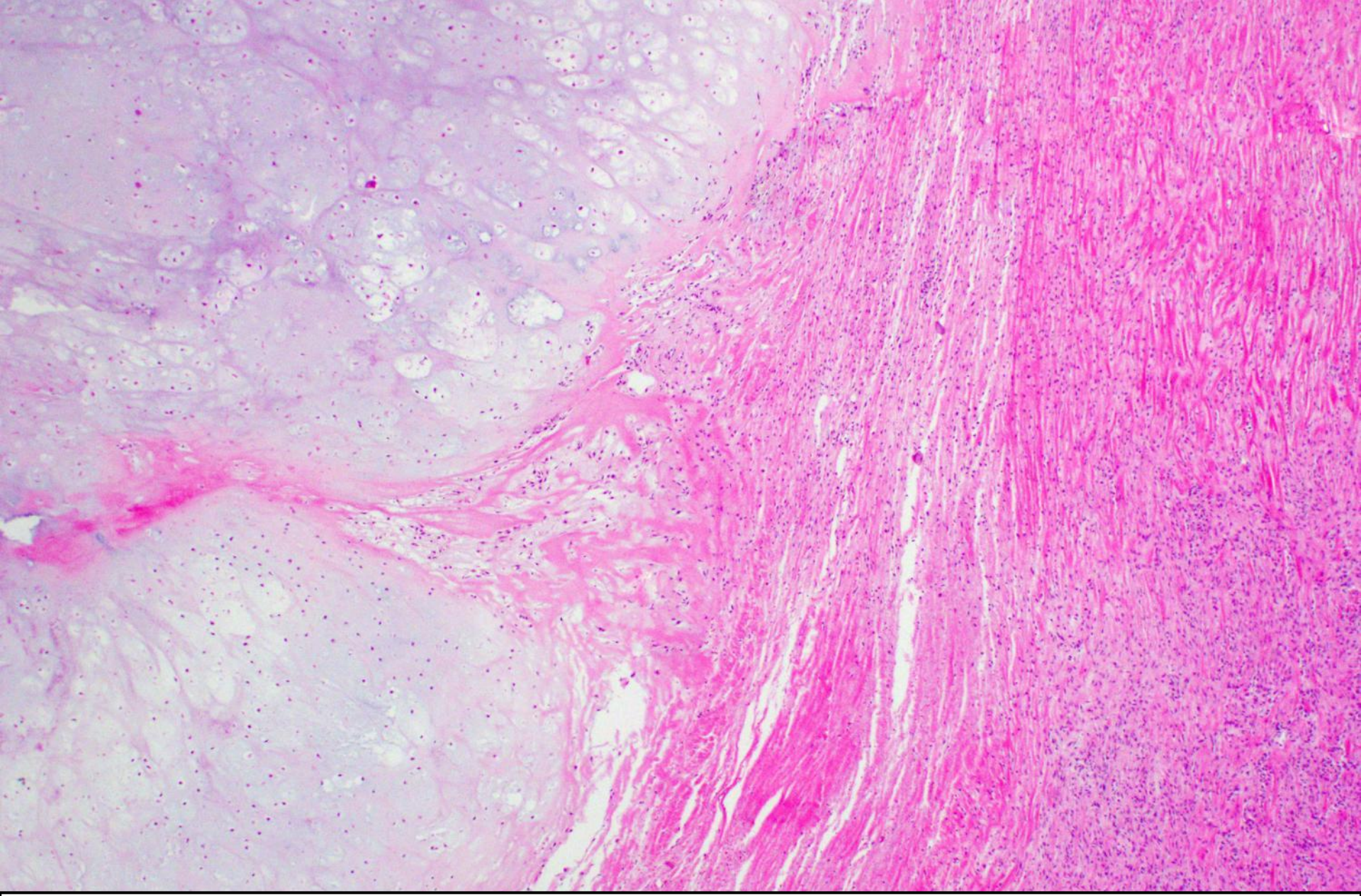
63-year-old man with increasing hip pain x 1 month; proximal femur lesion with soft tissue extension

Undifferentiated Malignant Neoplasm with Osteoclast-like Giant Cells

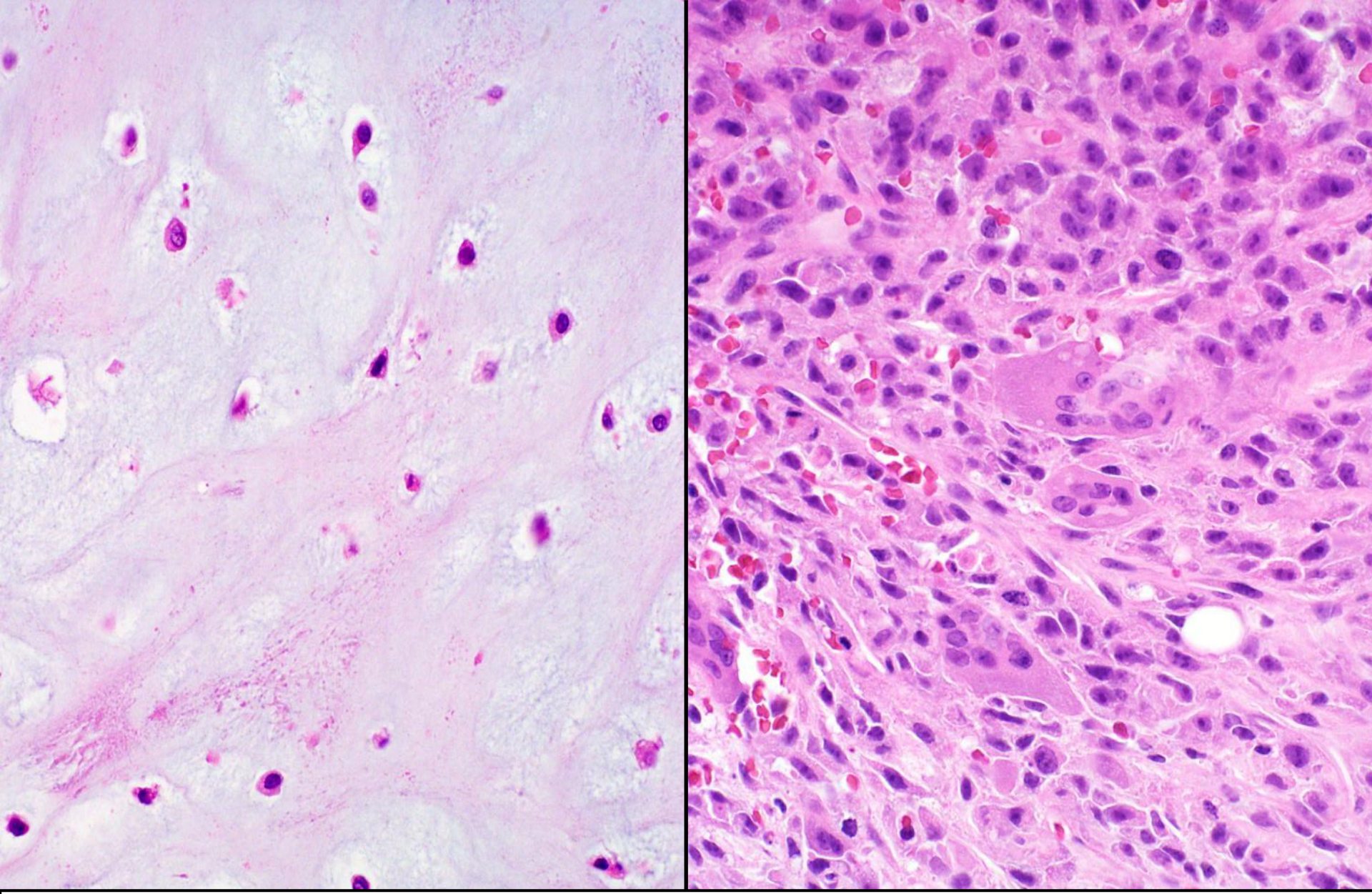
- Undifferentiated/anaplastic carcinoma
  - Keratin AE1/AE3
  - CDX2, PAX8, TTF-1
- Osteosarcoma
  - SATB2
- Leiomyosarcoma
  - Desmin, SMA, caldesmon



Keratin AE1/AE3+ (desmin, SMA -)
Conclusion: Favor undifferentiated carcinoma



Musculoskeletal radiologist sug. presence of chondroid matrix (MRI);
Subsequent femoral head resection for pathologic fracture



Abrupt transition from WD chondrosarcoma to undifferentiated neoplasm → dedifferentiated chondrosarcoma

Keratin-Positive Soft Tissue Tumors

- Chondroid lipoma
- Pleomorphic liposarcoma
- Desmoplastic fibroblastoma
- Solitary fibrous tumor
- Inflammatory myofibroblastic tumor
- Myxoinflammatory fibroblastic sarcoma
- **Leiomyosarcoma**
- Rhabdomyosarcoma
- Schwannoma (cross-reactivity with GFAP)
- Epithelioid hemangioma
- Pseudomyogenic hemangioendothelioma
- Epithelioid hemangioendothelioma
- **Angiosarcoma**
- Gastrointestinal stromal tumor
- Sclerosing perineurioma
- Dermal nerve sheath myxoma
- Epithelioid MPNST
- Ectopic hamartomatous thymoma
- Ossifying fibromyxoid tumor
- Myoepithelial tumors of soft tissue
- **Synovial sarcoma**
- **Epithelioid sarcoma**
- Desmoplastic small round cell tumor
- Extrarenal rhabdoid tumor
- Undifferentiated/unclassified sarcoma
- Chondroblastoma
- **Dedifferentiated chondrosarcoma**
- Conventional osteosarcoma
- Ewing sarcoma
- Chordoma
- Adamantinoma
- Osteofibrous dysplasia

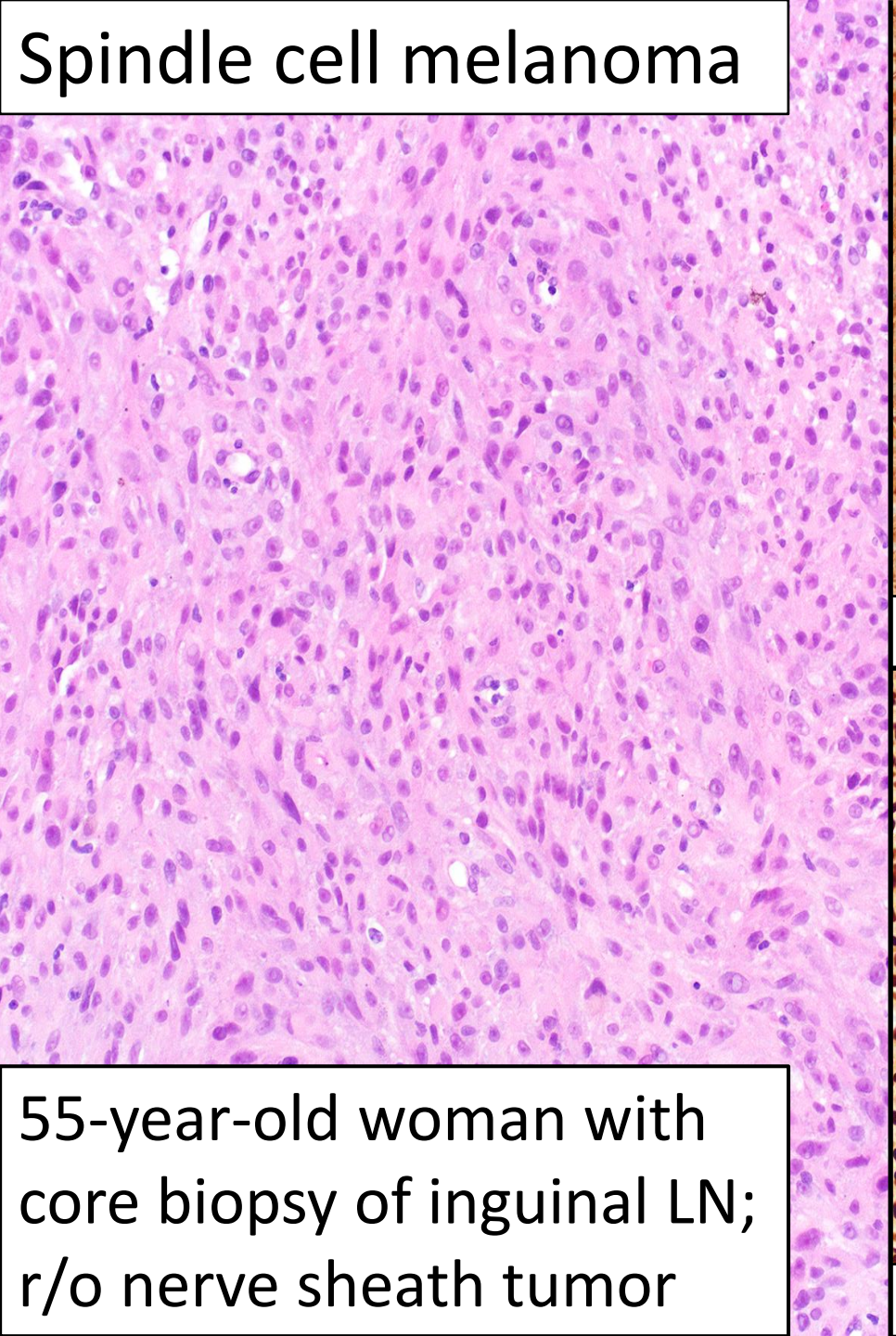
EMA-Positive Soft Tissue Tumors

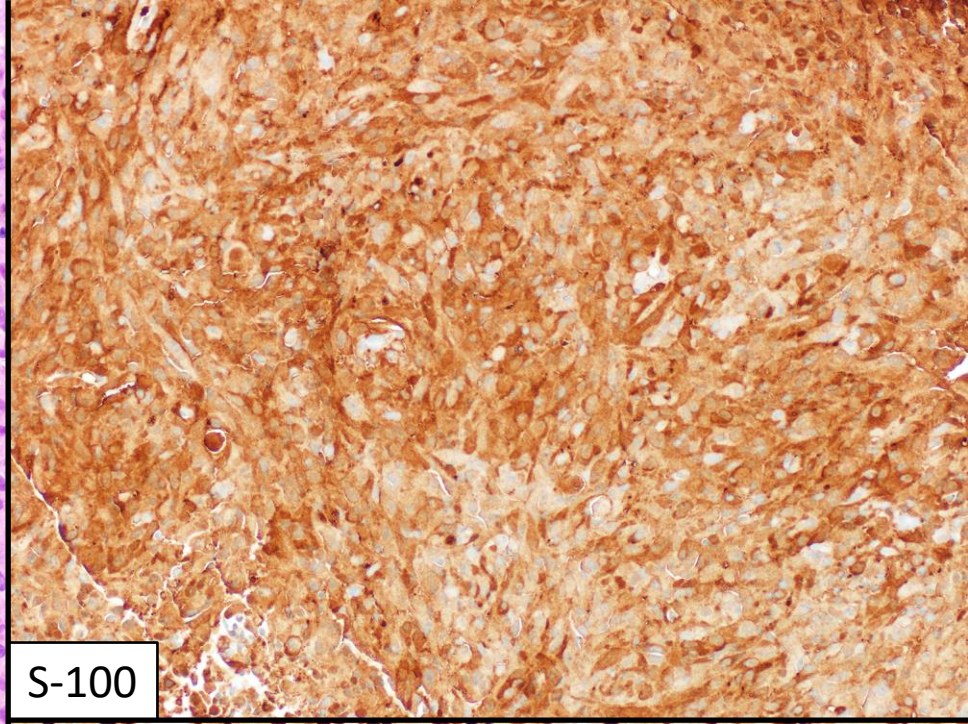
- Pleomorphic liposarcoma
- Calcifying aponeurotic fibroma
- Lipofibromatosis
- Dermatofibrosarcoma protuberans
- Solitary fibrous tumor
- **Low-grade fibromyxoid sarcoma**
- Sclerosing epithelioid fibrosarcoma
- **Leiomyosarcoma**
- Pleomorphic rhabdomyosarcoma
- Epithelioid hemangioma
- Epithelioid hemangioendothelioma
- **Angiosarcoma**
- Neurofibroma
- **Perineurioma**
- Dermal nerve sheath myxoma
- Solitary circumscribed neuroma
- **Meningioma**
- Hybrid nerve sheath tumor
- Acral fibromyxoma
- Angiomatoid fibrous histiocytoma
- Myoepithelial tumors of soft tissue
- **Synovial sarcoma**
- Epithelioid sarcoma
- Desmoplastic small round cell tumor
- Extrarenal rhabdoid tumor
- Undifferentiated/unclassified sarcoma
- Conventional osteosarcoma
- Chordoma
- Epithelioid hemangioma
- Adamantinoma

Non-Canonical Expression of Broad Tumor Class Screening Markers

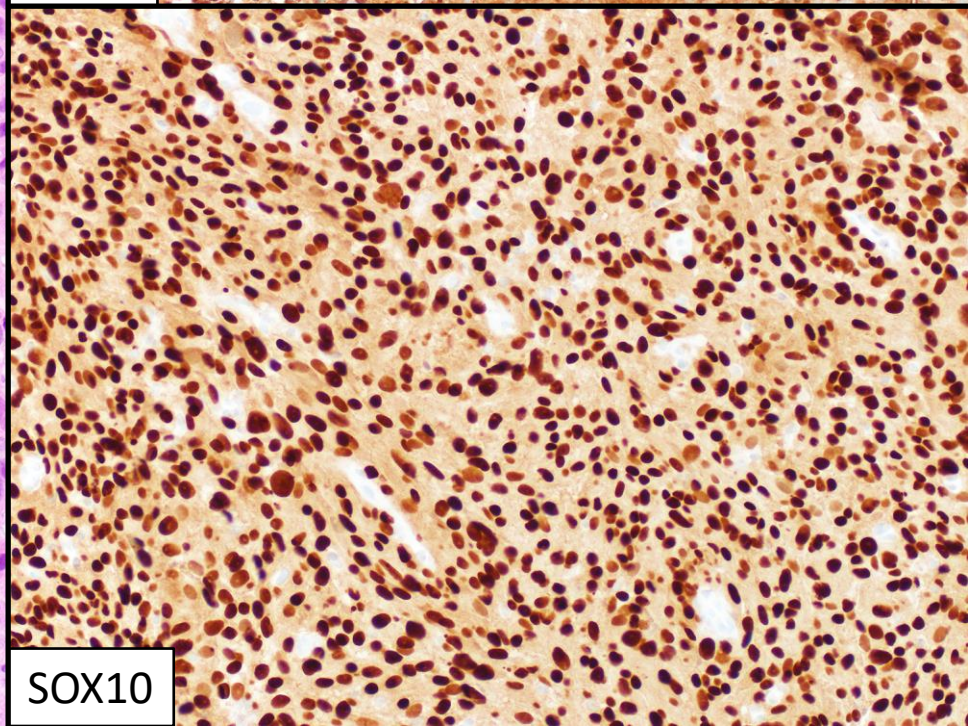
Marker Category	Non-Canonical Expressors
Broad-Spectrum Epithelial Markers	• **Sarcomas with epithelioid cytomorphology, small round blue cell sarcomas, leiomyosarcoma (30-40% keratin and/or EMA-positive)** • **EMA-positivity in plasma cell neoplasms (most), ALCL (50-95%), DLBCL variants (T-cell/histiocyte-rich, ALK+, plasmablastic, primary effusion lymphoma)**, NLPHL, FDCS • **Up to 25% of metastatic melanomas** (keratin probably>EMA) • **Embryonal carcinoma, yolk sac tumor, choriocarcinoma usually broad-spectrum keratin-positive**; seminoma rarely positive
Melanoma Markers	• **S-100 in 10-40% of carcinomas**, especially salivary gland, breast, and cutaneous adnexal tumors (when using a polyclonal antibody) • **SOX10** in tumors with myoepithelial differentiation, including most **TNBC** • Melan A (clone A103) in adrenal cortical tumors, sex-cord stromal tumors, t(6;11) translocation renal cell carcinomas; clear cell sarcoma, PEComa • MiTF in cutaneous fibrohistiocytic lesions (e.g., dermatofibroma) and undifferentiated pleomorphic sarcoma
Hematolymphoid Markers	• **"CD45 Never Lies"** • **CD138 (syndecan-1) expressed by ≥40% of carcinomas** • CD5/CD7 frequently expressed by GI tract tumors • MUM1 expressed by nearly all melanomas (but not spindle cell/desmoplastic)

Spindle cell melanoma



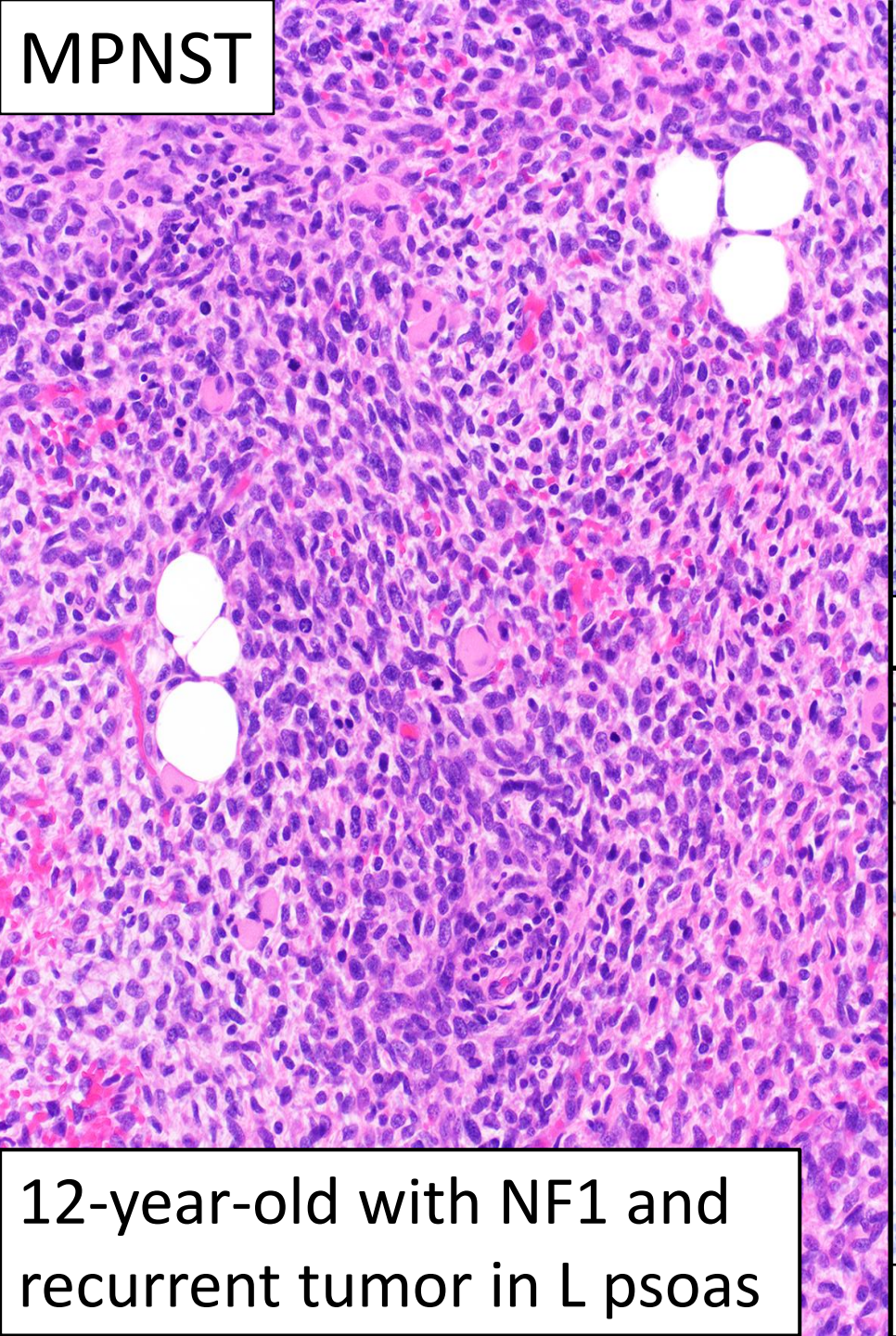


S-100

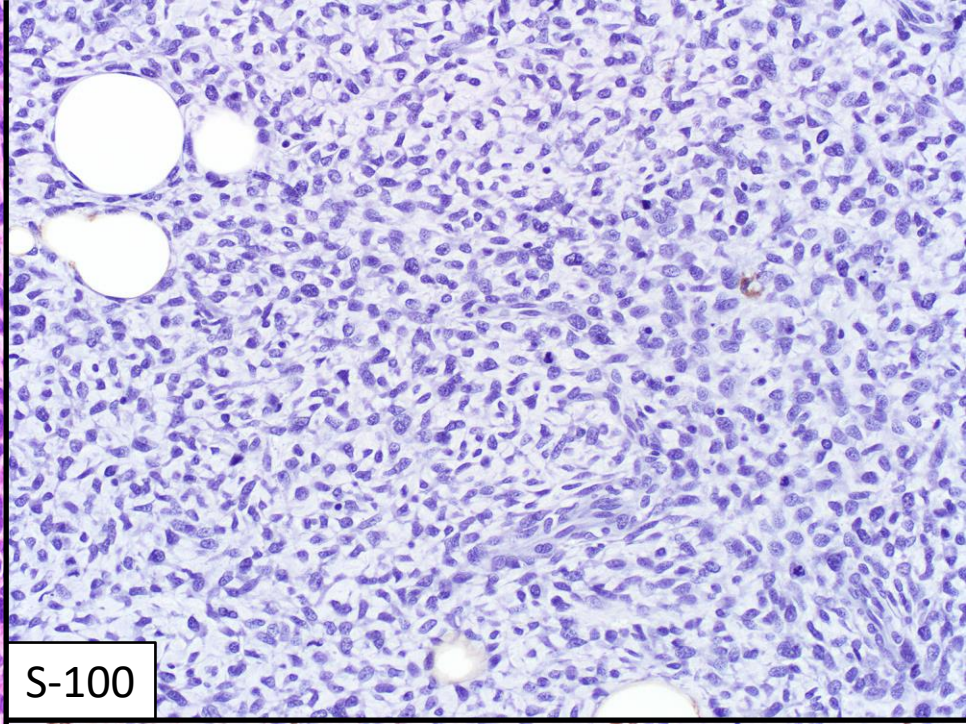


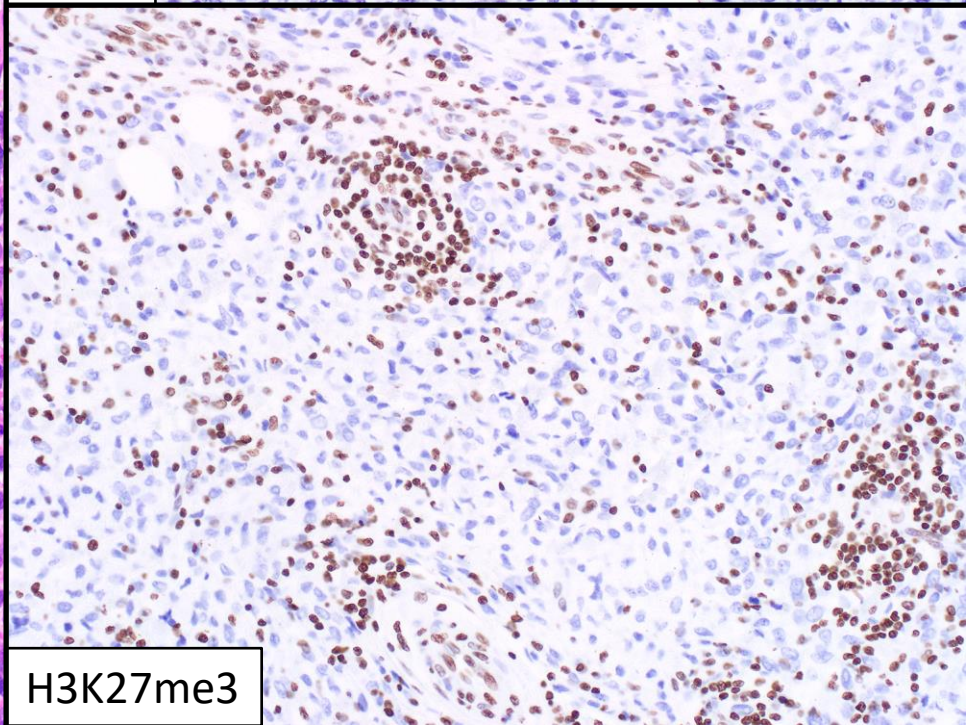
SOX10

55-year-old woman with core biopsy of inguinal LN; r/o nerve sheath tumor



12-year-old with NF1 and recurrent tumor in L psoas





Melanoma Markers in Variants

	Conventional	Spindle Cell	Desmoplastic
S-100	**95%**	**91%**	**96%**
SOX10	**97%**	**100%**	**92%**
Melan A	**85%**	44%	19%
HMB-45	**85%**	46%	9%
MiTF	**89%**	60%	9%
Tryrosinase	**93%**	46%	18%
MUM1	**92%**	67%	0%
BRAFV600E	50%	31%	5%

Weissinger SE, et al. *Mod Pathol*. 2014 Apr;27(4):524-34. PMID: 24051699

S-100 versus SOX10

S-100-, SOX10+

- Some MPNSTs, melanomas, and carcinomas with myoepithelial differentiation (sensitivity issue)

S-100+, SOX10+

- Melanoma
- Nerve Sheath Tumors
- Neoplasms with myoepithelial differentiation

S-100-, SOX10-

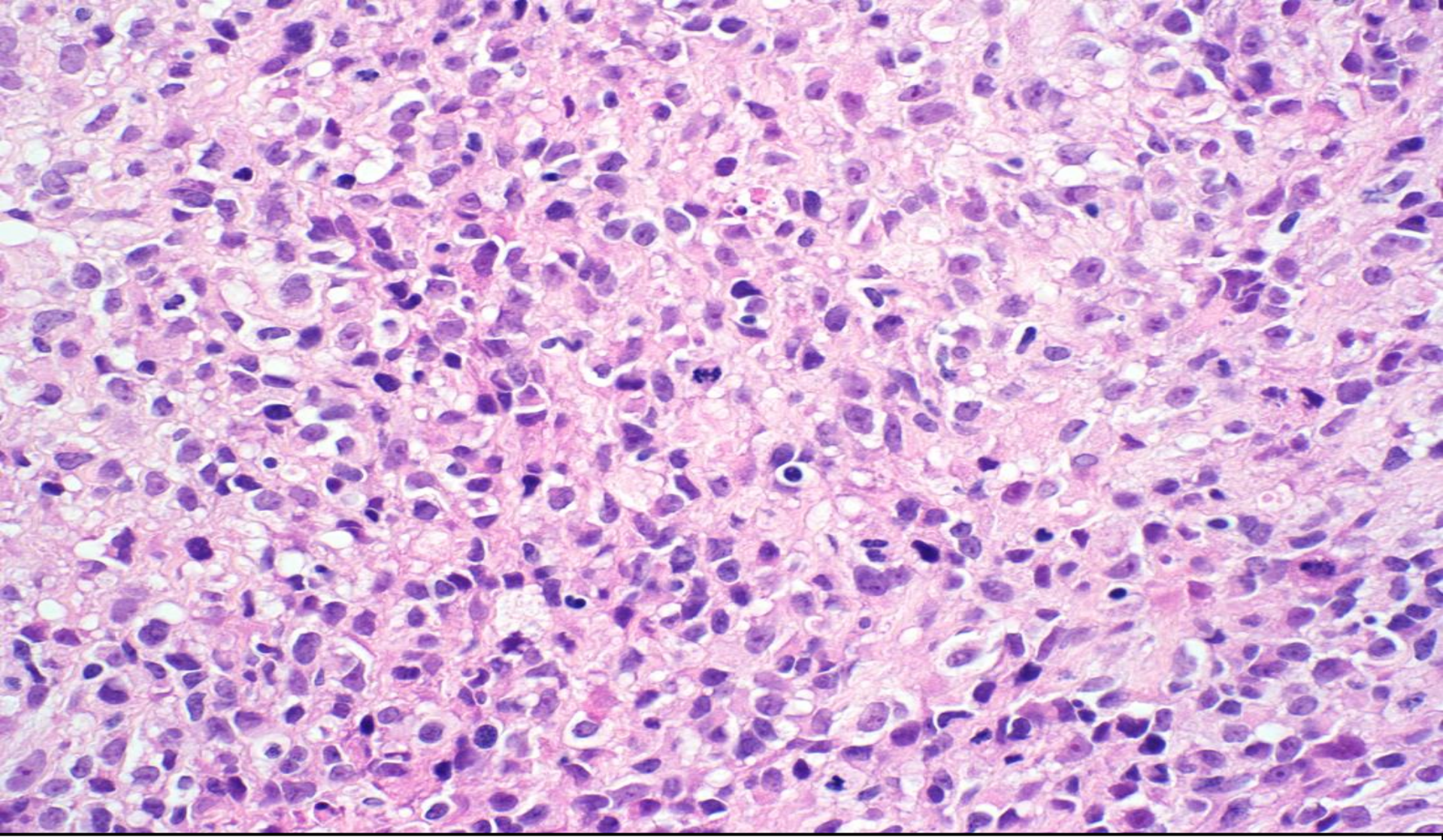
- Most carcinomas, sarcomas, lymphomas
- Mesothelioma
- Germ cell tumor
- Pheochromocytoma/paraganglioma (though both exp. By sustentacular cells)

S-100+, SOX10-

- Tumors of adipocytic/chondroid lineage
- Chordoma
- Ossifying fibromyxoid tumor
- Biphenotypic sinonasal sarcoma
- Lipofibromatosis-like neural tumor
- Infantile fibrosarcoma-like tumor
- Rare cases of Ewing, RMS, SS
- S-100+ carcinomas without myoepithelial differentiation (S-100A1, S-100A6-exp.)
- S-100+ histiocytic/dendritic cell tumors (Langerhans cell histiocytosis, Rosai-Dorfman, interdigitating dendritic cell tumor (100%); histiocytic sarcoma, Erdheim-Chester, blastic plamacytoid dendritic cell tumors (30%); follicular dendritic cell sarcoma, juvenile xanthogranuloma (occ.)

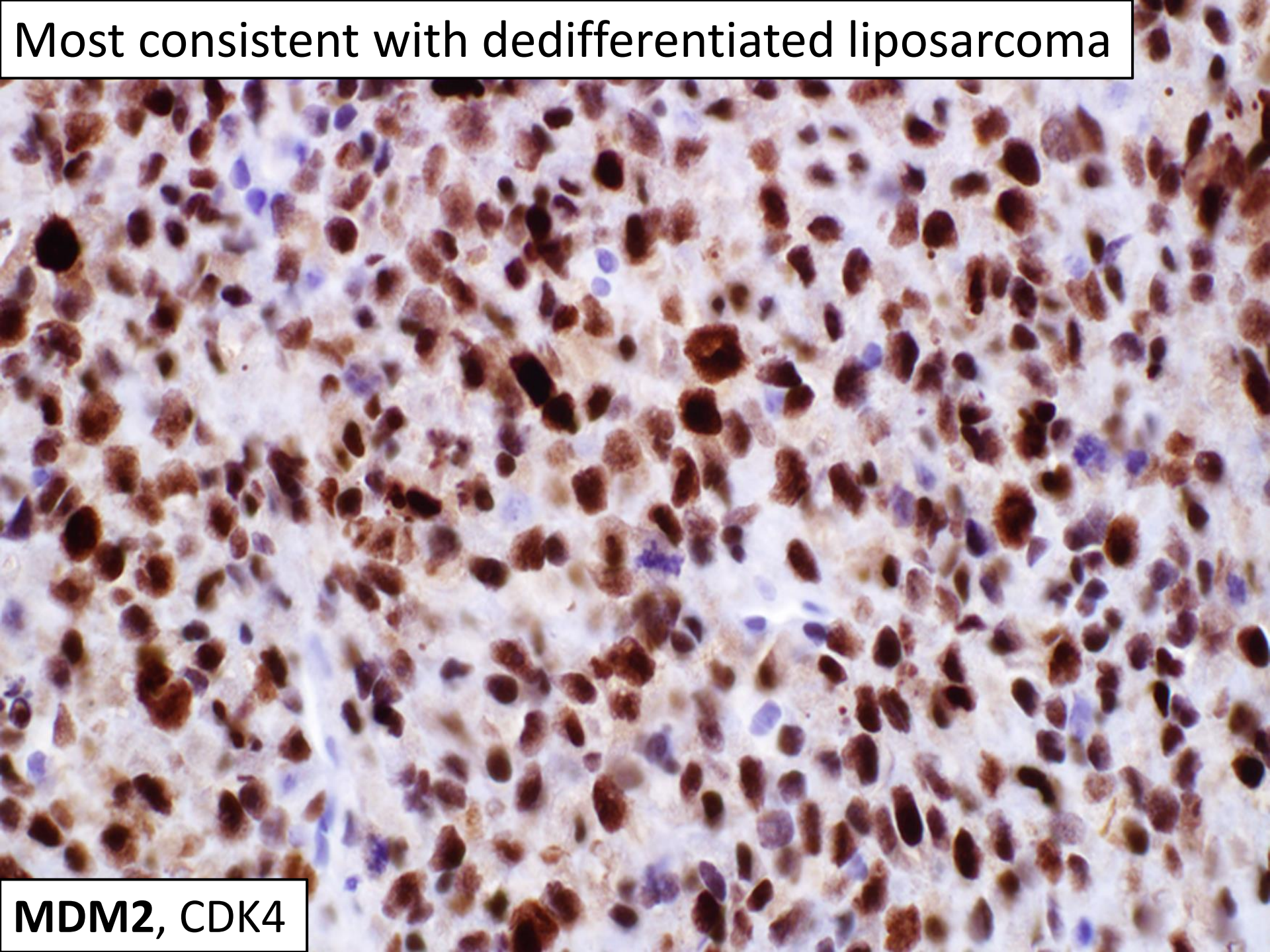
Polyclonal S-100
(S100B>>S100A1>>S100A6)


Parish



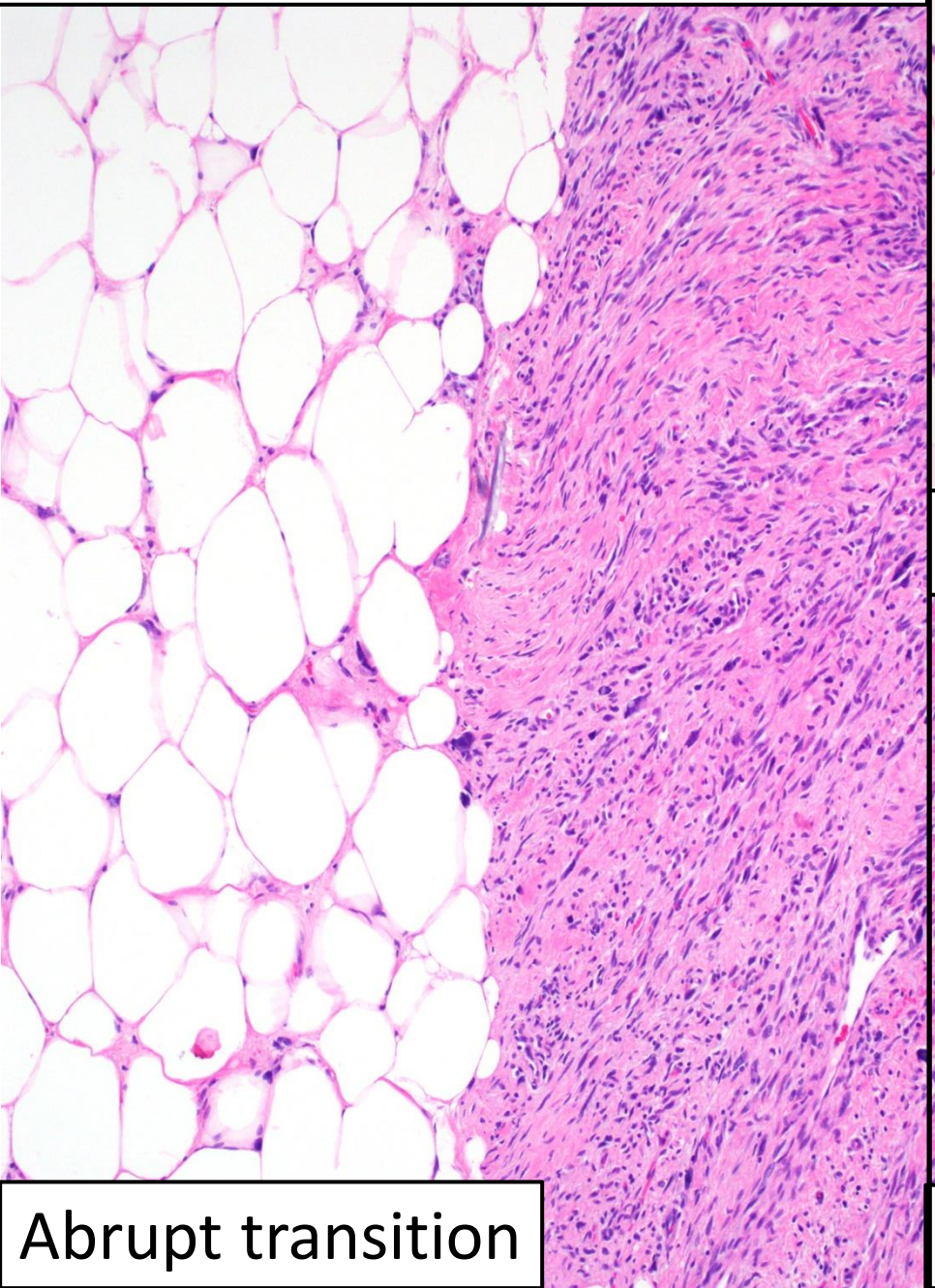
Core biopsy from a 40 cm **retroperitoneal tumor** demonstrates undifferentiated neoplasm composed of sheets of epithelioid cells. After performing 17 immunostains a diagnosis of “malignant neoplasm” indeterminate for sarcoma, carcinoma, or lymphoma was rendered.

Most consistent with dedifferentiated liposarcoma

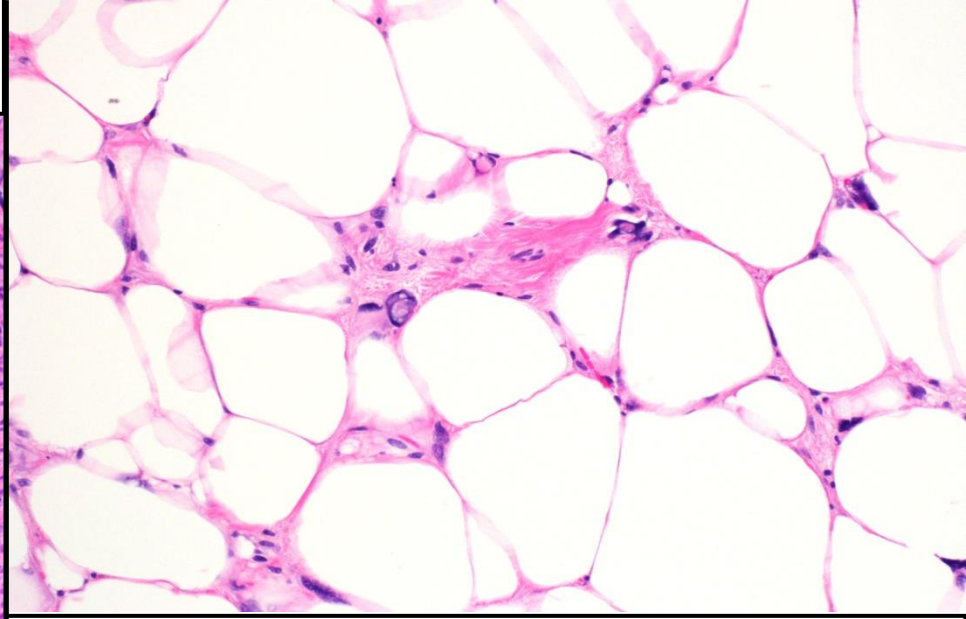


MDM2, CDK4

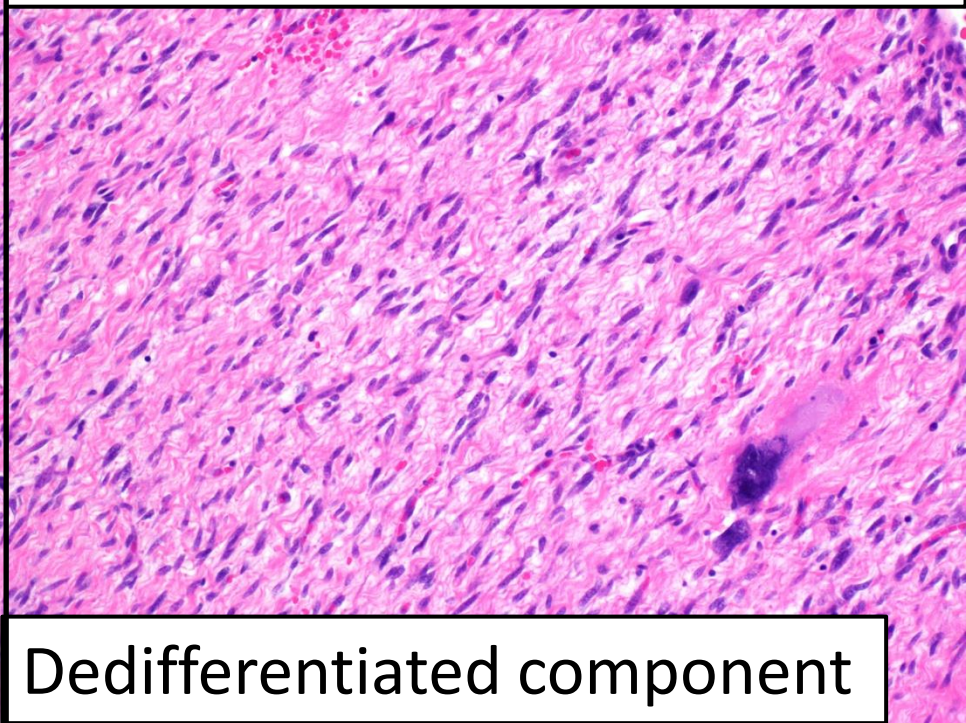
Resection of similar case



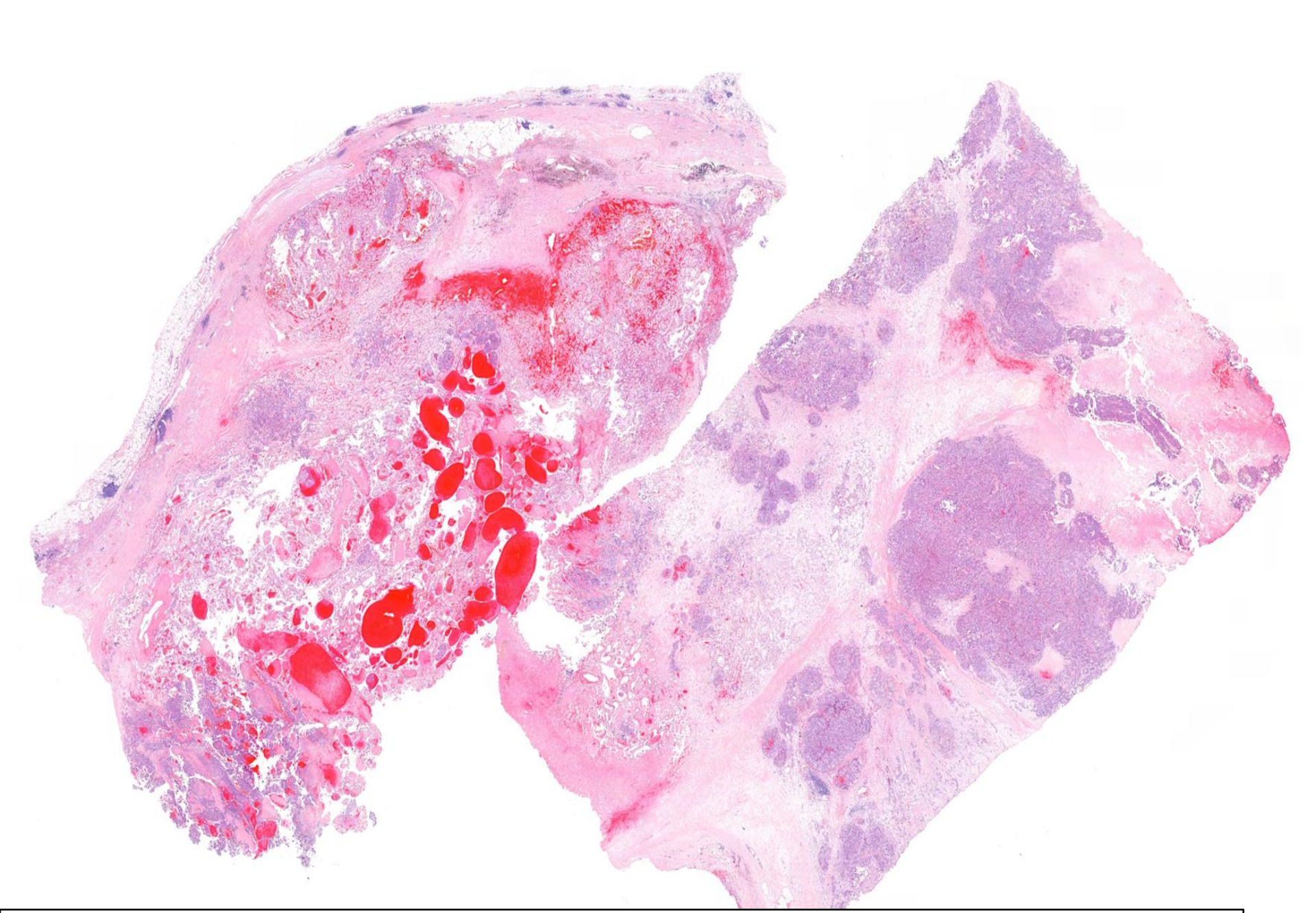
Abrupt transition



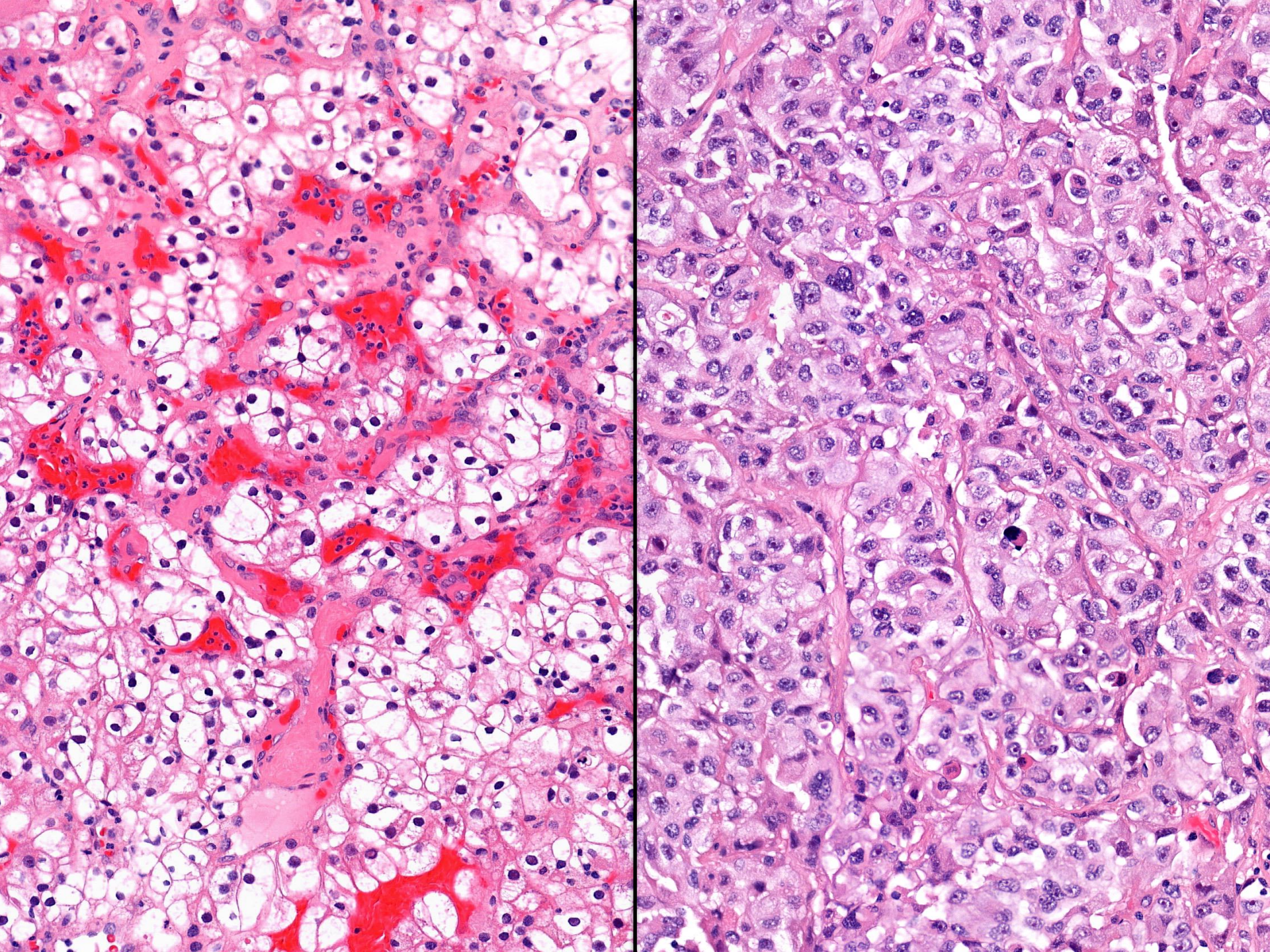
Well-differentiated component

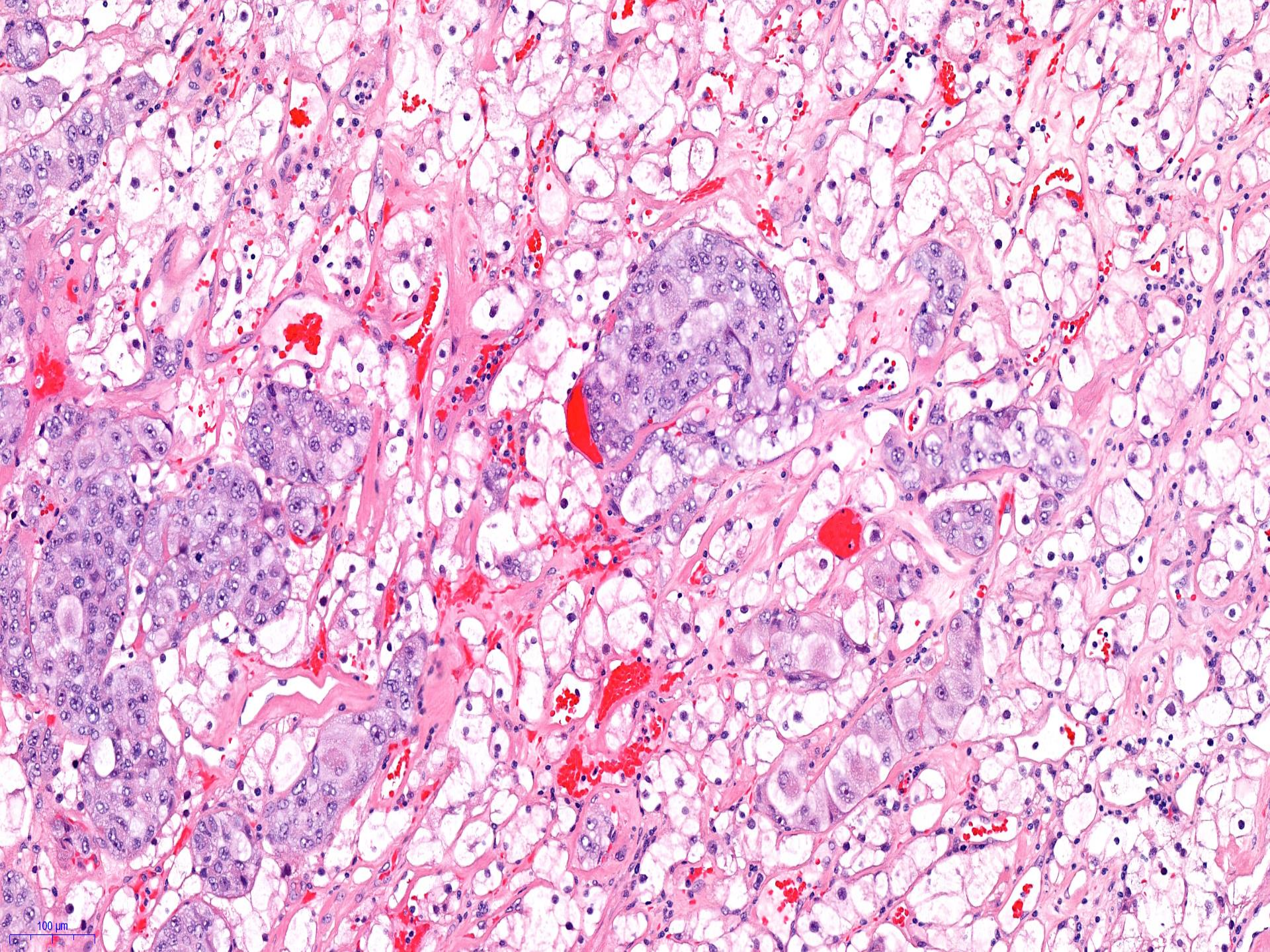


Dedifferentiated component

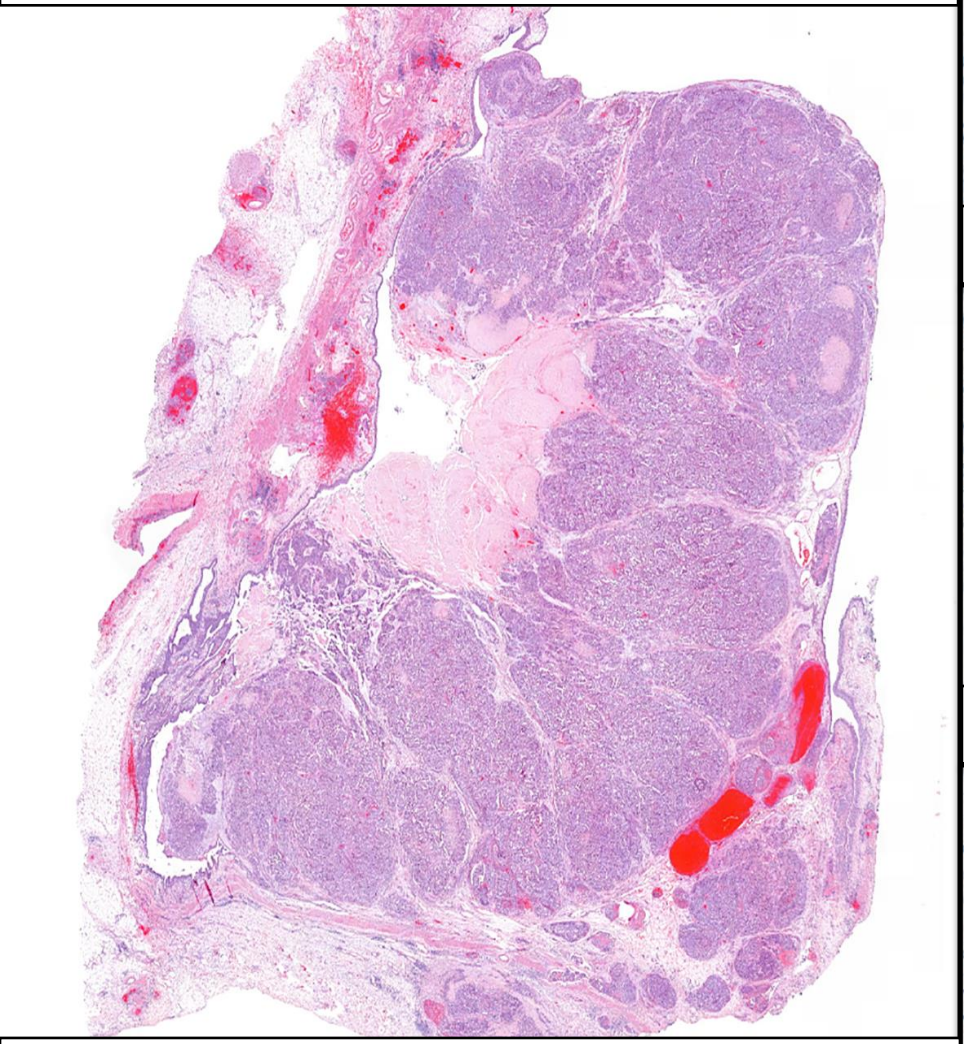


68-year-old woman with large left kidney tumor (12 cm)

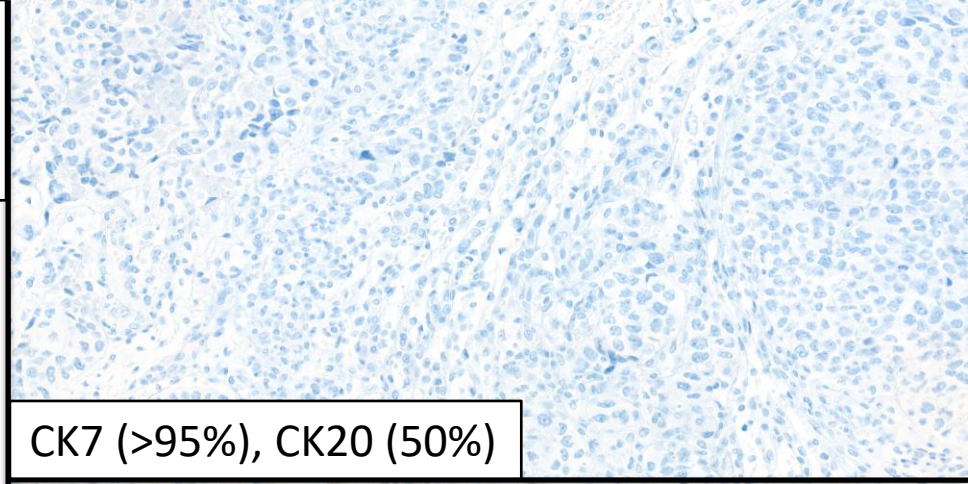



100 µm

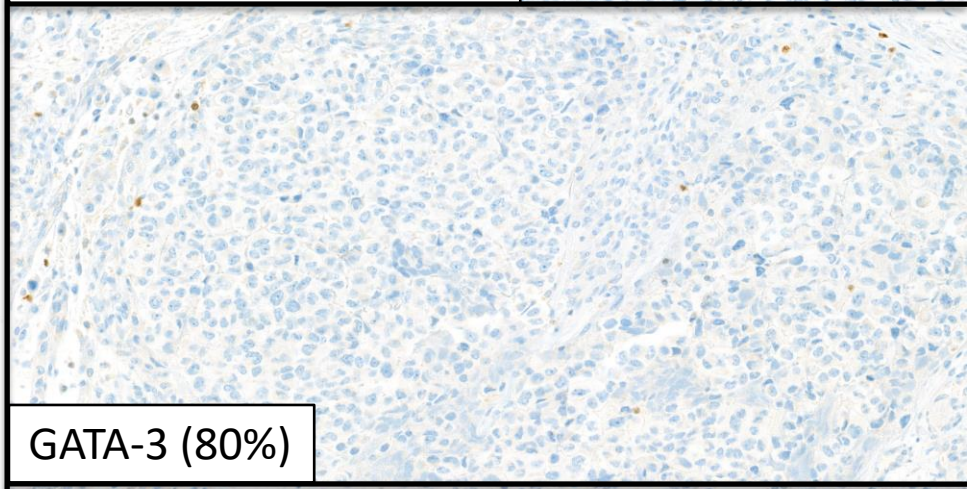
Original Dx: Clear cell RCC and Urothelial Carcinoma



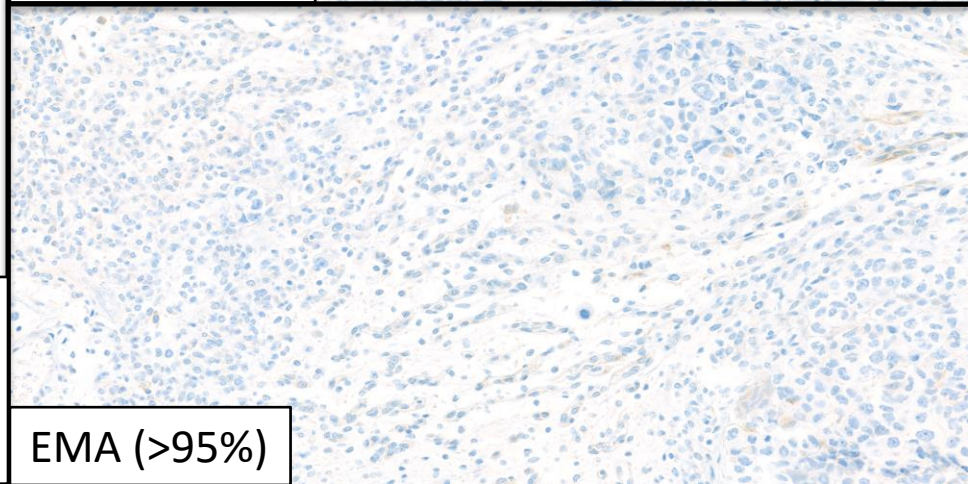
High-grade component involved renal pelvis



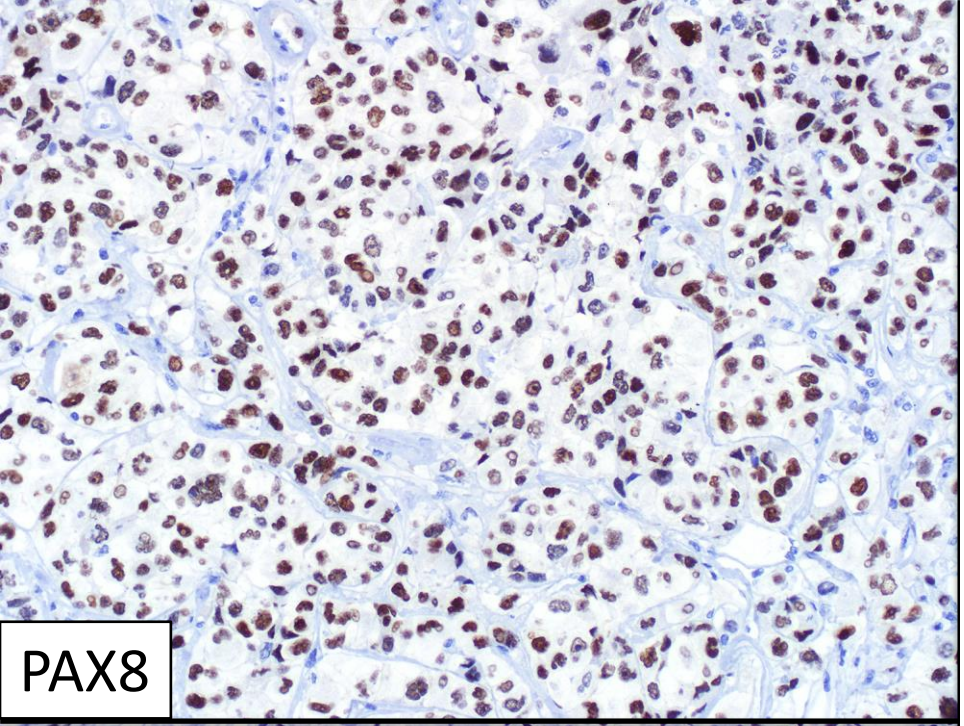
CK7 (>95%), CK20 (50%)

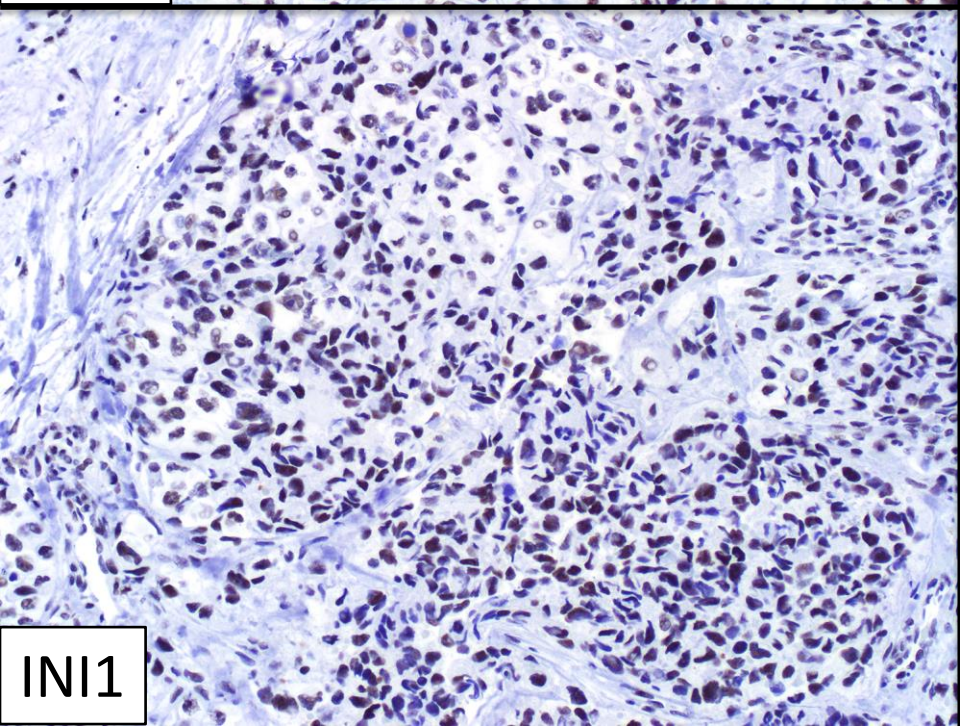


GATA-3 (80%)

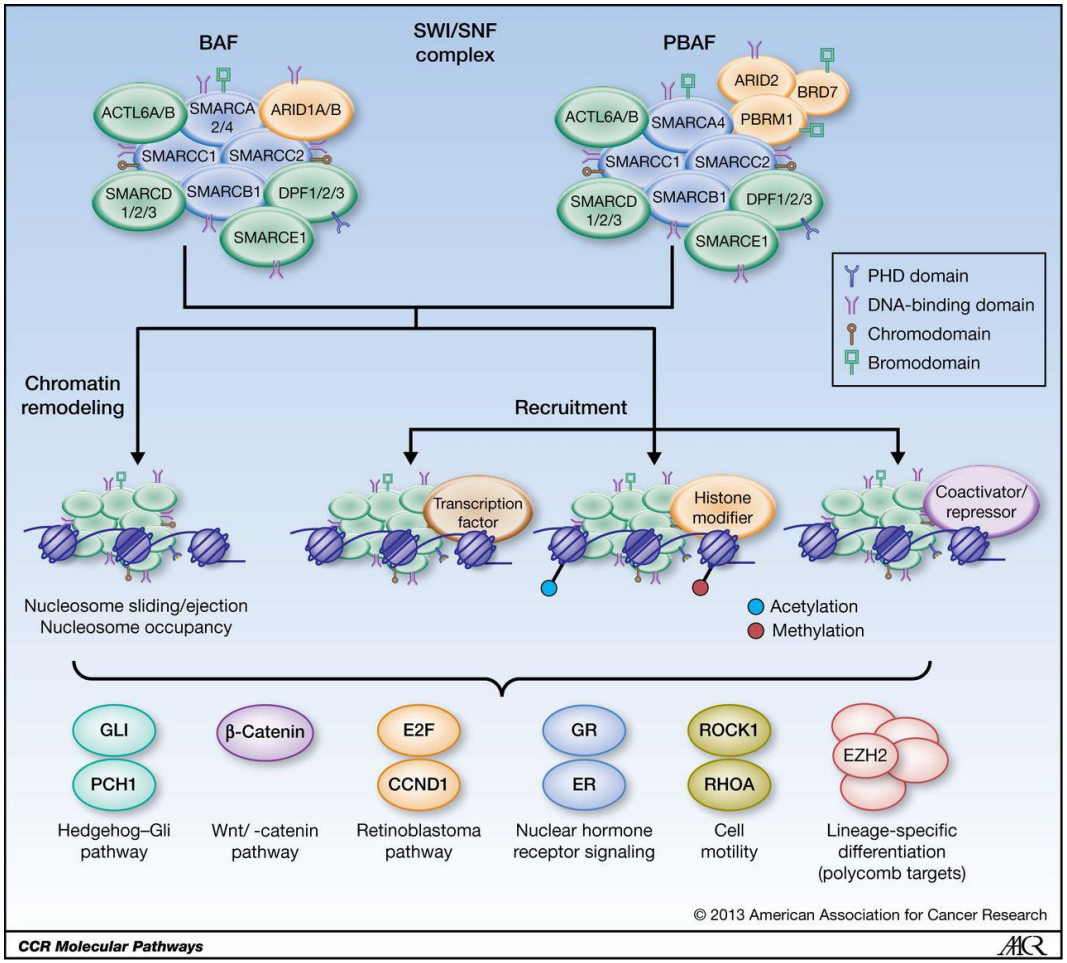


EMA (>95%)


PAX8


INI1

Inactivation of SWI/SNF Subunits is Ubiquitous in Cancer and is often seen in Dedifferentiated Carcinoma



- **SWI**tch/**S**ucrose **N**on-**F**ermentable
- SMARCxy=SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily x member y
- Catalytic ATPase subunits
  - SMARCA4 (BRG1)
  - SMARCA2 (BRM)
- Core subunits
  - SMARCB1 (INI1)
  - SMARCC1
  - SMARCC2
- Lineage-restricted subunits

Wang X, et al. *Clin Cancer Res*. 2014 Jan 1;20(1):21-7. PMID: 24122795

SWI/SNF Inactivation Underlies Diverse Tumor Types

INI1 (SMARCB1)-Inactivated Tumors
Malignant rhabdoid tumor (100%)
Epithelioid sarcoma (90%)
Renal medullary carcinoma (90%)
Epithelioid MPNST (70%)
Epithelioid schwannoma (40%)
Myoepithelial carcinoma (10-40%)
Extraskeletal myxoid chondrosarcoma (20%)
Poorly differentiated chordoma (100%)
INI1-inactivated carcinoma*

ORIGINAL ARTICLE

SMARCA4 (BRG1) Loss of Expression Is a Useful Marker for the Diagnosis of Ovarian Small Cell Carcinoma of the Hypercalcemic Type (Ovarian Rhabdoid Tumor)

A Comprehensive Analysis of 116 Rare Gynecologic Tumors, 9 Soft Tissue Tumors, and 9 Melanomas

Marie Karanian-Philippe, MD,†‡ Valérie Velasco, BSc,* Michel Longy, MD,* Anne Floquet, MD,§ Laurent Arnould, MD,‖ Jean-Michel Coindre, MD,*†‡ Cécile Le Naoures-Méar, MD,¶ Gerlinde Averous, MD,# Frédéric Guyon, MD,** Gaëtan MacGrogan, MD,*† and Sabrina Croce, MD*†*

Abstract: Ovarian small cell carcinoma of the hypercalcemic type (SCCOHT)/ovarian rhabdoid tumor is a rare and highly malignant tumor that typically occurs in young women. Up until now the diagnosis has been made on the basis of morphology without any specific immunohistochemical (IHC) markers. However, several authors have shown recently that SCCOHTs are characterized by inactivation of the *SMARCA4* gene (encoding the BRG1 protein) resulting in a loss of BRG1 protein expression in IHC. We evaluated BRG1 and INI1 expression in 12 SCCOHTs and in a series of 122 tumors that could mimic SCCOHT morphologically: 9 juvenile granulosa cell tumors, 47 adult granulosa cell tumors, 33 high-grade ovarian serous carcinomas, 9 desmoplastic round cell tumors, 13 Ewing sarcomas (5 from the pelvis and 8 from soft tissues), 1 round cell sarcoma associated with *CIC-DUX4* translocation from soft tissue (thigh), 1 case of high-grade endometrial stromal sarcoma of the ovary, and 9 melanomas. Forty-four adult granulosa cell tumors were interpretable by IHC. All 12 SCCOHTs were devoid of BRG1 expression and expressed INI1. All other interpretable 119 tumors showed BRG1 nuclear positivity, with variable staining proportions, ranging from 10% to 100% of positive cells (mean: 77%, median: 80%), variable intensities (weak: 5%, moderate: 37%, strong: 58%), and distributions: diffuse in 82 cases (70%) and heterogenous in 36 cases (30%). BRG1 positivity was heterogenous in desmoplastic round cell tumors and adult granulosa cell tumors. Overall, BRG1 is a useful diagnostic marker in SCCOHT, showing the absence of expression in SCCOHT. Nevertheless, the possible heterogeneity and the variable intensity of this staining warrant caution in the interpretation of BRG1 staining in biopsy specimens.

Key Words: rhabdoid tumor, small cell carcinoma hypercalcemic type, *SMARCA4*/BRG1, INI1/*SMARCB1*, ovary

(*Am J Surg Pathol* 2015;39:1197–1205)

Ovarian small cell carcinoma of the hypercalcemic type (SCCOHT) is a rare and highly malignant tumor that typically occurs in young women,[1] representing the most common type of undifferentiated carcinoma in women under 40 years. Although this tumor is often discovered at early stages I/II, it has poor clinical outcomes.[2,3]

Despite the name "carcinoma," SCCOHTs are classified as "miscellaneous ovarian tumors" by the World Health Organization (WHO).[1] Recently, several authors have shown that SCCOHTs are characterized by an inactivation of the *SMARCA4* gene,[4–6] which, like *SMARCB1*, is a member of the SWI/SNF chromatin-remodeling gene complex, which is mutated in several different cancers.[7,8] *SMARCA4* germline mutation has been reported in a tumor predisposition syndrome resulting in cranial or extracranial atypical rhabdoid tumors.[9,10]

Histologically, SCCOHT shows a sheet-like arrangement of small monomorphic cells, with scanty cytoplasm and round, ovoid, or, in rare cases, spindle, small nuclei containing single small nucleoli. Mitotic figures and necrosis are frequent. A follicle-like pattern is often present.[1,2,11–13] The main differential diagnoses are granulosa cell tumors of the adult or juvenile type, desmoplastic round cell tumors, Ewing sarcomas, metastatic melanomas, small cell carcinomas of the pulmonary type, or high-grade poorly differentiated carcinomas.[12,13]

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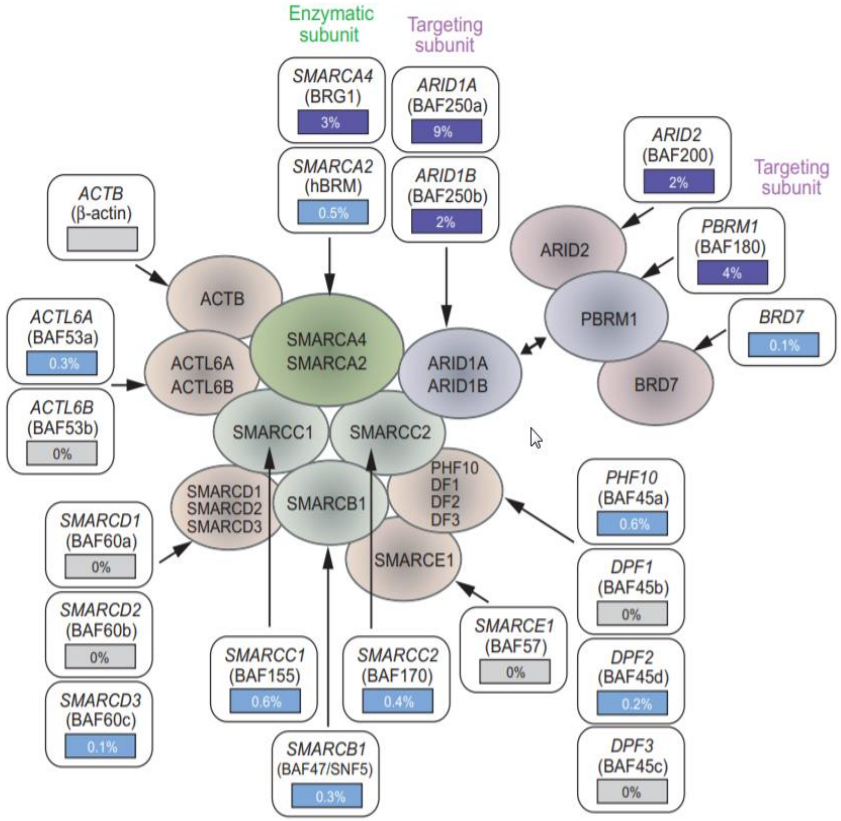
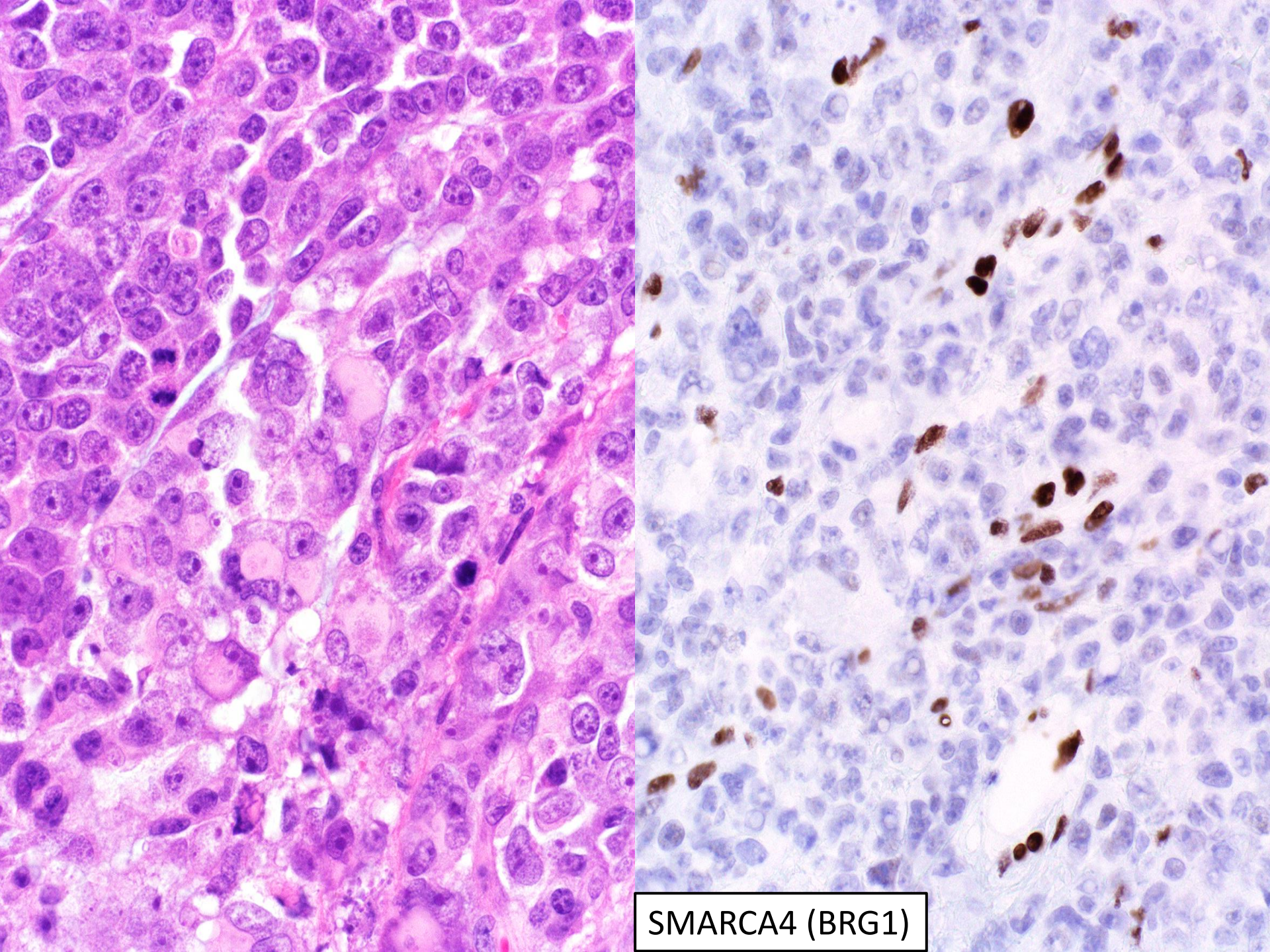
Frequency of SWI/SNF Inactivation

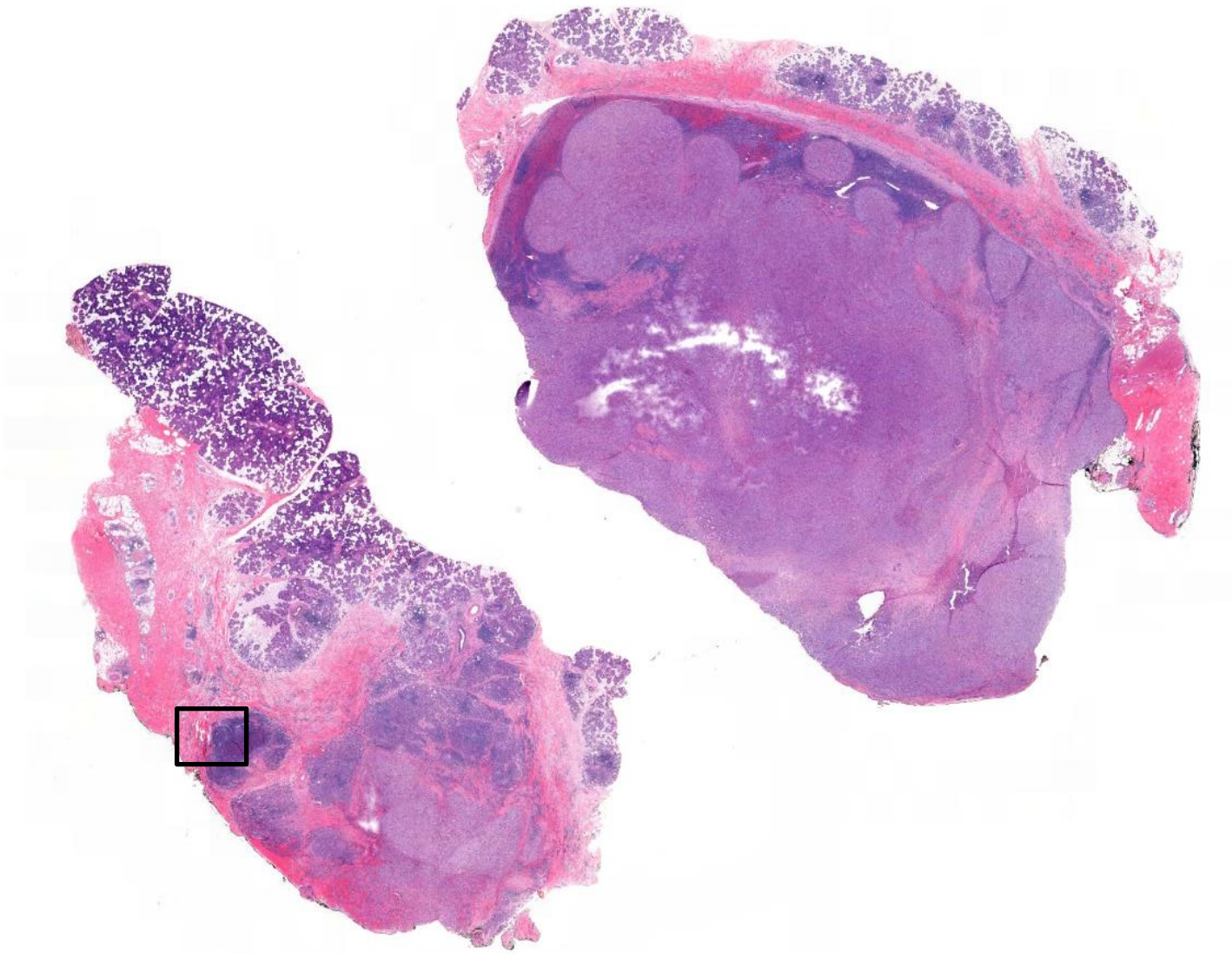
SWI/SNF inactivation found to be a molecular genetic underpinning of undifferentiated/rhabdoid carcinomas of:

- Ovary (SCCHT)
- Sinonasal tract (SNUC)
- Lung
- Tubal gut
- Kidney
- Bladder
- Endometrium

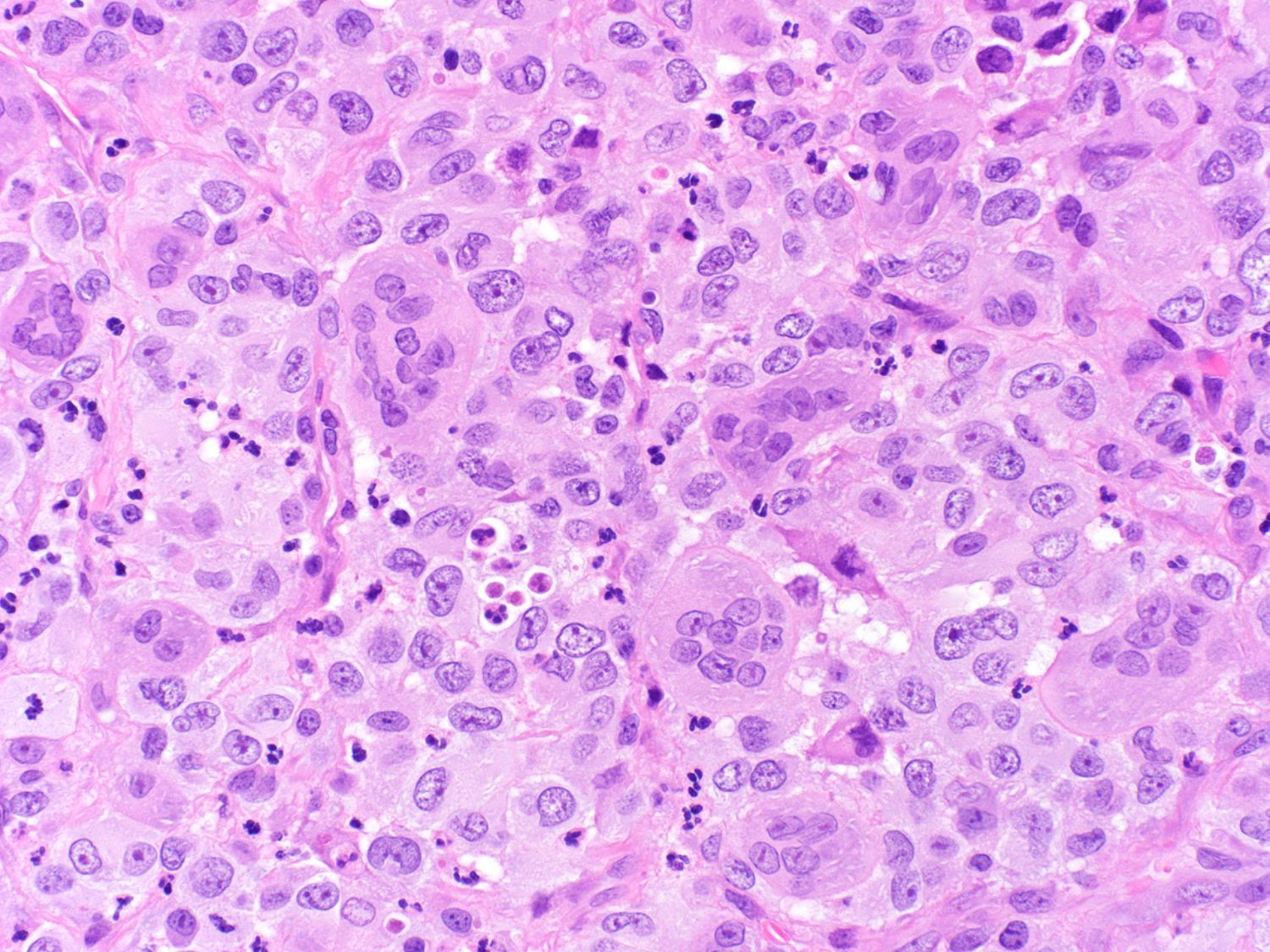


Kadoch C, et al. *Nat Genet*. 2013 Jun;45(6):592-601. PMID: 23644491
Shain AJ, Pollack JR. *PLoS One*. 2013;8(1):e55119. PMID: 23355908


SMARCA4 (BRG1)

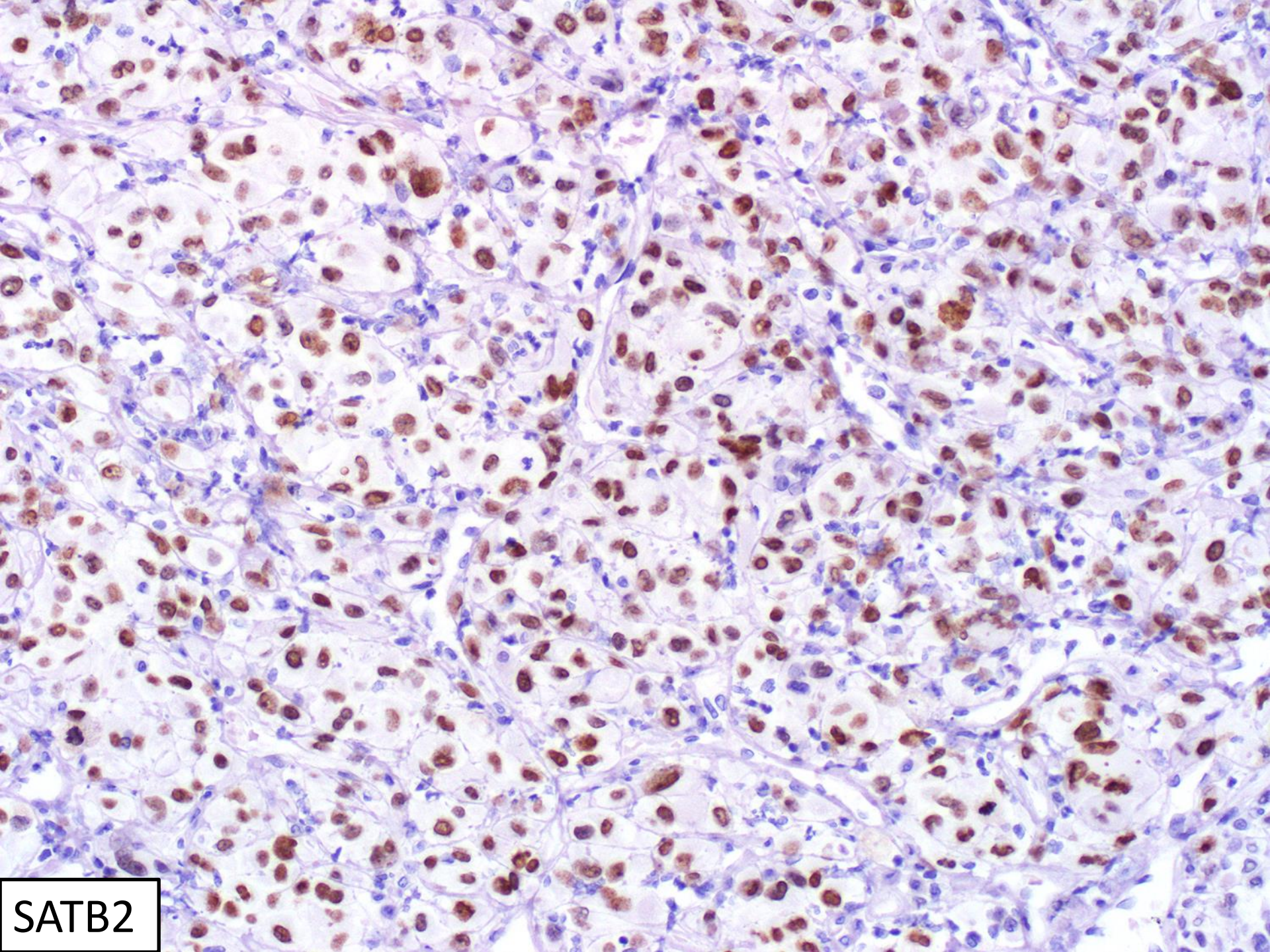


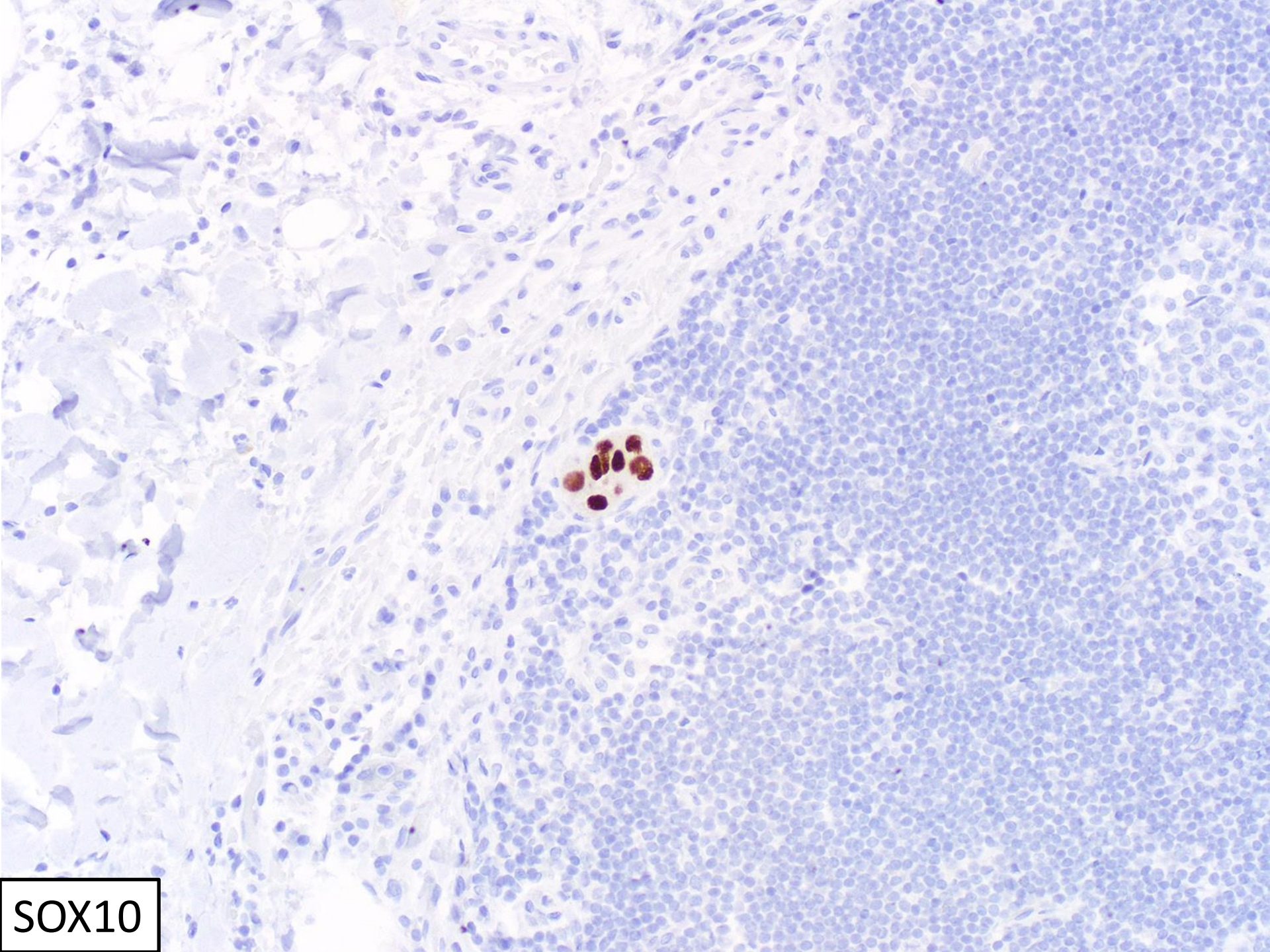
39-year-old man with large parotid mass. I inherited this case after an exhaustive immunopanel was performed.

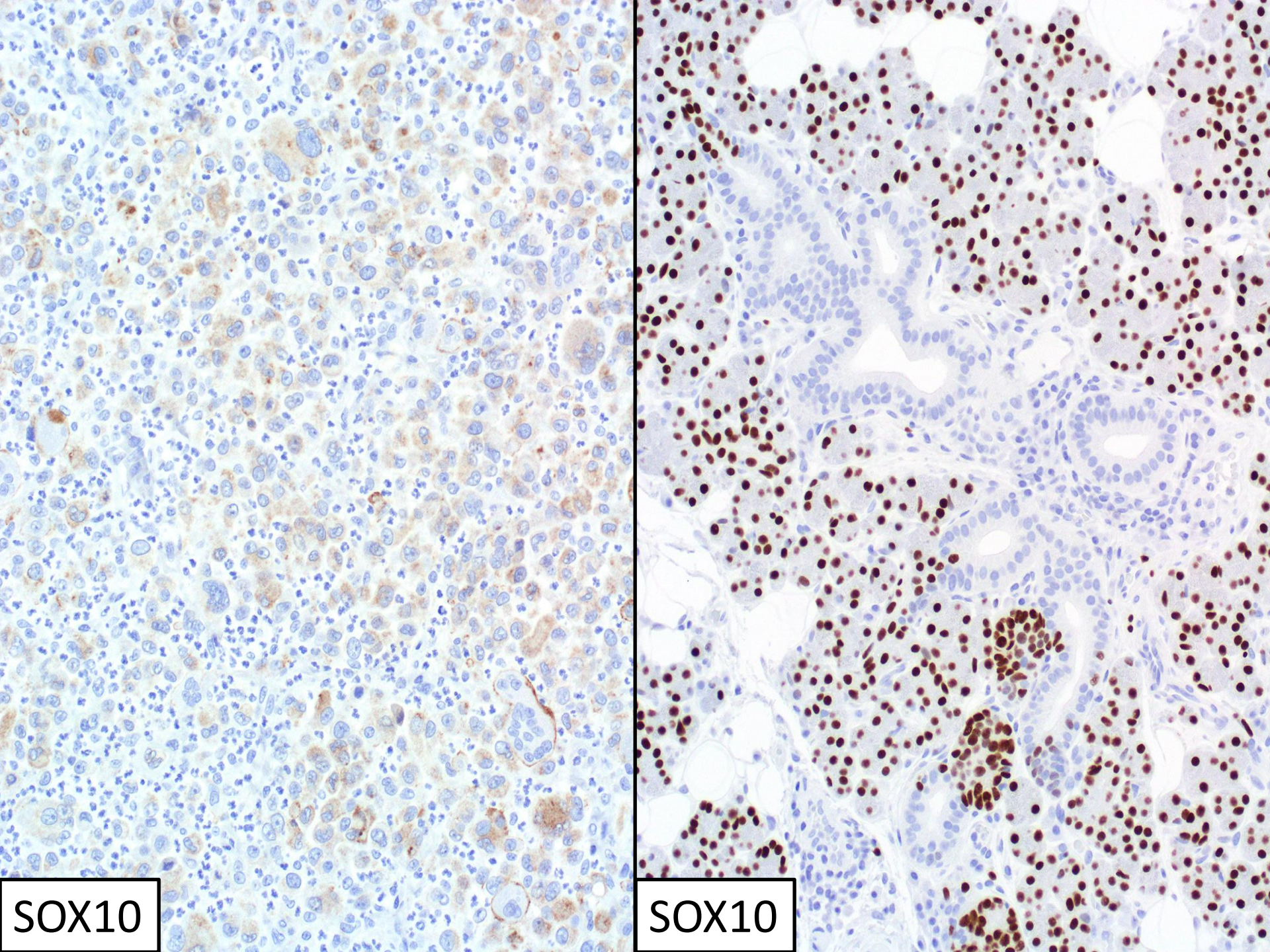


Undifferentiated Malignant Neoplasm with Osteoclast-like Giant Cells

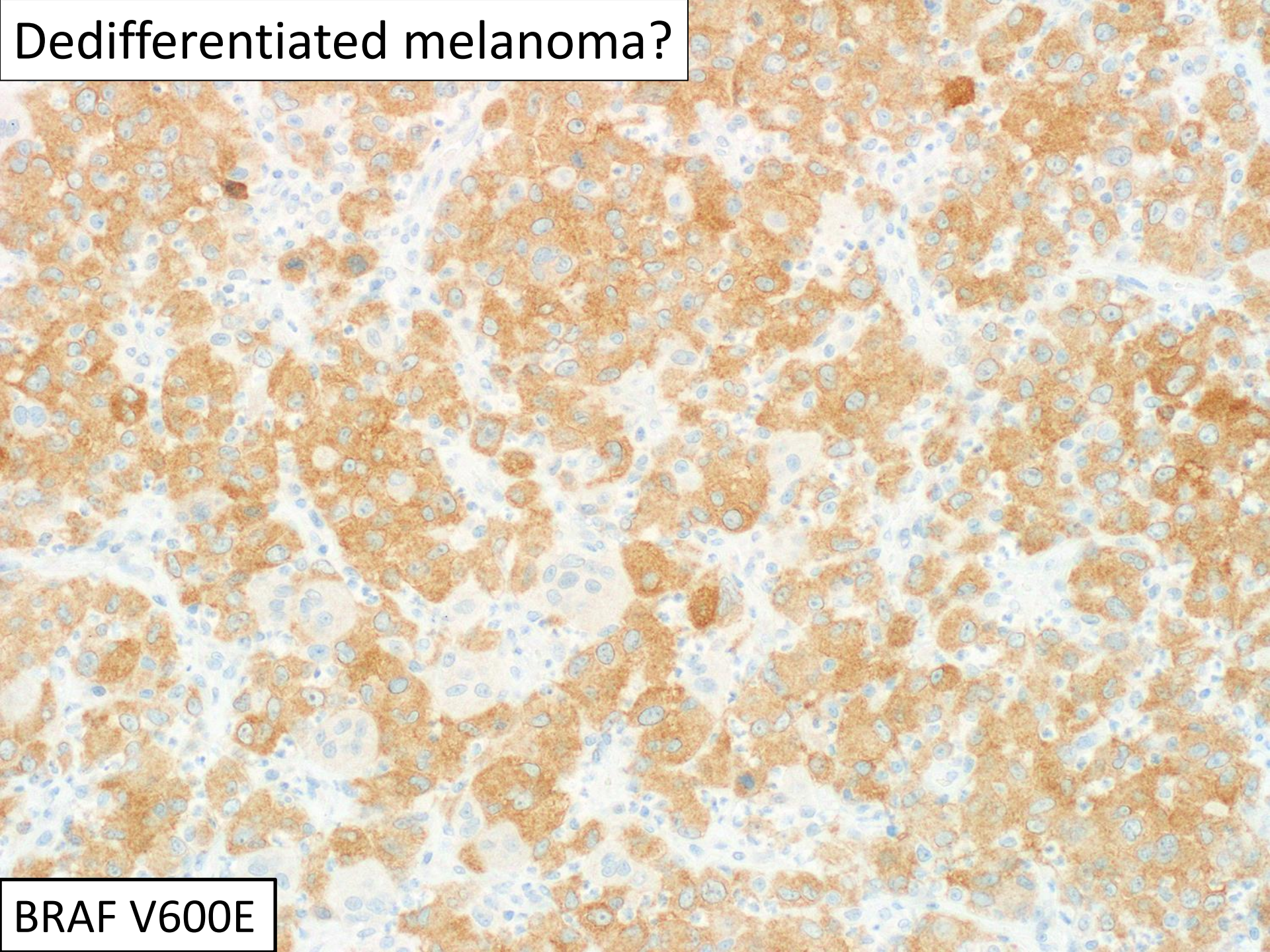
- Undifferentiated/anaplastic carcinoma
  - Keratin AE1/AE3
  - CDX2, PAX8, TTF-1
- Osteosarcoma
  - SATB2
- Leiomyosarcoma
  - Desmin, SMA, caldesmon


SATB2

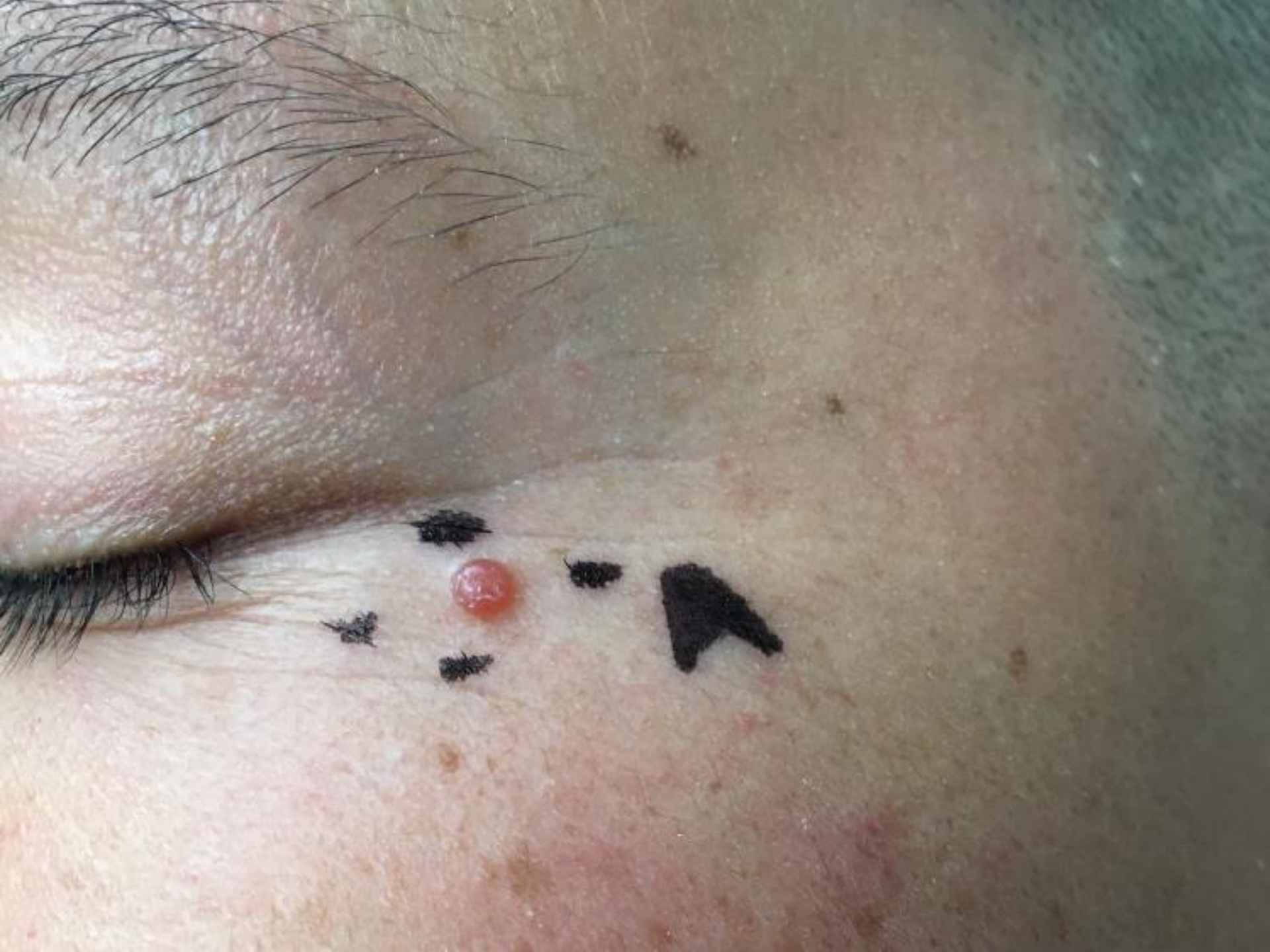

SOX10

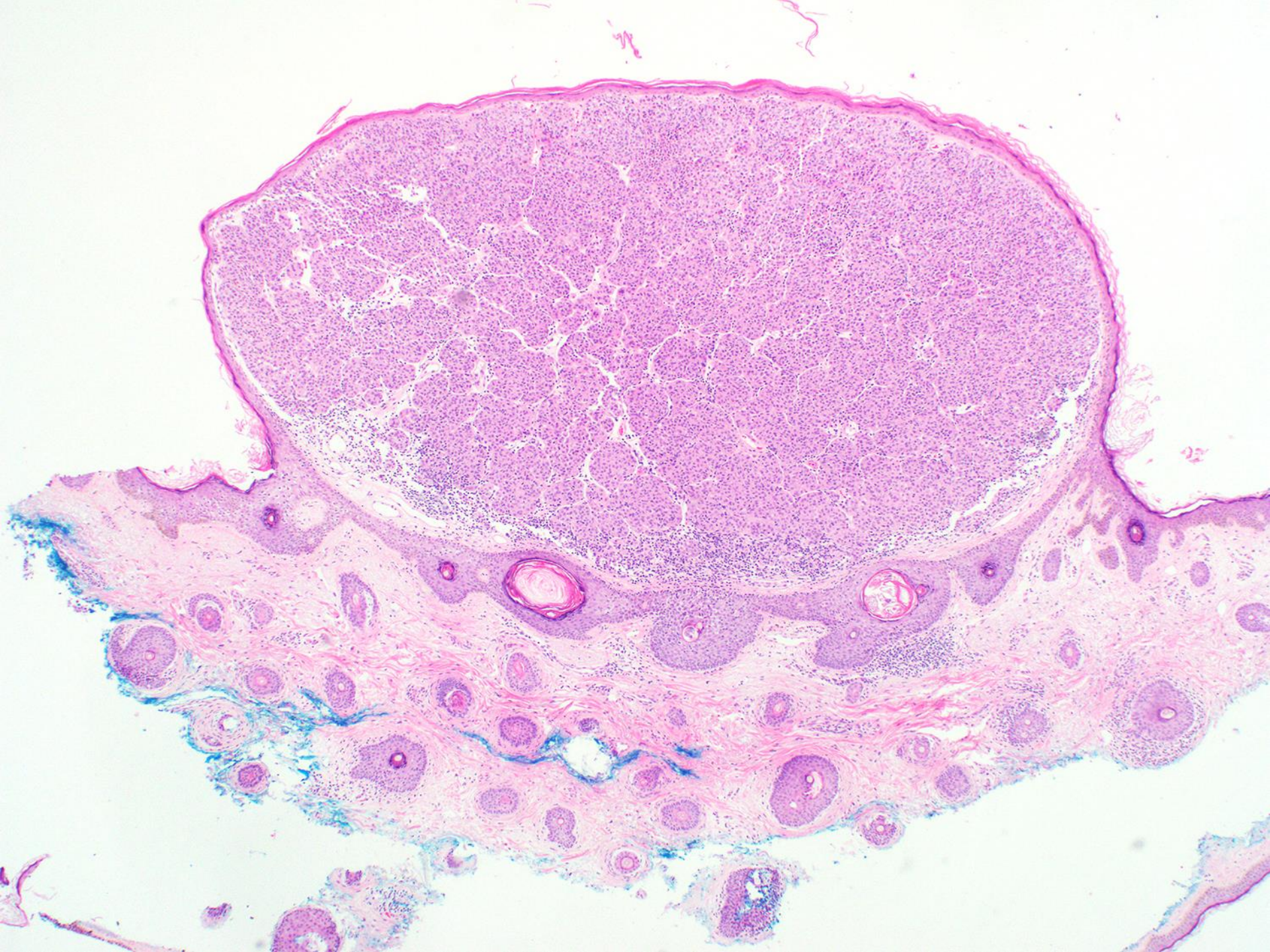

SOX10
SOX10

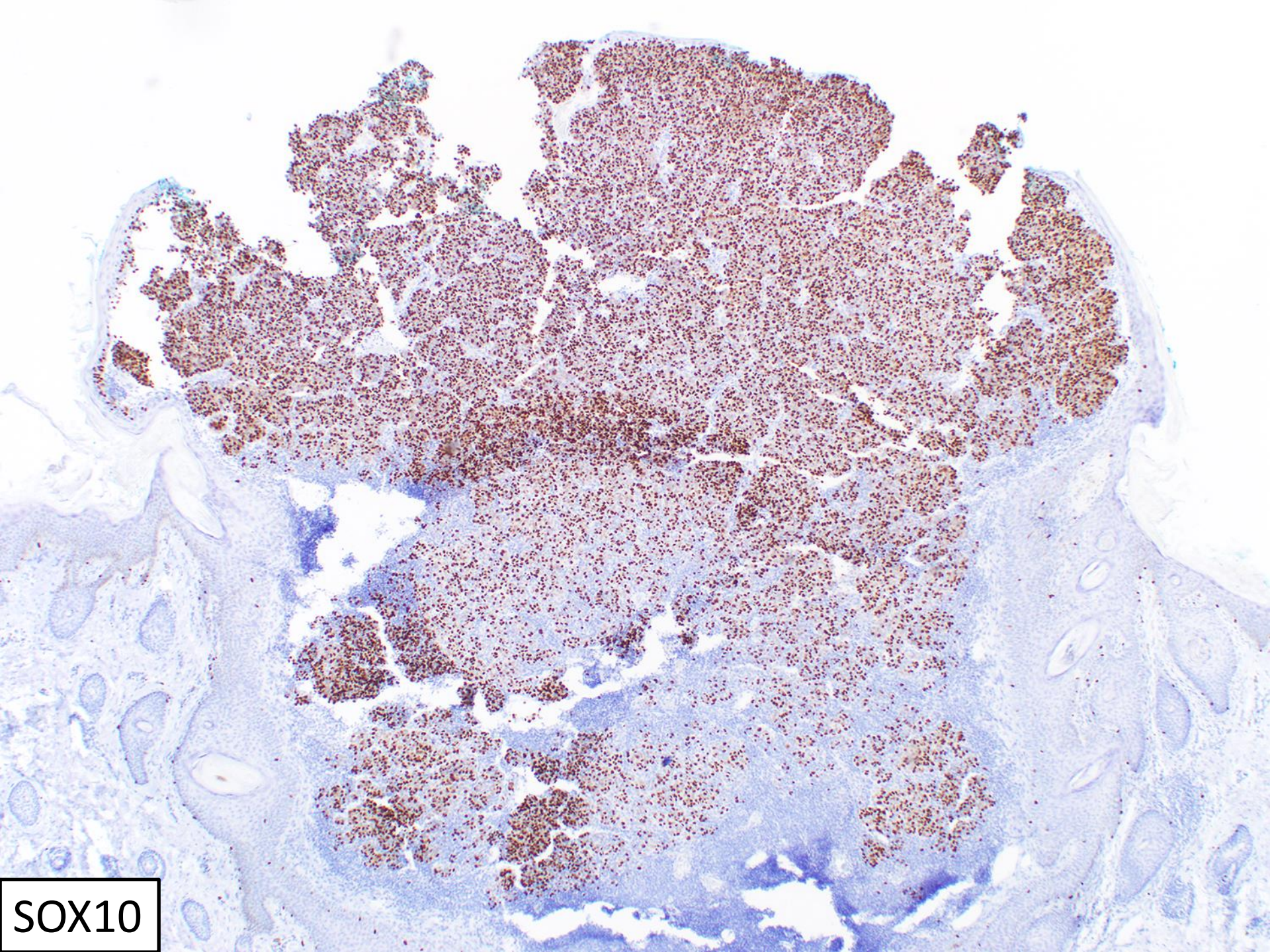
Dedifferentiated melanoma?



BRAF V600E






SOX10

Everything Can Dedifferentiate!

accuracy reflect a demand for high analytical precision in the HbA_{1c} assay due to patients achieving more stable glycohemoglobin values.

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S100 Protein and HMB-45 Negative "Rhabdoid" Malignant Melanoma: A Totally Dedifferentiated Malignant Melanoma?

To the Editor—We read with great enthusiasm the excellent article by Chang and colleagues[1] from Barnes Hospital reviewing their experience with the sparsely documented malignant melanoma with "rhabdoid" phenotype. We recently encountered an aggressive malignant tumor with "rhabdoid" phenotype occurring in a 60-year-old male 13 years after removal of a Clark level IV malignant melanoma that we believe expands the current data on the ultrastructural and immunohistochemical spectrum of malignant melanoma with "rhabdoid" features.

At the age of 47, this White male had excised from his sternal region a malignant melanoma invading into the reticular dermis (Clark's level IV) with a 1.1 mm depth of penetration (Breslow level). The patient was treated with adjuvant BCG (bacille Calmette-Guerin), and later underwent axillary lymph node dissection that showed no evidence of malignancy. At the age of 60, he presented with sudden synchronous development of large subcutaneous tumors in the left pectoral region, right lateral buttock, and right paraspinal region. Biopsy of the left chest mass revealed a malignant tumor with "rhabdoid" features. Despite chemotherapy including etoposide, iphosphamide, and doxorubicin, the patient succumbed to progressive disease, but request for autopsy was not granted. Sections of the left chest wall mass revealed a highly cellular, invasive neoplasm composed of dyshesive polygonal cells with unequivocal "rhabdoid" features including an ovoid nucleus with one or two prominent eosinophilic nucleoli and a prominent paranuclear cytoplasmic inclusion (Fig. 1). No pigment deposition or cross-striations were noted within the cells. Immunohistochemically, the cells displayed strong paranuclear immunoreactivity with the vimentin and desmin immunostain (Fig. 2). Neuron-specific enolase diffusely stained the cytoplasm of a majority of cells, while EMA and synaptophysin highlighted the periphery of scattered cells. The cells failed to stain with cytokeratins AE1/3 and CAM 5.2, S100 protein, HMB-45, HHF-35, myoglobin, and LCA. Ultrastructural analysis demonstrated whorled paranuclear aggregates of intermediate filaments entrapping rough endoplasmic reticulum, mitochondria, and lipid, but no evidence of basal lamina, primitive cell junctions, tonofilaments, melanosomes, neurosecretory granules, or glandular or skeletal muscle differentiation. Sections of the original tumor disclosed an invasive melanoma with a superficial spreading radial growth component arising in a nevus.

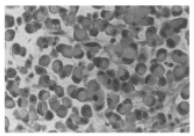

FIG. 1. High magnification of rhabdoid tumor cells showing dyshesive polygonal cells with ovoid to reniform nuclei and prominent paranuclear inclusions. (hematoxylin and eosin, ×400).

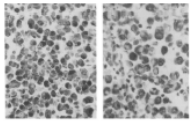

FIG. 2. Immunohistochemical findings. Paranuclear reactivity with desmin (Left) and vimentin (Right). (Avidin-biotin-peroxidase method. ×200).

Metastatic Malignant Melanoma With Complete Loss of Differentiation Markers (Undifferentiated/Dedifferentiated Melanoma)

Analysis of 14 Patients Emphasizing Phenotypic Plasticity and the Value of Molecular Testing as Surrogate Diagnostic Marker

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Bruno Märkl, MD,§ Gerald Niedobitek, MD,‖ Melanie Straub, MD,† Thomas Hager, MD,¶
*Anna-Carinna Reis, MD,¶ Bastian Schilling, MD,# Regine Schneider-Stock, PhD,**
Arndt Hartmann, MD, and Thomas Mentzel, MD***

Abstract: Metastatic malignant melanoma is notorious for its phenotypic diversity and loss of differentiation markers. We herein summarized our experience with 14 metastatic melanomas showing complete loss of immunohistochemical melanocytic markers (with or without heterologous differentiation). Patients included 11 men and 3 women aged 24 to 78 years (median, 67 y). Thirteen patients had histologically confirmed primary skin melanoma, and 1 had metastatic melanoma of unknown primary. Undifferentiated metastasis was diagnosed synchronous to primary tumor (n = 1), following skin melanoma by 3 months to 9 years (n = 11) and preceding it by 1 year (n = 1). Sites of undifferentiated metastases were axillary (3), inguinal (1), or submandibular (1) lymph nodes, digestive tract (2), bone/soft tissue (2), lung/pleura (2), and disseminated (n = 3). Histology of metastases mimicked undifferentiated pleomorphic or spindle cell sarcoma with variable myxoid and giant cell areas (n = 10) and cytokeratin-positive undifferentiated small cell sarcoma (n = 1). Three cases showed heterologous dedifferentiation: pleomorphic rhabdomyosarcoma (n = 1), teratocarcinosarcoma-like with prominent rhabdomyoblasts (n = 1), and adenocarcinoma-like with metaplastic bone (n = 1). All cases were negative for S100, melanoma cocktail, HMB45, Melan A, and SOX10. Other markers showed following results: smooth muscle actin (1/14), p16 (1/14), TP53 (2/12), pancytokeratin (4/14), desmin (5/14), h-caldesmon (0/9), and MDM2/CDK4 (0/5). SMARCB1 was intact in 8/8 cases. Genotyping showed *BRAF*^{V600E} mutation (5/14), *NRAS* mutation (5/14), and *BRAF*/*NRAS* wild-type (4/14). In conclusion, undifferentiated/dedifferentiated metastatic melanoma is likely underrecognized and frequently mistaken for undifferentiated sarcoma or other neoplasms. Diagnosis of undifferentiated sarcoma at sites where melanoma metastasis are frequent (eg, inguinal and axillary region) should be made with great caution and warrants exploration of the remote history. Genotyping is a helpful surrogate marker in classifying such difficult cases. In the light of available targeted therapies, recognition of undifferentiated/dedifferentiated metastatic melanoma is mandatory for appropriate treatment.

Key Words: undifferentiated melanoma, dedifferentiation, rhabdomyosarcoma, *BRAF*, heterologous, *NRAS*, teratocarcinosarcoma

(*Am J Surg Pathol* 2016;40:181–191)

Malignant melanoma represents a common and highly aggressive skin cancer. Diagnosis is mainly based on a combination of topographical, histomorphologic, and immunohistochemical features.[1] The great phenotypic diversity of primary and metastatic malignant melanoma is well appreciated.[1–3] Although recognition of the melanocytic nature of primary cutaneous melanoma is usually straightforward and is essentially a hematoxylin and eosin (H&E)–based diagnosis, occasional melanoma cases may present with unexpected and unusual phenotypes that mimic a variety of nonmelanocytic neoplasms and thus pose a great diagnostic confusion.[3–6] This is particularly true for metastatic melanomas, as pathologists infrequently consider melanoma in nonmelanocytic-looking metastatic neoplasms. However, recent developments in diagnostic immunohistochemistry and molecular pathology have identified new phenotypic and genotypic markers that proved to be valuable in confirming melanoma diagnosis in unusual-looking and undifferentiated/dedifferentiated cases. Among the latter,

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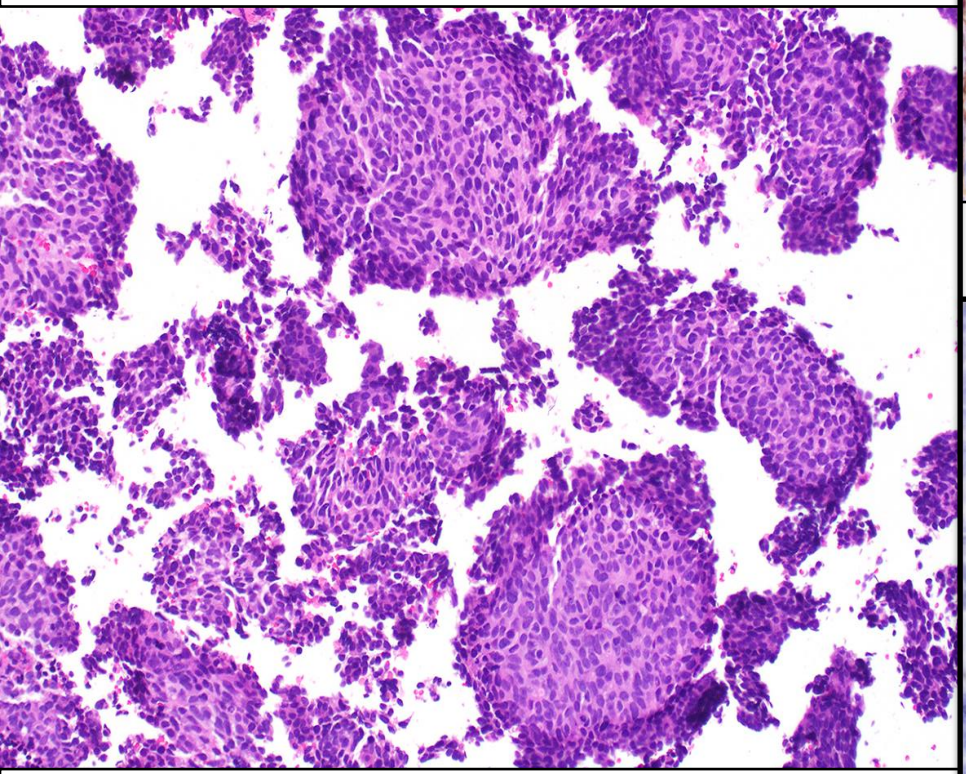
Dynamic Differentiation States Leading to Diagnostic Uncertainty

State of Differentiation	Tumor Type	Mechanism (Unknown for most)
Dedifferentiation	Sarcoma, carcinoma, melanoma	MSI-H, SWI-SNFi (carcinoma)
Undifferentiated	Carcinoma, sarcoma	MSI-H (carcinoma)
High-grade transformation	Lymphoma	p53, MYC
Grade progression	Carcinoma, sarcoma, lymphoma	
Divergent differentiation	Carcinoma (sarcomatoid carcinoma, MiNEN)	
Transdifferentiation	Lymphoma, carcinoma	

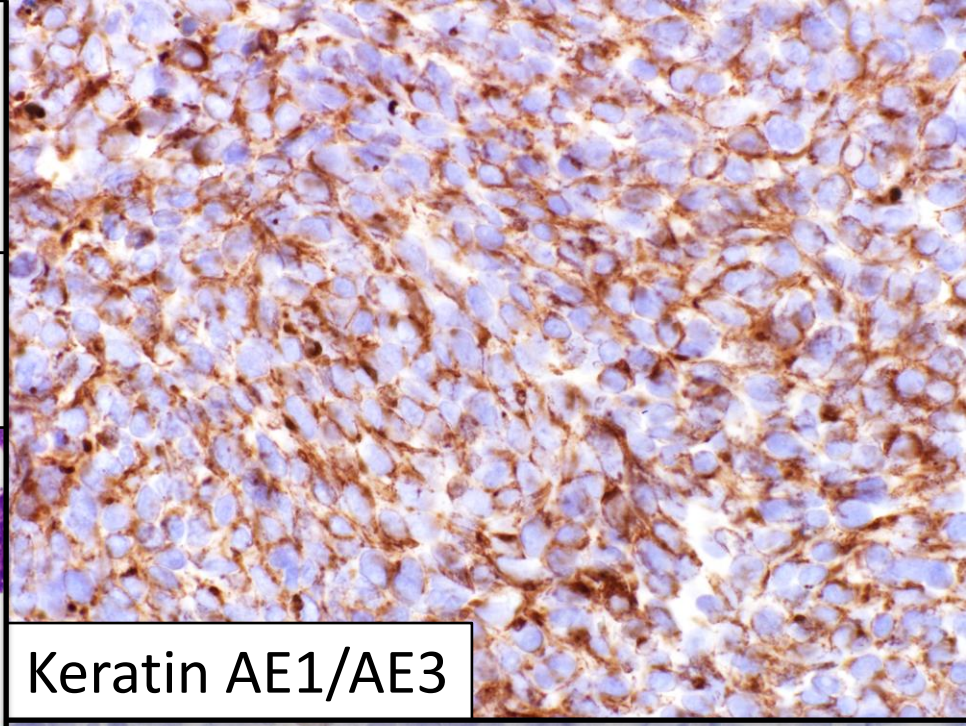

GMullins

Tumor did NOT express CgA, synaptophysin, PSA, PSAP, GATA-3, TTF-1; KIT, DOG1, or CK5/6

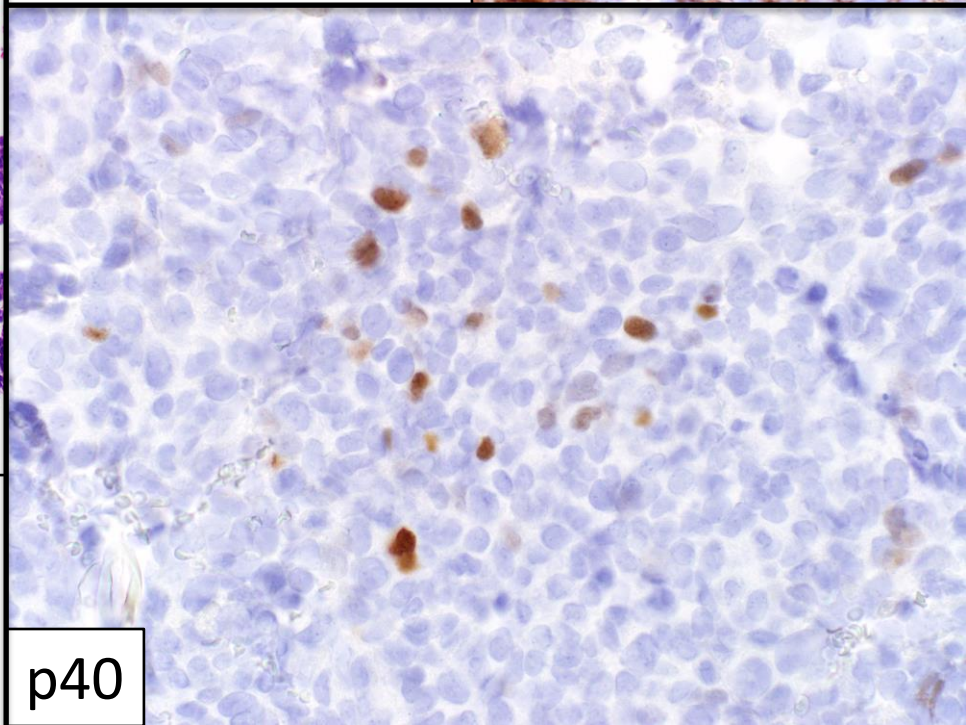
Diagnosis: Poorly differentiated carcinoma with squamous features



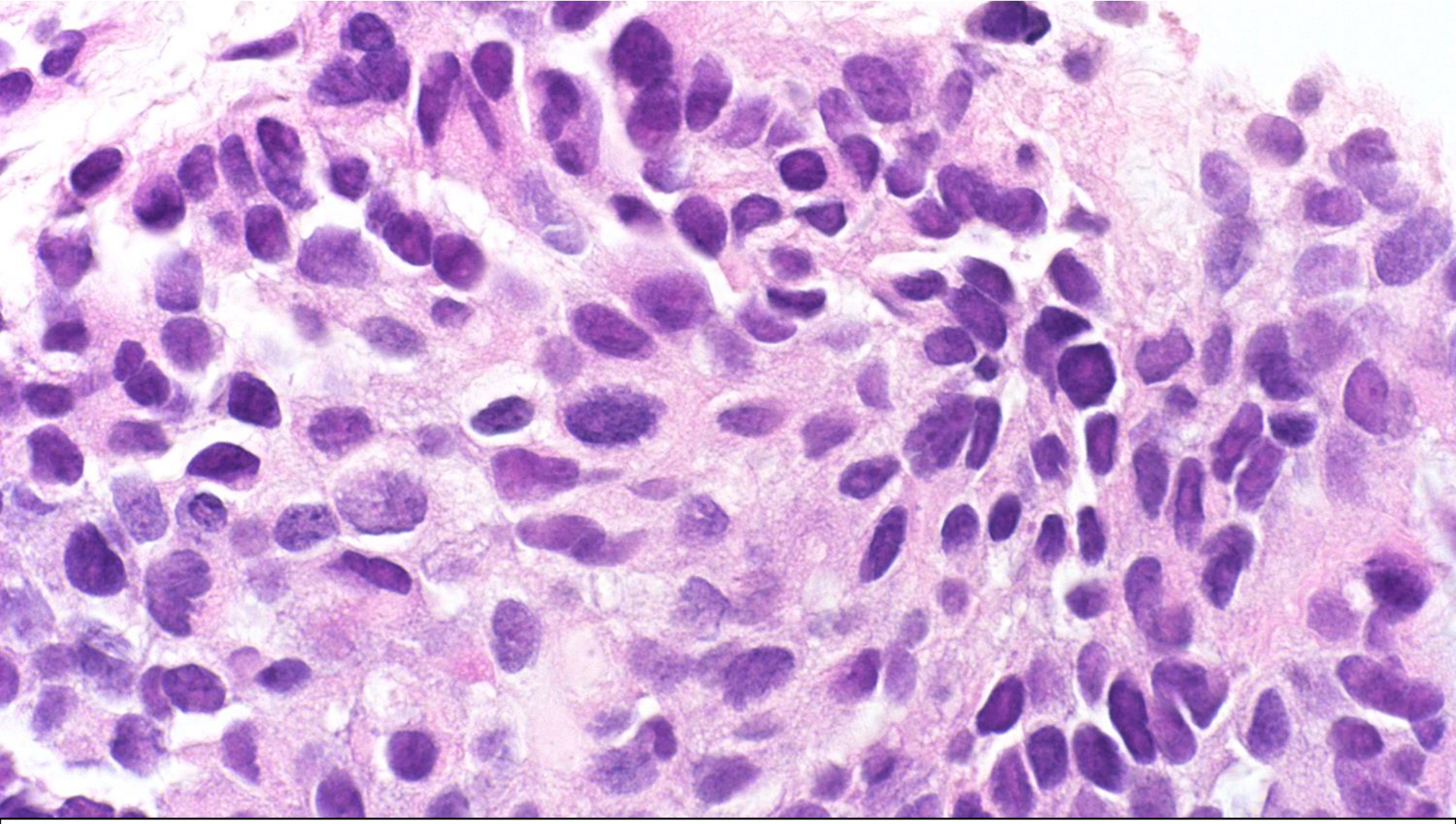
61-year-old man 1-year s/p kidney transplant with large abdominal mass (perigastric/retroperitoneal)



Keratin AE1/AE3



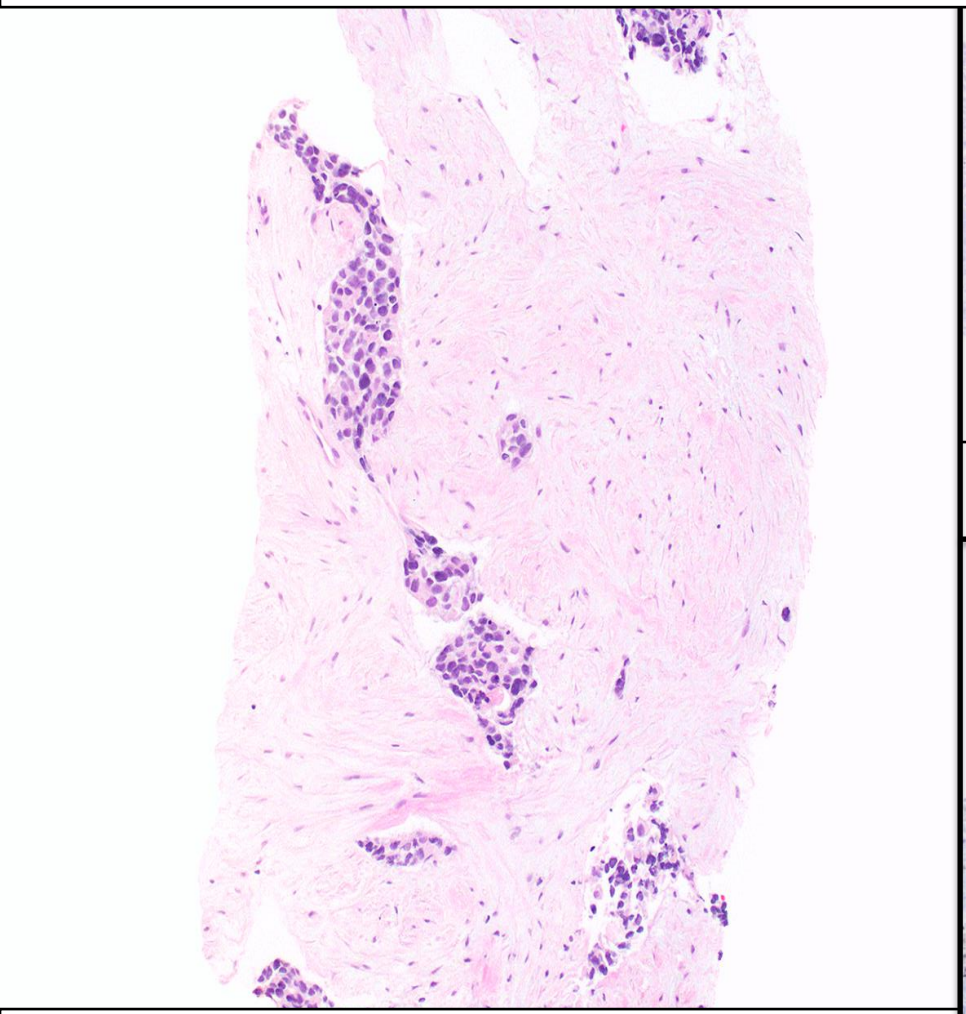
p40



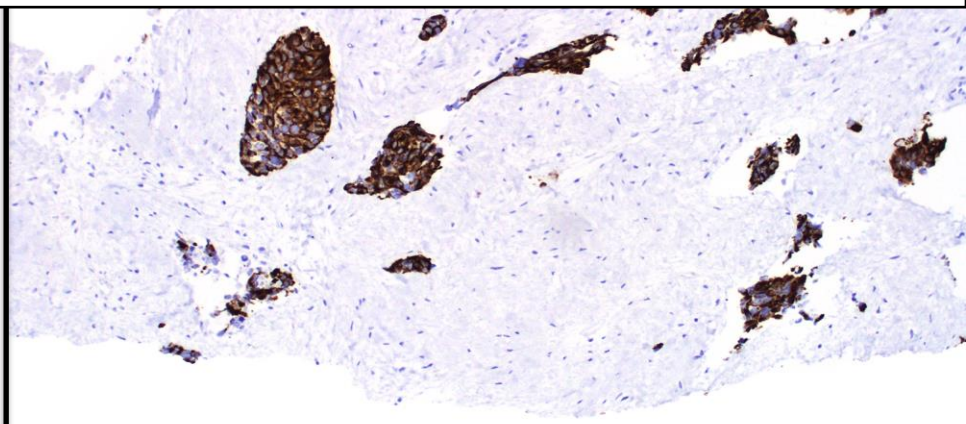
Patient was rebiopsied for PD-L1 (negative), MMR (intact), and HER2 (negative) testing; additional diagnostic IHC was performed and tumor did NOT express CK7, CK20, CDX2, CDH17, HepPar1, glypican-3, inhibin A, calretinin, CD30, SALL4, or NUT

EWSR1-WT1 rearrangement detected on NGS, confirming the diagnosis:

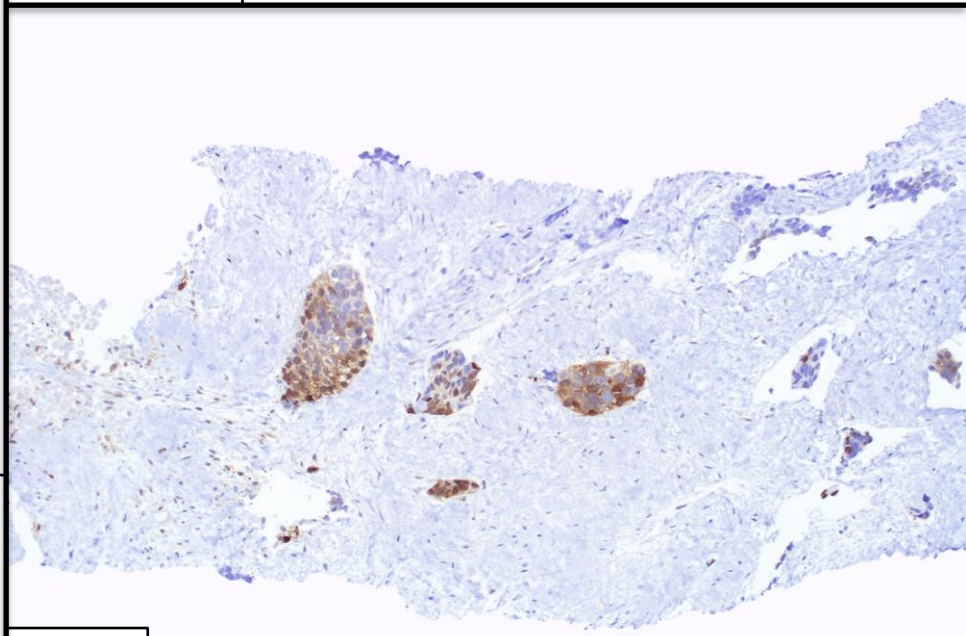
Desmoplastic small round cell tumor



Based on this appearance
I recommended CD99, desmin, myogenin, NSE, Rb, SATB2, WT-1



Desmin



NSE

Initial Panel in a Small Round Blue Cell Tumor

Marker	Expressed by	Also Expressed by
CD99	Ewing sarcoma	Lymphoblastic lymphoma; mesenchymal chondrosarcoma
NKX2.2	Ewing sarcoma	Olfactory neuroblastoma
Desmin	Rhabdomyosarcoma; Desmoplastic small round cell tumor	Triton tumor
Myogenin	Rhabdomyosarcoma (ARMS>>ERMS)	Atrophic skeletal muscle
CD45	Lymphoma	
TdT	Lymphoblastic lymphoma	
INSM1 (CgA/Syn)	Neuroendocrine carcinoma; neuroblastoma; ?DSRCT	Extraskeletal myxoid chondrosarcoma
Pan-K	Carcinoma; Desmoplastic small round cell tumor	PD synovial sarcoma; occ. aberrantly expressed by sarcoma, melanoma
SOX10	Melanoma, MPNST (<50%)	Tumors with myoepithelial differentiation

Final Diagnoses in 41 "Ewing-Like Sarcomas" initially found to be negative/non-informative for *EWSR1*-rearrangement

Tumor Type	Freq.*	Diagnostic Markers
Ewing sarcoma	41%	CD99, NKX2.2, *EWSR1*
***CIC*-rearranged**	29%	**WT-1**, ETV4, DUX4, *CIC*
***BCOR*-associated**	13%	**BCOR**, SATB2, *BCOR, CCNB3, YWHAE*
Neuroblastoma	8%	CgA, Syn, INSM1, **PHOX2B**, TH, GATA-3
Malignant rhabdoid tumor	8%	**INI1 (SMARCB1)**
Lymphoblastic lymphoma	4%	TdT
Clear cell sarcoma	4%	SOX10, *EWSR1*
Small cell carcinoma	4%	INSM1
Rhabdomyosarcoma	4%	Desmin, myogenin, *FOXO1* (ARMS)
DSRCT	4%	Pan-K, desmin, **NSE**, WT-1 (-COOH), *EWSR1*
MPNST	4%	**H3K27me3**, SOX10
PD synovial sarcoma	4%	**TLE1**, Pan-K, *SS18*
GIST	4%	**DOG1, KIT**
SMARCA4-deficient sarc.	4%	BRG1 (SMARCA4)

Machado I, et al. *Ann Diagn Pathol*. 2018 Jun;34:1-12. PMID: 29661713

nature medicine

TECHNICAL REPORTS

Anchored multiplex PCR for targeted next-generation sequencing

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We describe a rapid target enrichment method for next-generation sequencing, termed anchored multiplex PCR (AMP), that is compatible with low nucleic acid input from formalin-fixed paraffin-embedded (FFPE) specimens. AMP is effective in detecting gene rearrangements (without prior knowledge of the fusion partners), single nucleotide variants, insertions, deletions and copy number changes. Validation of a gene rearrangement panel using 319 FFPE samples showed 100% sensitivity (95% confidence limit: 96.5–100%) and 100% specificity (95% confidence limit: 99.3–100%) compared with reference assays. On the basis of our experience with performing AMP on 986 clinical FFPE samples, we show its potential as both a robust clinical assay and a powerful discovery tool, which we used to identify new therapeutically important gene fusions: *ARHGEF2-NTRK1* and *CHTOP-NTRK1* in glioblastoma, *MSN-ROS1*, *TRIM4-BRAF*, *VAMP2-NRG1*, *TPM3-NTRK1* and *RUFY2-RET* in lung cancer, *FGFR2-CREB5* in cholangiocarcinoma and *PPL-NTRK1* in thyroid carcinoma. AMP is a scalable and efficient next-generation sequencing target enrichment method for research and clinical applications.

Next-generation sequencing has been instrumental in the advancement of genomic research and clinical molecular diagnostics in recent years. Although the cataloguing of complete genomes and their variation is an important endeavor for reference building and discovery, the use of whole-human-genome sequencing outside of this context is impractical with respect to cost and efficiency[1]. Certain applications such as cancer genotyping for somatic mutations require selective deep sequencing to achieve the desired analytical sensitivity for clinical utility[2]. At the present time, clinical sequencing is most feasible for assays based on targeted gene panels. The emerging need for a rapid and focused confirmation sequencing strategy to validate variants also remains to be addressed. Currently, there is need for a rapid and efficient technique for gene rearrangement detection by next-generation sequencing.

For clinical molecular diagnostics, we developed AMP to address the escalating demand for gene rearrangement testing of the *ALK* (encoding anaplastic lymphoma receptor tyrosine kinase), *RET* (encoding ret proto-oncogene) and *ROS1* (encoding ROS proto-oncogene 1) genes, all of which are associated with response to targeted therapy in lung cancer[3–5]. Fluorescence *in situ* hybridization (FISH) lacks scalability for high-volume multitarget testing and requires diagnostic expertise. Immunohistochemistry is used to detect expressed fusion genes as a surrogate marker for gene rearrangements; however, the technique relies on the availability of good-quality antibodies and on qualitative scoring. Neither FISH nor immunohistochemistry provide fusion partner breakpoint precision, which may partially explain heterogeneous treatment responses[3,6,7]. Reverse-transcription PCR may yield such information but requires knowledge of all fusion partner variants for primer design and demonstrates limited scalability in the setting of multiple heterologous partners and their involved exons. For example, *ROS1* rearrangements in lung cancer pose a challenge due to potential involvement with at least eleven different fusion partners and variable splicing[8].

RESULTS

Targeted RNA sequencing

Our initial motivation for designing AMP was to tackle the current deficiencies of clinical gene rearrangement detection noted above by employing a targeted RNA sequencing (RNA-seq) strategy. AMP is in theory similar to the technique known as rapid amplification of cDNA ends (RACE)[9], specifically in its ability to uncover unknown sequences adjacent to a known DNA sequence. Briefly, double-stranded cDNA undergoes end repair, adenylation and ligation, as previously described[10–12], with a new universal half-functional adapter. The resulting half-functional library by itself is insufficient for downstream bridge amplification, emulsion PCR or sequencing. The library is rendered fully functional at the end of two rounds of nested low-cycle PCR, which represent the core steps for target enrichment. The second PCR step uses nested primers that are 5′ tagged with a common sequencing adapter. In combination with the first half-functional universal adapter, the resulting target amplicons are functionalized for clonal amplification (for example, emulsion PCR or bridge PCR) and sequencing. Nontarget fragments remain

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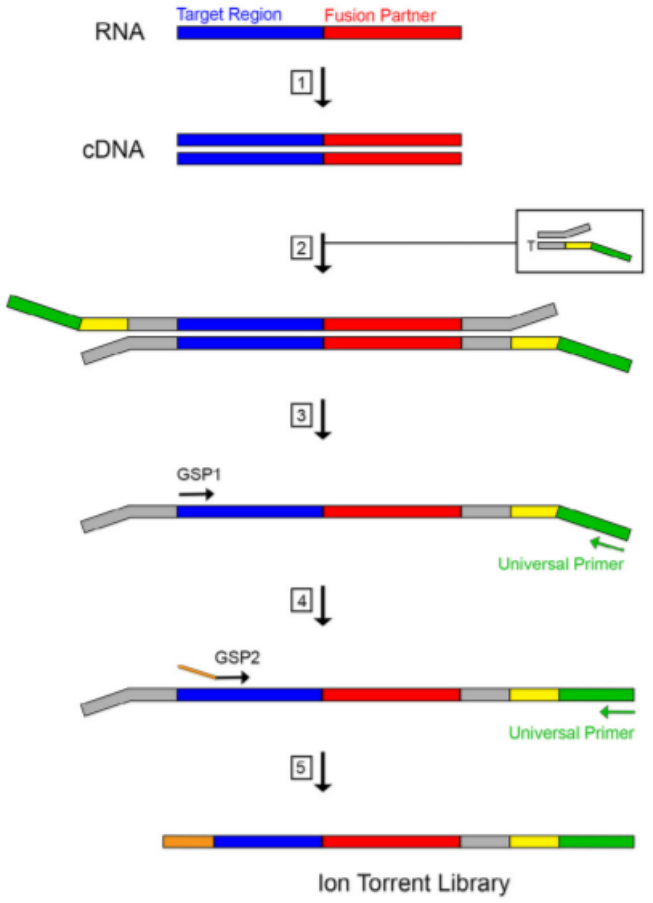
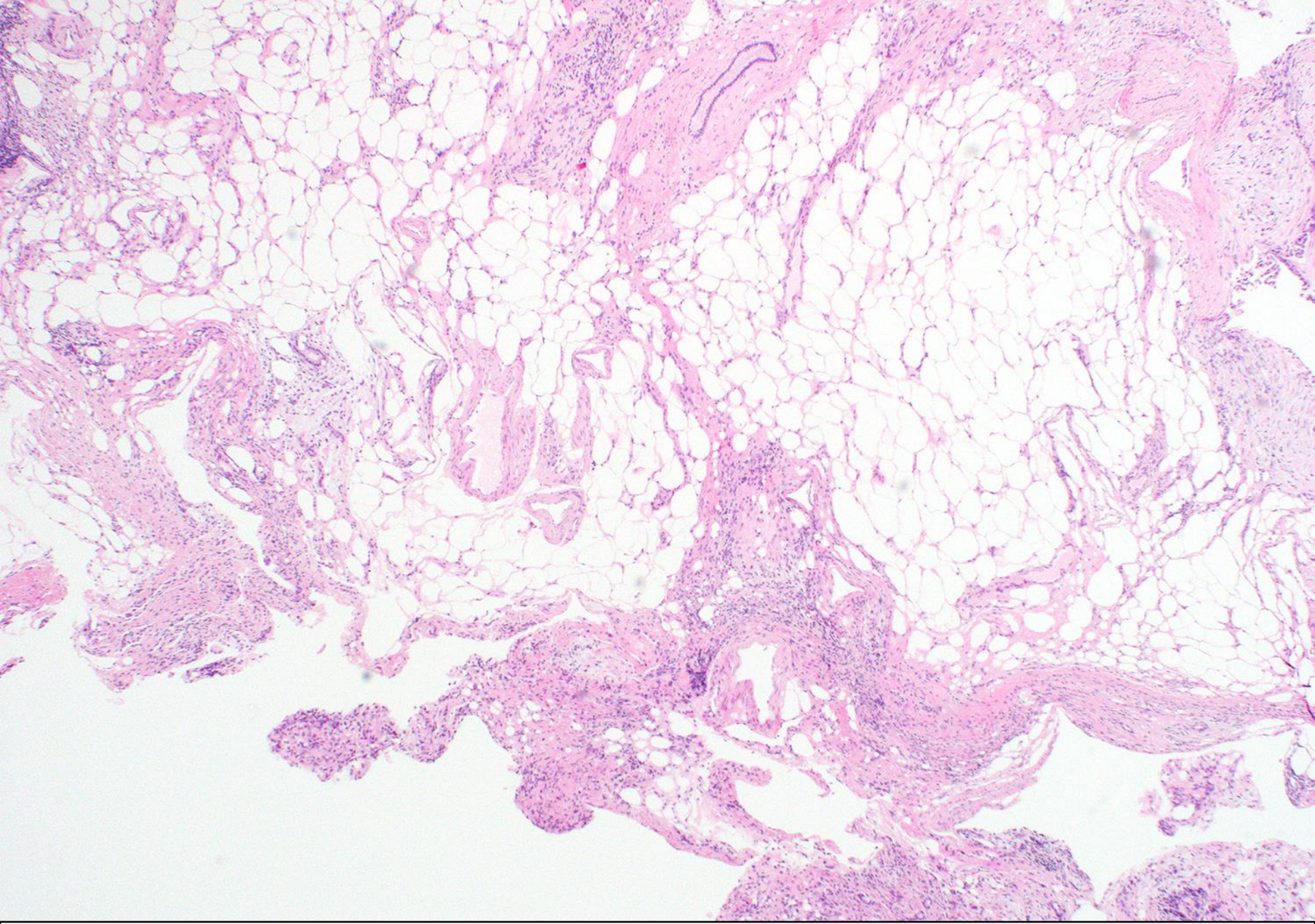
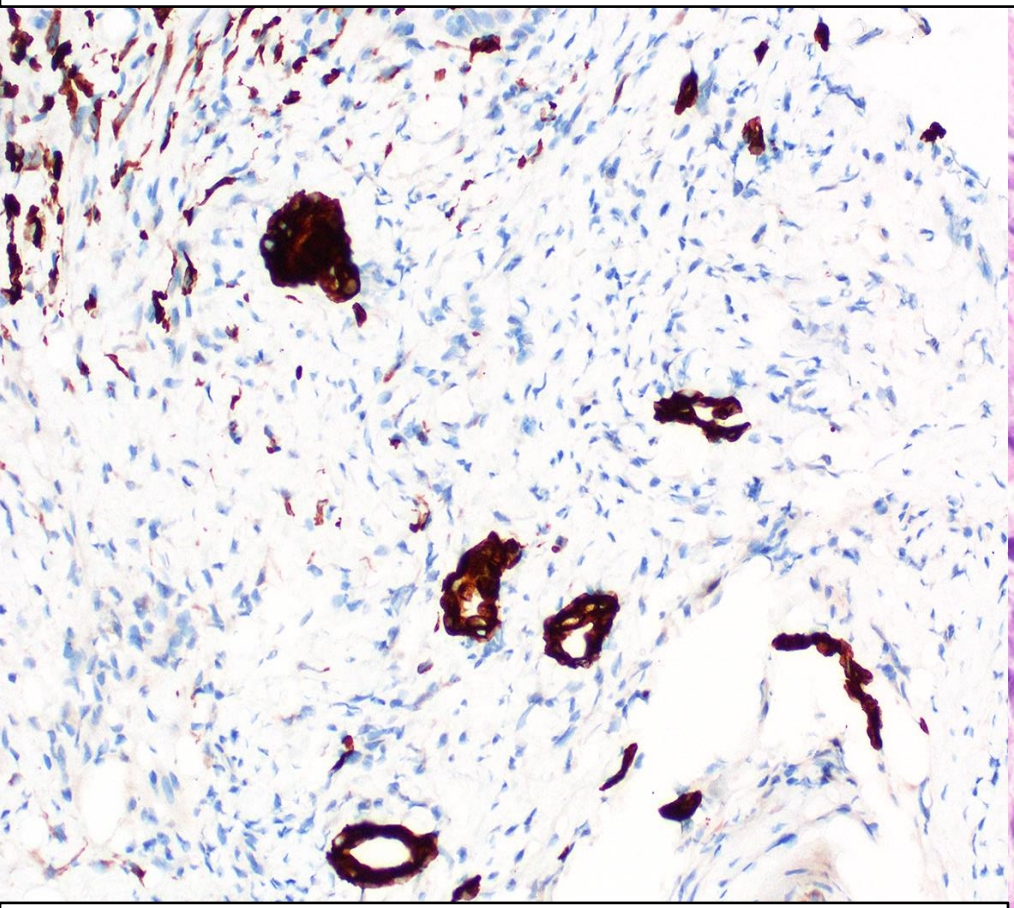


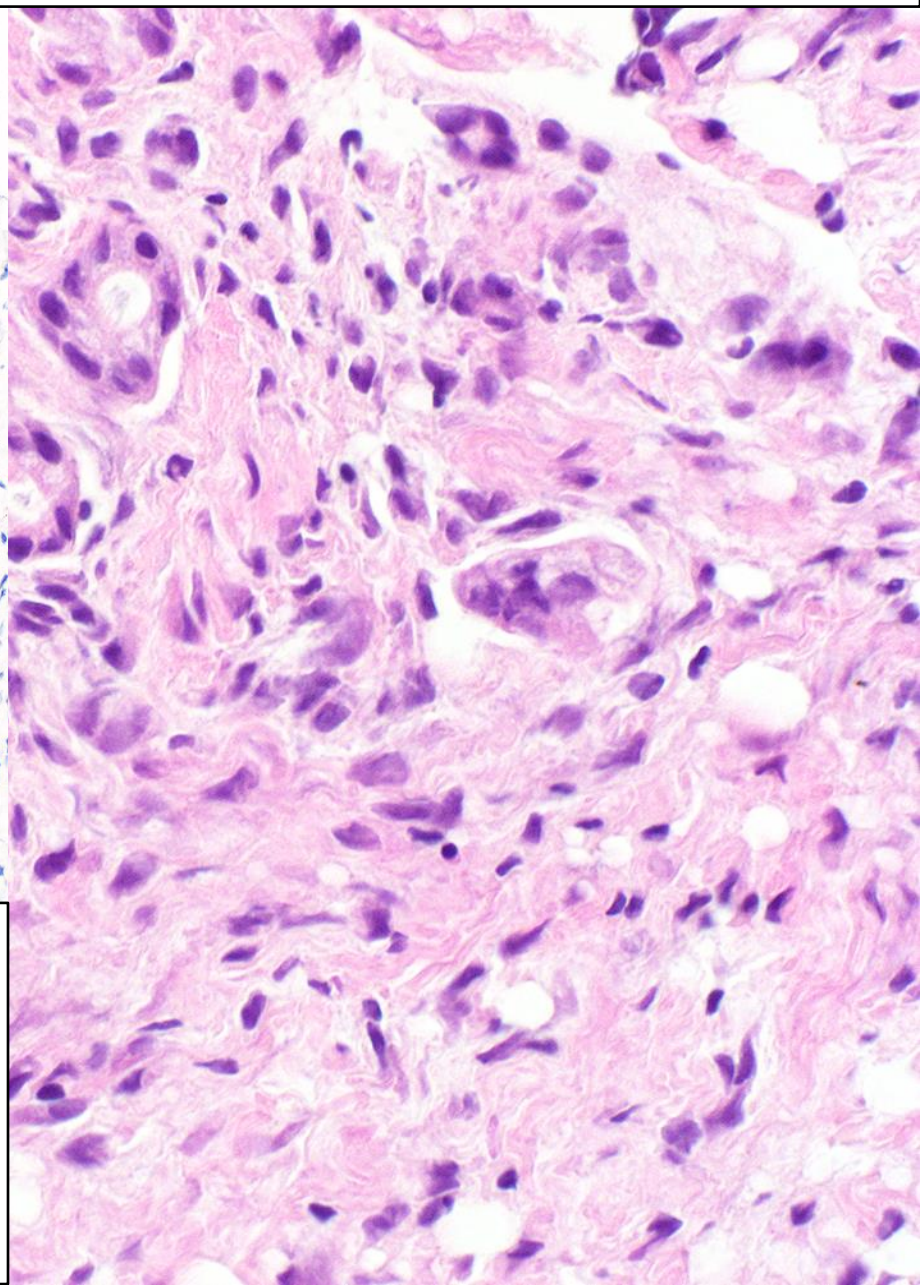
Gene	Tumor	Gene	Tumor
ALK	Inflammatory myofibroblastic tumor; epithelioid fibrous histiocytoma	***NCOA2***	Mesenchymal chondrosarcoma; angiofibroma of soft tissue; uterine tumor resembling ovarian sex cord tumor (rare)
CAMTA1	Epithelioid hemangioendothelioma	***NTRK3***	Infantile fibrosarcoma; congenital mesoblastic nephroma
CCNB3	*BCOR*-rearranged sarcoma; clear cell sarcoma of kidney;	***PDGFB***	Giant cell fibroblastoma; dermatofibrosarcoma protuberans
CIC	*CIC*-rearranged sarcoma	***PLAG1***	Lipoblastoma; myoepithelioma of soft tissue; myxoid leiomyosarcoma
~~*EPC1*~~	Low-grade endometrial stromal sarcoma (rare); ossifying fibromyxoid tumor (rare)	***ROS1***	Inflammatory myofibroblastic tumor
EWSR1	Ewing sarcoma; clear cell sarcoma; desmoplastic small round cell tumor; angiomatoid fibrous histiocytoma; myoepithelial tumors of soft tissue; extraskeletal myxoid chondrosarcoma; myxoid liposarcoma; sclerosing epithelioid fibrosarcoma	***SS18***	Synovial sarcoma
FOXO1	Alveolar rhabdomyosarcoma	***STAT6***	Solitary fibrous tumor
FUS	Myxoid liposarcoma; low-grade fibromyxoid sarcoma; sclerosing epithelioid fibrosarcoma; Ewing sarcoma; angiomatoid fibrous histiocytoma; extraskeletal myxoid chondrosarcoma	***TFE3***	Alveolar soft part sarcoma; PEComa; epithelioid hemangioendothelioma; ossifying fibromyxoid tumor
GLI1	Plexiform fibromyxoma; gastroblastoma; pericytoma with t(7;12)	***TCF12***	Extraskeletal myxoid chondrosarcoma
HMGA2	Lipoma; deep angiomyxoma	***TAF15***	Extraskeletal myxoid chondrosarcoma
JAZF1	Endometrial stromal nodule; low-grade endometrial stromal sarcoma	***TFG***	Extraskeletal myxoid chondrosarcoma; inflammatory myofibroblastic tumor
~~*MEAF6*~~	Low-grade endometrial stromal sarcoma (rare); ossifying fibromyxoid tumor (rare)	***USP6***	Nodular fasciitis; aneurysmal bone cyst; fibro-osseus pseudotumor of digits; myositis ossificans
MKL2	Chrondroid lipoma; Ectomesenchymal chrondromyxoid tumor	***YWHAE***	High-grade endometrial stromal sarcoma; *YWHAE*-rearranged sarcoma



69-year-old with abdominal discomfort found to have peritoneal discomfort

“Possible primary sites include but are not limited to lung, breast, upper GI, and pancreatobiliary; clinical and radiographic correlation is needed”

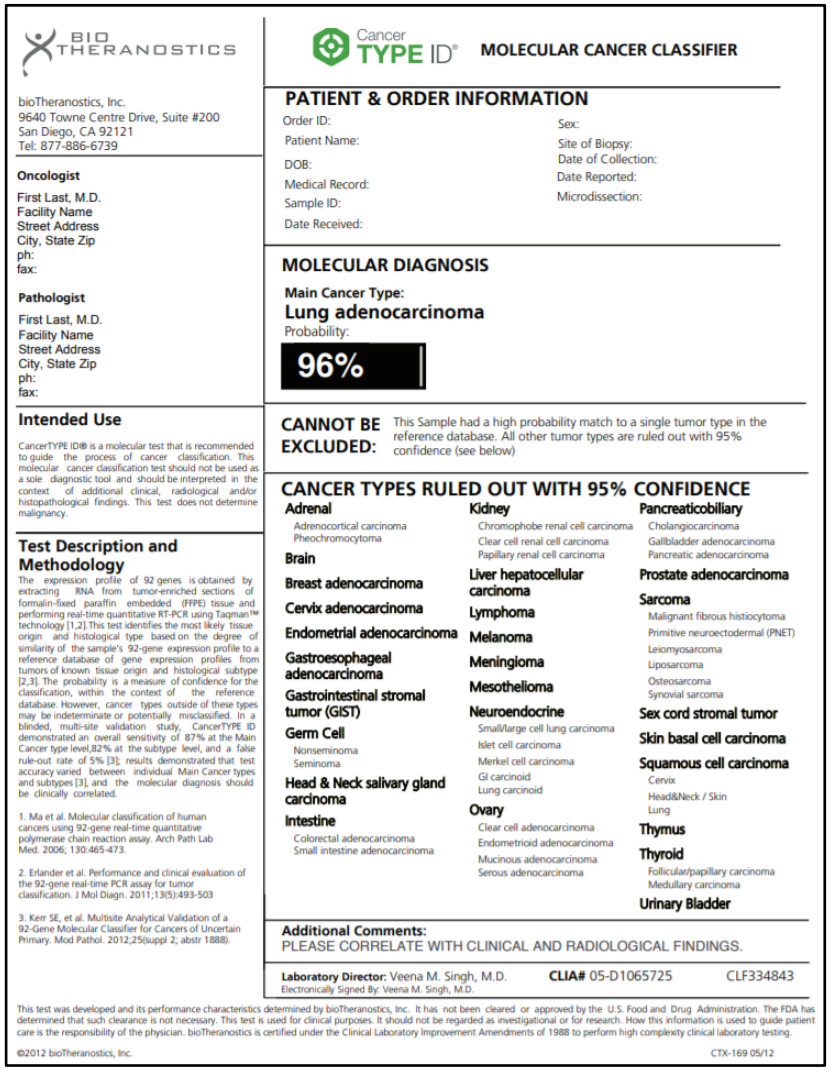




CK7+; CK20, CDX2, TTF-1, GATA-3, PAX8, calretinin, (SATB2, ER, albumin ISH,) pan-TRK, PD-L1-

What Happens When We Abdicate Responsibility

- "The tumor was sent for genetic testing that returned an ovarian origin at >90% probability."
- "The patient has been receiving neoadjuvant carboplatin/taxol"



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ph:
fax:

Intended Use

CancerTYPE ID® is a molecular test that is recommended to guide the process of cancer classification. This molecular cancer classification test should not be used as a sole diagnostic tool and should be interpreted in the context of additional clinical, radiological and/or histopathological findings. This test does not determine malignancy.

Test Description and Methodology

The expression profile of 92 genes is obtained by extracting RNA from tumor-enriched sections of formalin-fixed paraffin embedded (FFPE) tissue and performing real-time quantitative RT-PCR using Taqman™ technology [1,2].This test identifies the most likely tissue origin and histological type based on the degree of similarity of the sample's 92-gene expression profile to a reference database of gene expression profiles from tumors of known tissue origin and histological subtype [2,3]. The probability is a measure of confidence for the classification, within the context of the reference database. However, cancer types outside of these types may be indeterminate or potentially misclassified. In a blinded, multi-site validation study, CancerTYPE ID demonstrated an overall sensitivity of 87% at the Main Cancer type level,82% at the subtype level, and a false rule-out rate of 5% [3]; results demonstrated that test accuracy varied between individual Main Cancer types and subtypes [3], and the molecular diagnosis should be clinically correlated.

1. Ma et al. Molecular classification of human cancers using 92-gene real-time quantitative polymerase chain reaction assay. Arch Path Lab Med. 2006; 130:465-473.

2. Erlander et al. Performance and clinical evaluation of the 92-gene real-time PCR assay for tumor classification. J Mol Diagn. 2011;13(5):493-503

3. Kerr SE, et al. Multisite Analytical Validation of a 92-Gene Molecular Classifier for Cancers of Uncertain Primary. Mod Pathol. 2012;25(suppl 2; abstr 1888).

Cancer TYPE ID® **MOLECULAR CANCER CLASSIFIER**

PATIENT & ORDER INFORMATION

Order ID:
Patient Name:
DOB:
Medical Record:
Sample ID:
Date Received:
Sex:
Site of Biopsy:
Date of Collection:
Date Reported:
Microdissection:

MOLECULAR DIAGNOSIS

Main Cancer Type:
Lung adenocarcinoma
Probability:
96%

CANNOT BE EXCLUDED: This Sample had a high probability match to a single tumor type in the reference database. All other tumor types are ruled out with 95% confidence (see below)

CANCER TYPES RULED OUT WITH 95% CONFIDENCE

Adrenal
Adrenocortical carcinoma
Pheochromocytoma

Brain

Breast adenocarcinoma

Cervix adenocarcinoma

Endometrial adenocarcinoma

Gastroesophageal adenocarcinoma

Gastrointestinal stromal tumor (GIST)

Germ Cell
Nonseminoma
Seminoma

Head & Neck salivary gland carcinoma

Intestine
Colorectal adenocarcinoma
Small intestine adenocarcinoma

Kidney
Chromophobe renal cell carcinoma
Clear cell renal cell carcinoma
Papillary renal cell carcinoma

Liver hepatocellular carcinoma

Lymphoma

Melanoma

Meningioma

Mesothelioma

Neuroendocrine
Small/large cell lung carcinoma
Islet cell carcinoma
Merkel cell carcinoma
GI carcinoid
Lung carcinoid

Ovary
Clear cell adenocarcinoma
Endometrioid adenocarcinoma
Mucinous adenocarcinoma
Serous adenocarcinoma

Pancreaticobiliary
Cholangiocarcinoma
Gallbladder adenocarcinoma
Pancreatic adenocarcinoma

Prostate adenocarcinoma

Sarcoma
Malignant fibrous histiocytoma
Primitive neuroectodermal (PNET)
Leiomyosarcoma
Liposarcoma
Osteosarcoma
Synovial sarcoma

Sex cord stromal tumor

Skin basal cell carcinoma

Squamous cell carcinoma
Cervix
Head&Neck / Skin
Lung

Thymus

Thyroid
Follicular/papillary carcinoma
Medullary carcinoma

Urinary Bladder

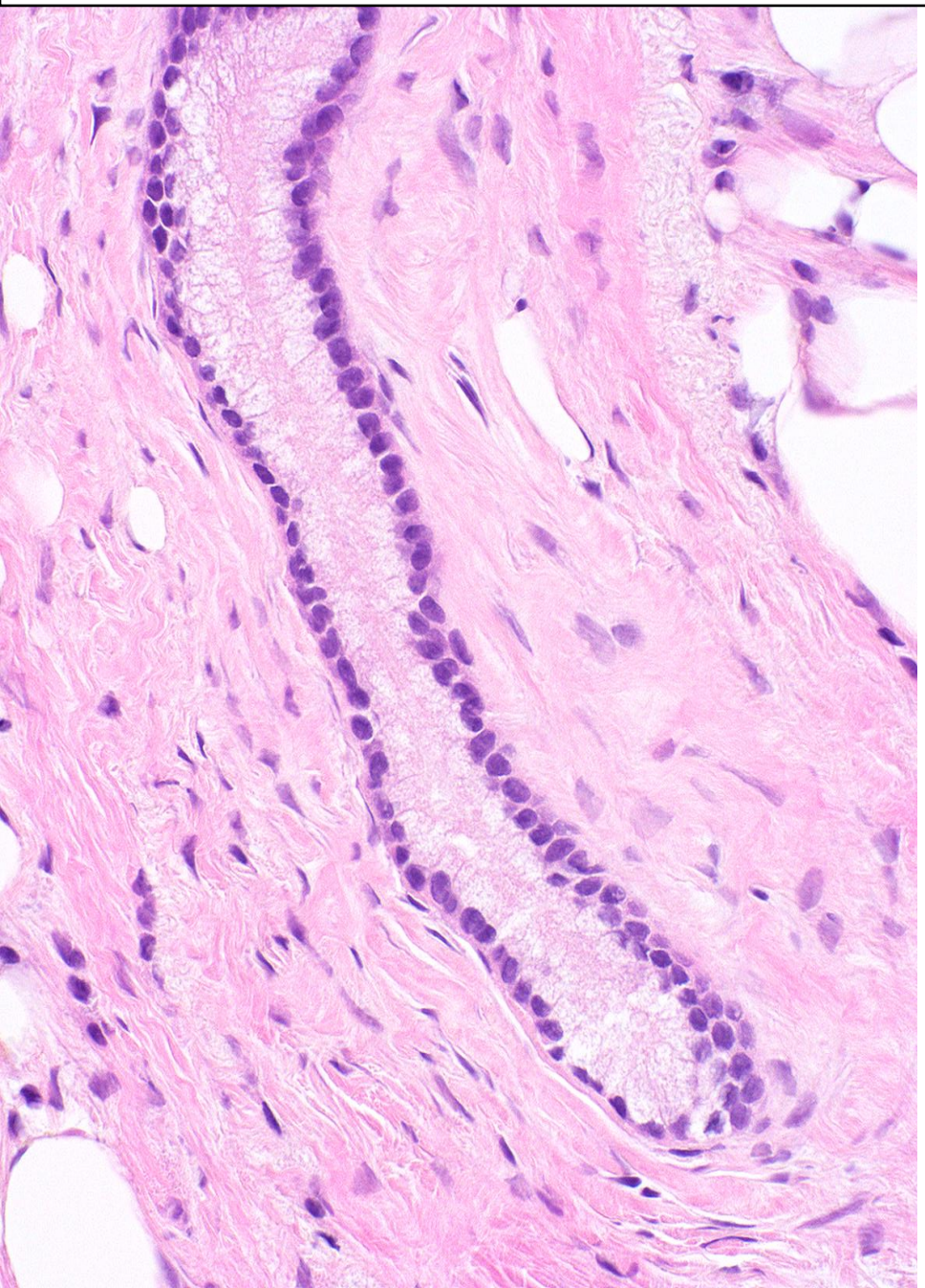
Additional Comments:
PLEASE CORRELATE WITH CLINICAL AND RADIOLOGICAL FINDINGS.

Laboratory Director: Veena M. Singh, M.D. **CLIA#** 05-D1065725 CLF334843
Electronically Signed By: Veena M. Singh, M.D.

This test was developed and its performance characteristics determined by bioTheranostics, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. How this information is used to guide patient care is the responsibility of the physician. bioTheranostics is certified under the Clinical Laboratory Improvement Amendments of 1988 to perform high complexity clinical laboratory testing.

CTX-169 05/12

I requested a block for SMAD4 and MMR IHC



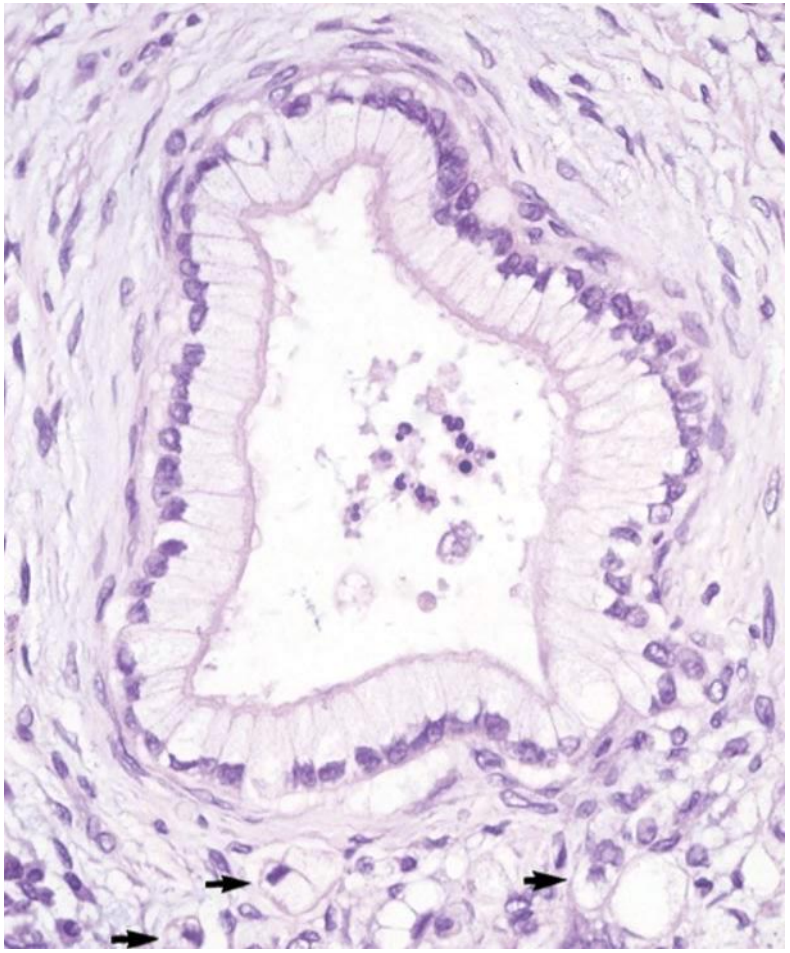
ORIGINAL ARTICLES

Foamy Gland Pattern of Pancreatic Ductal Adenocarcinoma

A Deceptively Benign-Appearing Variant

Adsay, Volkan M.D.; Logani, Sanjay M.D.; Sarkar, Fazlul Ph.D.; Crissman, John M.D.; Vaitkevicius, Vainitus M.D. **Author Information**

The American Journal of Surgical Pathology: April 2000 - Volume 24 - Issue 4 - p 493-504




BIO THERANOSTICS
Specimen Shipping Kit
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#UIHCStrong

Multiclass cancer diagnosis using tumor gene expression signatures

Sridhar Ramaswamy*†, Pablo Tamayo*, Ryan Rifkin*‡, Sayan Mukherjee*‡, Chen-Hsiang Yeang*§, Michael Angelo*, Christine Ladd*, Michael Reich*, Eva Latulippe¶, Jill P. Mesirov*, Tomaso Poggio‡, William Gerald¶, Massimo Loda†||, Eric S. Lander*,**, and Todd R. Golub*†††‡‡

*Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research, Cambridge, MA 02138; Departments of †Adult and ††Pediatric Oncology, Dana–Farber Cancer Institute/Harvard Medical School, Boston, MA 02115; ||Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115; ¶Department of Pathology, Memorial Sloan–Kettering Cancer Center, New York, NY 10021; and Departments of **Biology, §McGovern Institute, Center for Brain and Computational Learning, and ‡Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139

Contributed by Eric S. Lander, October 23, 2001

The optimal treatment of patients with cancer depends on establishing accurate diagnoses by using a complex combination of clinical and histopathological data. In some instances, this task is difficult or impossible because of atypical clinical presentation or histopathology. To determine whether the diagnosis of multiple common adult malignancies could be achieved purely by molecular classification, we subjected 218 tumor samples, spanning 14 common tumor types, and 90 normal tissue samples to oligonucleotide microarray gene expression analysis. The expression levels of 16,063 genes and expressed sequence tags were used to evaluate the accuracy of a multiclass classifier based on a support vector machine algorithm. Overall classification accuracy was 78%, far exceeding the accuracy of random classification (9%). Poorly differentiated cancers resulted in low-confidence predictions and could not be accurately classified according to their tissue of origin, indicating that they are molecularly distinct entities with dramatically different gene expression patterns compared with their well differentiated counterparts. Taken together, these results demonstrate the feasibility of accurate, multiclass molecular cancer classification and suggest a strategy for future clinical implementation of molecular cancer diagnostics.

Cancer classification relies on the subjective interpretation of both clinical and histopathological information with an eye toward placing tumors in currently accepted categories based on the tissue of origin of the tumor. However, clinical information can be incomplete or misleading. In addition, there is a wide spectrum in cancer morphology and many tumors are atypical or lack morphologic features that are useful for differential diagnosis (1). These difficulties can result in diagnostic confusion, prompting calls for mandatory second opinions in all surgical pathology cases (2). In the aggregate, these are significant limitations that may hinder patient care, add expense, and confound the results of clinical trials.

Molecular diagnostics offer the promise of precise, objective, and systematic human cancer classification, but these tests are not widely applied because characteristic molecular markers for most solid tumors have yet to be identified (3). Recently, DNA microarray-based tumor gene expression profiles have been used for cancer diagnosis. However, studies have been limited to few cancer types and have spanned multiple technology platforms complicating comparison among different datasets (4–10). The feasibility of cancer diagnosis across all of the common malignancies based on a single reference database has not been explored. In addition, comprehensive gene expression databases have yet to be developed, and there are no established analytical methods capable of solving complex, multiclass, gene expression-based classification problems.

To address these challenges, we created a gene expression database containing the expression profiles of 218 tumor samples representing 14 common human cancer classes. By using an innovative analytical method, we demonstrate that accurate multiclass cancer classification is indeed possible, suggesting the feasibility of molecular cancer diagnosis by means of comparison with a comprehensive and commonly accessible catalog of gene expression profiles.

Materials and Methods

Snap-frozen human tumor and normal tissue specimens, spanning 14 different tumor classes, were obtained from the National Cancer Institute/Cooperative Human Tissue Network, Massachusetts General Hospital Tumor Bank, Dana–Farber Cancer Institute, Brigham and Women's Hospital, Children's Hospital (all in Boston), and Memorial Sloan-Kettering Cancer Center (New York). Tissue was collected and studied under an anonymous discarded tissue protocol approved by the Dana–Farber Cancer Institute Institutional Review Board.

Initial diagnoses were made at university hospital referral centers by using all available clinical and histopathological information. Tissues underwent centralized clinical and pathology review at the Dana–Farber Cancer Institute and Brigham and Women's Hospital (by M.L.) or Memorial Sloan-Kettering Cancer Center (by E.L. and W.G.) to confirm initial diagnosis of site of origin and histological type. All tumors were biopsy specimens from primary sites (except where noted) obtained before any treatment and were enriched in malignant cells (>50%) but otherwise unselected. Normal tissue RNA (Biochain, Hayward, CA) was from snap-frozen autopsy specimens collected through the International Tissue Collection Network.

"Hybridization targets" were prepared with RNA from whole tumors by using published methods (4). Targets were hybridized sequentially to oligonucleotide microarrays [Hu6800 and Hu35KsubA GeneChips (Affymetrix, Santa Clara, CA)] containing a total of 16,063 probe sets representing 14,030 GenBank and 475 The Institute for Genomic Research (TIGR) accession nos., and arrays were scanned by using standard Affymetrix protocols and scanners. For subsequent analysis, each probe set was considered as a separate gene. Expression values for each gene were calculated by using Affymetrix GENECHIP analysis software.

Of 314 tumor and 98 normal tissue samples processed, 218 tumor and 90 normal tissue samples passed quality control criteria and were used for subsequent data analysis. The remaining 104 samples either failed quality control measures of the amount and quality of RNA, as assessed by spectrophotometric measurement of OD and agarose gel electrophoresis, or yielded

Abbreviations: SVM, support vector machine; OVA, one vs. all; S2N, signal to noise.

††To whom reprint requests should be addressed at: Dana-Farber Cancer Institute, 44 Binney Street, Dana 640, Boston, MA 02115. E-mail: golub@genome.wi.mit.edu.

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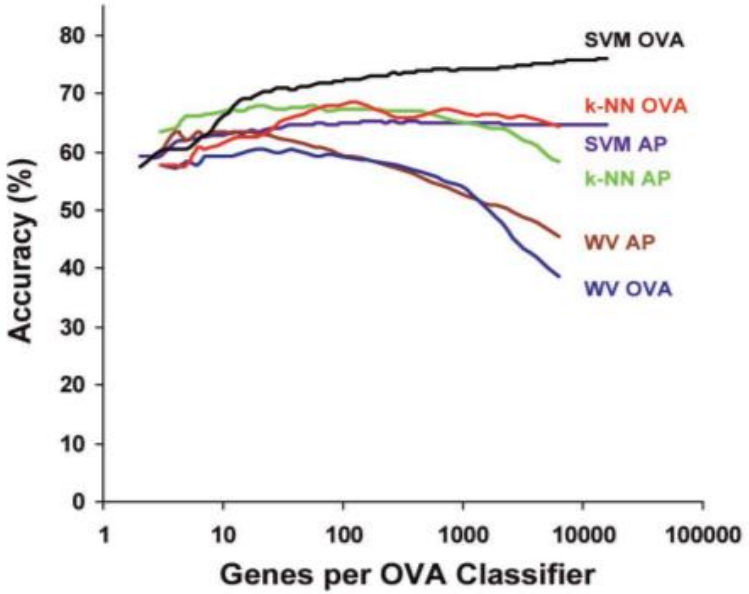
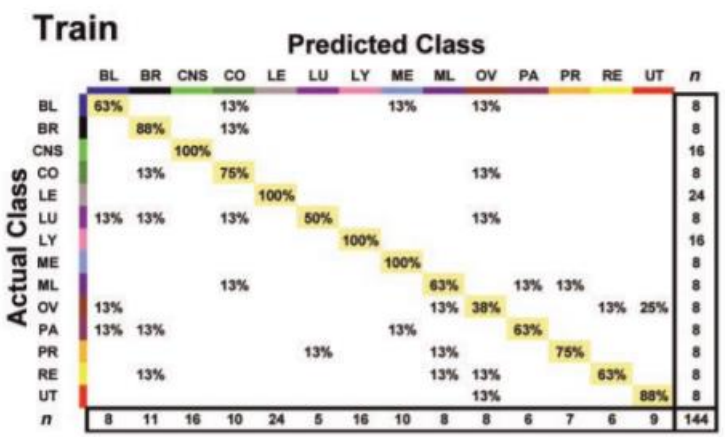


Fig. 5. Multiclass classification as a function of gene number. Training and test datasets were combined (190 tumors; 14 classes), then were randomly split into 100 training and test sets of 144 and 46 samples (all primary tumors) in a class-proportional manner. SVM OVA prediction was performed, and mean classification accuracy for the 100 splits was plotted as a function of number of genes used by each of the 14 OVA classifiers, showing decreasing prediction accuracy with decreasing gene number. Results using other algorithms (*k*-NN, *k*-nearest neighbors; WV, weighted voting) and classification schemes (AP, all-pairs) are also shown.



Biotheranostics CancerTYPE ID

- Real time reverse-transcriptase PCR assay analyzing 87 tumor-associated and 5 reference genes
- Recognizes 54 tumor types
  - One of the tumor types is “sarcoma”
  - There are 113 distinct soft tissue neoplasms
- Compares gene expression to a reference database of >2,000 tumors
- Provides a probability score based on closeness of match
- Sample: FFPE – same as for IHC

Development of Cancer Type ID

- Started with 22,000-gene microarray
- 578 tumors from 39 tumor types
- Biostatistical methods to arrive at a “compact” classifier (wanted to work with a 96-well plate)
- All genes in classifier expressed by multiple tumor types (no magic bullets)

Ma XJ, et al. *Arch Pathol Lab Med*. 2006;130:465-473.

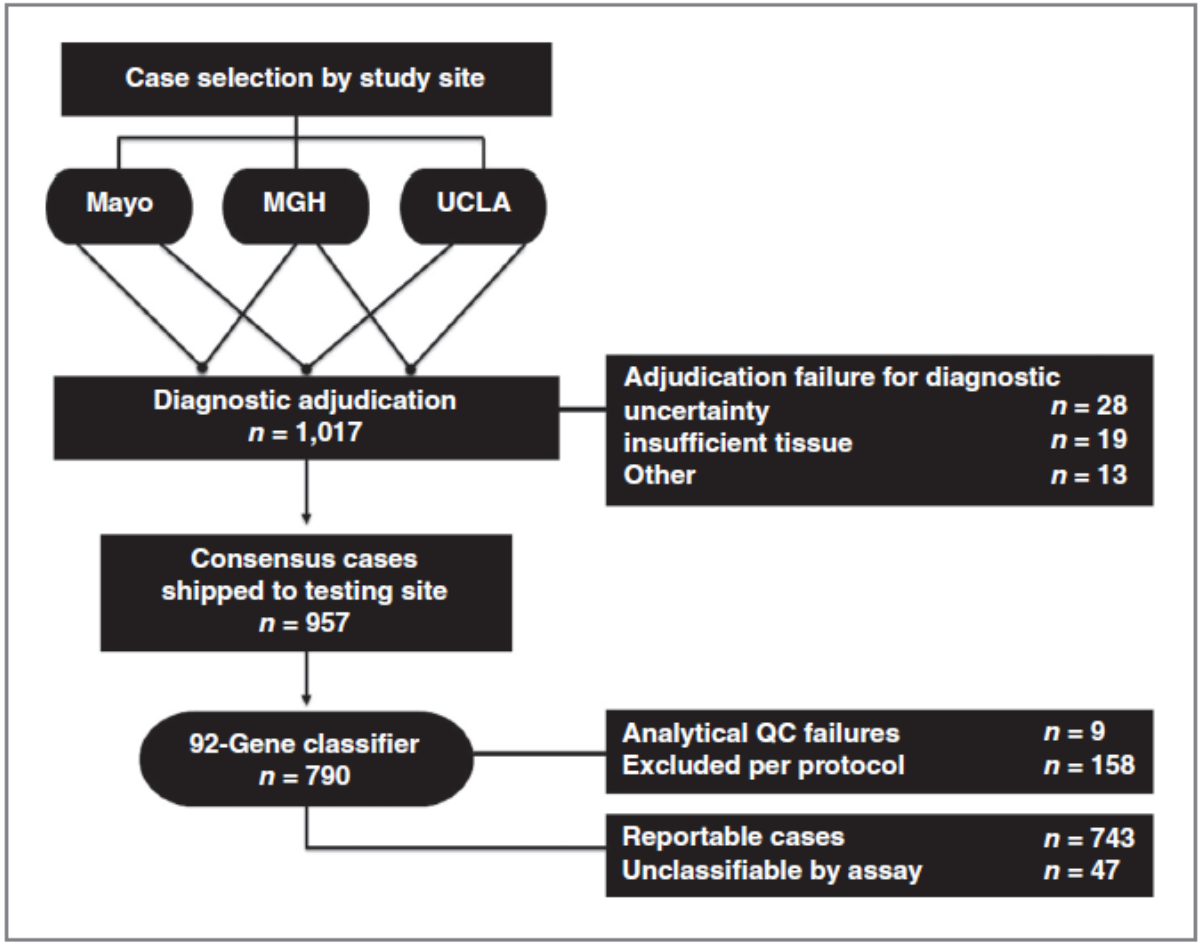
Table 3. List of Selected 87 Genes

Accession	Gene Symbol	Description*
AA456140	*PANX3*	Pannexin 3
AA745593	*BATF*	Basic leucine zipper transcription factor, ATF-like
AA765597	*SPRED2*	Sprouty-related, EVH1 domain containing 2
AA782845	*SLC35F3*	Solute carrier family 35, member F3
AA865917		Hypothetical LOC389142
AA946776	*FGF9*	Fibroblast growth factor 9 (glia-activating factor)
AA993639	*FLJ10748*	Hypothetical protein FLJ10748
AB038160	*TMPRSS3*	Transmembrane protease, serine 3
AF104032	*SLC7A5*	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 5
AF133587	*RTDR1*	Rhabdoid tumor deletion region gene 1
AF301598	*EMX2*	Empty spiracles homolog 2 (Drosophila)
AF332224	*CYorf15A*	Chromosome Y open reading frame 15A
AI041545	*KDELR2*	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2
AI147926	*CSF2RB*	Colony-stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
AI309080	*KCNJ11*	Potassium inwardly rectifying channel, subfamily J, member 11
AI341378	*CPEB2*	Cytoplasmic polyadenylation element binding protein 2
AI457360	*ERN2*	Endoplasmic reticulum to nucleus signalling 2
AI620495	*MEIS1*	Meis1, myeloid ecotropic viral integration site 1 homolog (mouse)
AI632869	*UPK1B*	Uroplakin 1B
AI683181	*PRDM6*	PR domain containing 6
AI685931	*KIBRA*	KIBRA protein
AI802118	*SLC6A13*	Solute carrier family 6 (neurotransmitter transporter, GABA), member 13
AI804745		
AI952953		
AI985118	*C14orf105*	Chromosome 14 open reading frame 105
AJ000388	*CAPN6*	Calpain 6
AK025181	*LOC91464*	RAX-like homeobox
AK027147	*TITF1*	Hypothetical protein LOC253970
AK054605	*FLJ11539*	Hypothetical protein FLJ11539
AL023657	*SH2D1A*	SH2 domain protein 1A, Duncan disease (lymphoproliferative syndrome)
AL039118	*FOXG1B*	Forkhead box G1A
AL110274		
AL157475	*C8orf13*	Chromosome 8 open reading frame 13
AW118445	*CELSR2*	Cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, *Drosophila*)
AW194680	*HOXD11*	Homeobox D11
AW291189		Hypothetical LOC388416
AW298545	*KIAA1904*	KIAA1904 protein
AW445220	*LY6K*	Lymphocyte antigen 6 complex, locus K
AW473119	*ESR1*	Estrogen receptor 1
AY033998	*ELAVL4*	ELAV (embryonic lethal, abnormal vision, *Drosophila*)–like 4 (Hu antigen D)
BC000045	*VGLL1*	Vestigial like 1 (*Drosophila*)
BC001293	*HOXC10*	Homeobox C10
BC001504	*PYCR1*	Pyrroline-5-carboxylate reductase 1
BC001639	*SLC43A1*	Solute carrier family 43, member 1
BC002551	*CDCA3*	Cell division cycle associated 3
BC004331	*HSDL2*	Hydroxysteroid dehydrogenase like 2
BC004453	*HTR3A*	5-hydroxytryptamine (serotonin) receptor 3A
BC005364	*C10orf59*	Chromosome 10 open reading frame 59
BC006537	*HOXA9*	Homeobox A9
BC006881	*PPARG*	Peroxisome proliferative activated receptor, gamma
BC006819	*S100P*	S100 calcium binding protein P
BC008764	*KIF2C*	Kinesin family member 2C
BC008765	*SDC1*	Syndecan 1
BC009084	*SELENBP1*	Selenium binding protein 1
BC009237	*TSHR*	Thyroid-stimulating hormone receptor
BC010626	*KIF12*	Kinesin family member 12
BC011949	*CA2*	Carbonic anhydrase II
BC012926	*EPS8L3*	EPS8-like 3
BC013117	*RGS17*	Regulator of G-protein signalling 17
BC015754	*CADPS*	Ca^{2+}-dependent secretion activator
BC017586	*MGC26610*	Calcyphosine-like
BE552004		CDNA FLJ44317 fis, clone TRACH3000586
BE962007	*COX11*	COX11 homolog, cytochrome c oxidase assembly protein (yeast)
BF224381		Hypothetical LOC400951
BF437393		
BF446419	*PCANAP6*	Prostate cancer–associated protein 6
BF592799	*PRKCQ*	Protein kinase C, theta
BI493248	*IBSP*	Integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II)
H05388	*ZNF365*	Hypothetical protein LOC283045
H07885		Transcribed locus
H09748	*BCL11B*	B-cell CLL/lymphoma 11B (zinc finger protein)
M95585	*HLF*	Hepatic leukemia factor

Table 3. Continued

Accession	Gene Symbol	Description*
N64339	*GJB6*	Gap junction protein, beta 6 (connexin 30)
NM_000065	*C6*	Complement component 6
NM_001337	*CX3CR1*	Chemokine (C-X3-C motif) receptor 1
NM_003914	*CCNA1*	Cyclin A1
NM_004062	*CDH16*	Cadherin 16, KSP-cadherin
NM_004063	*CDH17*	Cadherin 17, LI cadherin (liver-intestine)
NM_004496	*FOXA1*	Forkhead box A1
NM_006115	*PRAME*	Preferentially expressed antigen in melanoma
NM_019894	*TMPRSS4*	Transmembrane protease, serine 4
NM_033229	*TRIM15*	Tripartite motif-containing 15
R15881	*CHRM3*	Cholinergic receptor, muscarinic 3
R45389		CDNA clone IMAGE:4797120
R61469		Transcribed locus, moderately similar to NP_775622.1 hypothetical protein LOC270028 [*Mus musculus*]
X69699	*PAX8*	Paired box gene 8
X96757	*MAP2K6*	Mitogen-activated protein kinase kinase 6

Performance of CancerTYPE ID



Exclusions

- Dx not in assay panel
- Necrosis
- Decalcified

Kerr SE, et al. *Clin Cancer Res*. 2012;18(14):3952-3960.

Performance of CancerTYPE ID

- 790 well-vetted diagnoses (based on clinicopathologic correlation, agreed upon by 3 study pathologists)
- Sensitivity 87%, specificity 96+% for tumor type

	Adrenal	Brain	Breast	Cervix adenocarcinoma	Endometrium	Gastroesophageal	Germ cell	GIST	Head-neck-salivary	Intestine	Kidney	Liver	Lung-adeno/large cell	Lymphoma	Melanoma	Meningioma	Mesothelioma	Neuroendocrine	Ovary	Pancreaticobiliary	Prostate	Sarcoma	Sexcordstromaltumor	Skin basal cell	Squamous	Thymus	Thyroid	Urinary bladder	Unclassified	Total
Adrenal	24																					1								25
Brain		24																				1								25
Breast			16						4																				5	25
Cervix adenocarcinoma				13		1													2	2									7	25
Endometrium				1	10														10										4	25
Gastroesophageal						13	1			2									1	3									5	25
Germ cell							19																					4	2	25
GIST							1	23														1								25
Head-neck-salivary			1						21									1							1				1	25
Intestine						2				17									1										5	25
Kidney											29				1															30
Liver												24								1										25
Lung-adeno/large cell				1					2	1			15					1							1			2	2	25
Lymphoma													1	21								3								25
Melanoma												1			22							2								25
Meningioma																25														25
Mesothelioma																	20			1		2							2	25
Neuroendocrine							1											49												50
Ovary			1	1						2	1								31										4	40
Pancreaticobiliary						1				1										21								1	6	30
Prostate																					25									25
Sarcoma	1										1									1		57								60
Sexcordstromaltumor	4													1									20							25
Skin basal cell																								25						25
Squamous				1						2								1							25				1	30
Thymus											1														5	18	1			25
Thyroid																									1		24			25
Urinary bladder										3															5			14	3	25
Total	29	24	18	17	10	17	22	23	27	28	32	25	16	22	23	25	20	52	45	29	25	67	20	25	38	18	25	21	47	790

Anatomic Site	Sensitivity
Endometrium	48%
Bladder	64%
GEA	65%
Cervix AdCA	72%

“A pathologist looking down the barrel of a light microscope at an H&E-stained slide is doing ‘subcortical integrative genomics.’”

Steven Mentzer, MD
Division of Thoracic Surgery
Brigham and Women’s Hospital

Fourteen Diagnoses to Consider Before Busting on a "Triple-Negative" Malignant Neoplasm

Tumor Type	Additional Diagnostic Markers
Sarcomatoid carcinoma	Add'l broad-spectrum epithelial markers, including HMW-keratin; p40
Poorly differentiated neuroendocrine ca	Chromogranin A, synaptophysin, INSM1, TTF-1, Rb
Adrenal cortical carcinoma	SF1, melan A, synaptophysin, calretinin, inhibin A
Sarcoma	MDM2/CDK4 (esp. undiff. malignant neoplasm); SMA, desmin; CD34 (rarely expressed by carcinoma); add'l dictated by histology
Gastrointestinal stromal tumor	DOG1, KIT
Follicular dendritic cell sarcoma	CD21, CD23, CD35
Acute lymphoblastic leukemia/lymphoma	TdT, CD34, CD43
ALK-positive large cell lymphoma	ALK, CD30
Plasma cell neoplasm (anaplastic)	CD79a, CD138, MUM1, kappa/lambda light chains
Classical Hodgkin lymphoma	CD30, CD15, PAX5
Plasmablastic lymphoma	CD79a, CD138, MUM1, EBV EBER
Melanoma (dedifferentiated)	BRAF V600E; ?melan A, HMB-45
Germ cell tumor	SALL4 or PLAP
Pheochromocytoma/paraganglioma	Chromogranin A, synaptophysin, INSM1, GATA-3

Pearls of Pathology

- Next-generation immunostains include lineage-restricted transcription factors, protein correlates of molecular genetic events and/or lineage-restricted transcription factors
- **Broad-spectrum keratin/CD45/S-100** (though I prefer **SOX10**) has stood the test of time as the screening pattern in most "undifferentiated malignant neoplasms"
- Broad tumor classes include **carcinoma, lymphoma, melanoma, and sarcoma** BUT ALSO **germ cell tumor, mesothelioma, and pheochromocytoma/paraganglioma**
- Non-canonical expression of broad tumor class screening markers leads to diagnostic confusion
- Broad-spectrum epithelial markers are often expressed by sarcomas, especially those with epithelioid cytomorphology and occ. by small round blue cell sarcomas.

Pearls of Pathology

- **“EMA+ only”** (i.e., keratin and CD45-negative) **lymphomas** may be mistaken for carcinoma
- Melanomas are occasionally broad-spectrum keratin and/or EMA-positive (though EPCAM-negative)
- Most germ cell tumors (excluding seminoma) are broad-spectrum-epithelial marker-positive
- S-100 is sometimes expressed by carcinomas, while SOX10-expression appears restricted to carcinomas with myoepithelial differentiation
- MPNST is S-100/SOX10 weak-to-negative
- Spindle cell melanoma is often negative for melanoma “differentiation markers”

Pearls of Pathology

- **Initial panel in a small round blue cell tumor to include: CD99, desmin, myogenin, TdT, INSM1, pan-keratin, SOX10**
- Novel small round blue cell sarcoma markers include NKX2.2 (Ewing sarcoma), WT-1 (*CIC*-rearranged sarcoma), and BCOR or SATB2 (*BCOR*-associated)
- **Tumors of every broad class can dedifferentiate**
- Consider **dedifferentiated liposarcoma (DDLPS)** when facing an undifferentiated malignant neoplasm in the retroperitoneum, mediastinum, or paratestis
- **Carcinomas with undifferentiated/rhabdoid cytomorphology** occasionally demonstrate inactivation of one or more **SWI/SNF** subunits

Pearls of Pathology

- Most **“stains I hate”** are tumor-associated glycoproteins that have been supplanted by lineage-restricted transcription factors
- (pan)p63 is frequently expressed by adenocarcinomas and lymphomas; **p40** is the **clearly superior** marker of squamous, urothelial, and myoepithelial differentiation
- Vimentin . . . Boo Hiss . . .
- **Broad-spectrum-keratin/CD45/S-100 “triple-negative” neoplasms** include several **“can’t miss” diagnoses**, including seminoma (SALL4+), lymphomas (lymphoblastic lymphoma, Tdt+; several “EMA+ only” lymphomas, panel to include CD43, CD79a, MUM1, ALK, CD30), and neuroendocrine carcinoma (INSM1+)

- Most **“stains I hate”** are tumor-associated glycoproteins that have been supplanted by lineage-restricted transcription factors
- (pan)p63 is frequently expressed by adenocarcinomas and lymphomas; **p40** is the **clearly superior** marker of squamous, urothelial, and myoepithelial differentiation

Big Grove Brewery, Solon

Confluence Brewery, Des Moines

Sutliff Cider, Lisbon

Small Town and “No Town” Iowa is Breathtaking

Sutliff Bridge, Sutliff

Loess Hills State Forest


Backbone State Park

Messages Jason Details

Wed, Feb 24, 4:56 PM

Nice! Aidan can lighten your service load...

Give him the polyps to start.